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OF

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VOL. XIX, PART II

MADISON, WISCONSIN

1919

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ARTHUR BEATTY,
Secretary.

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CONTENTS

NORTH AMERICAN ASCOCHYTAE

A DESCRIPTIVE LIST OF SPECIES; COMPILED BY J. J. DAVIS.

A list of North American species of *Phyllosticta* was published by Ellis & Everhart in 1900 and a similar list of species of *Septoria, Phleospora, Rhabdospora* and *Phlyctaena* prepared by Dr. George Martin and Mr. J. B. Ellis was issued in the *Journal of Mycology,* vol. III[1887]. The present list enumerates species of another of the genera of the same group.

To the genus *Ascochyta* are referred species the sporules of which, as ordinarily observed, are uniseptate and so remain; those in which the sporules remain continuous nearly or quite to the time of full maturity becoming then uniseptate; species in which a majority of the sporules are continuous but a minority uniseptate. In the first two classes 2–3 septate sporules occur rarely. Species in which the sporules are uniseptate until maturity becoming then triseptate are referred to *Stagonospora. Ascochyta* is separated from *Phyllosticta* on the one side and *Stagonospora* on the other by somewhat shadowy lines. Some species that have been placed in this genus I am referring to *Stagonospora* and to *Marssonina.*

1 ASCOCHYTA ACHLYDIS Dearn. (Mycologia **8**: 101–2.)

Spots scattered, numerous small ones, 2mm., mostly sterile, and a few large ones 1 cm. or more in diameter with a central, sharply delimited, thin, arid, deciduous area surrounded by a diffuse dark red or purple brown border 1–5 mm. in width; pycnidia nearly concolorous with the arid area, epiphyllous although visible from the under side, 150–200μ; sporules hyaline, 2–3 guttulate, obscurely uniseptate, rounded at the ends, 14–20 x 5–6½μ. On leaves of *Achlys triphylla.*

2 ASCOCHYTA ACHLYICOLA Ell. & Evht.
Proc. Acad. Nat. Sci. Phila. 1894, p. 364.

Spots suborbicular or irregular with a sordid more or less deciduous center and a broad, shaded, purple margin, 3–15 mm. in diameter; pycnidia few, epiphyllous, innate-prominent, 75μ in diameter; sporules elliptical, hyaline, binucleate soon becoming uniseptate, 5–8 x 2½–3μ. On *Achlys triphylla.*

3 ASCOCHYTA ACTAEAE (Bres.) Davis.
Actinomena actaeae Allesch. *Stagonosporopsis actaeae* Died. *Marssonia actaeae* Bres.

On indefinite, blackened, dying areas of the leaves, the cuticle on the upper surface of which is sometimes wrinkled in dendritic lines; pycnidia mostly epiphyllous, scattered, succineous, globose, 100–130μ in diameter, the walls at first hyphal but at maturity formed of flat polygonal cells, without ostiolar thickening; sporules hyaline, cylindrical with rounded ends, straight or somewhat curved 1– (1–2) septate, 17–24 x 5–6μ (12–28 x 6–7μ). On leaves of *Actaea rubra.*

4 ASCOCHYTA AMPELINA Sacc. Mich.1 :158.

Spots epiphyllous, angular, dark bordered, becoming whitish; pycnidia scattered, punctiform, lenticular, ostiolate, 70μ in diameter; sporules oblong-fusoid, pale olivaceous, uniseptate, not constricted, 10 x 3μ, rarely 12–15 x 3–3½μ, 2–3 septate. On *Vitis.*

5 ASCOCHYTA ASCLEPIADIS Ell. & Evht.
Proc. Acad. Nat. Sci. Phila. 1894, p. 364.

Spots amphigenous, suborbicular, grayish with darker zones and a shaded dark brown border, ½–1 mm.; pycnidia epiphyllous-innate, ostiolate, black, 100–110μ in diameter; sporules oblong-elliptical to ovate-elliptical, hyaline, becoming faintly uniseptate, 6–8 x 3μ. On *Asclepias syriaca.*

6 ASCOCHYTA ASPIDISTRAE. Massee.
Diseases of Cultivated Plants (1910) p. 431, fig. 133.

Spots large, irregular, bleached; pycnidia grouped in blackish streaks which run across the leaf and not along its length; sporules narrow-fusiform, uniseptate, 10–11 x 3–4μ. On leaves of *Aspidistra lurida.*

7 ASCOCHYTA BACCAE Rostr.

Till. Groenl. Svampe, p. 625.

Pycnidia small, grayish brown; sporules hyaline, guttulate, uniseptate, constricted at the septum, 9–12 x 1½–2μ. On fruit of *Empetrum nigrum.*

8 ASCOCHYTA BOERHAAVIAE Tharp.

Mycologia **9**: 106.

Spots suborbicular, dirty brownish grey, 2–4 mm.; pycnidia epiphyllous-innate, dark brown, globose-depressed, ostiolate, 80–120 x 70–105μ; sporules hyaline, guttulate, uniseptate, 12–14 x 3½–4μ. On leaves of *Boerhaavia erecta.*

9 ASCOCHYTA BRESADOLAE Sacc. & Syd.

Sacc. Syll. Fung. **14**:948. *Ascochyta fagopyri* Bres. Hedwigia, **31**: 40.

Spots alutaceous with a darker border above, pale below, 5–9 mm. in diameter; pycnidia epiphyllous, scattered, subglobose-ovoid, ostiolate, 130–140μ; sporules cylindric-oblong, sometimes somewhat curved, uniseptate, constricted at the septum, 16–18 x 6–7μ. On leaves of *Fagopyrum esculentum.*

10 ASCOCHYTA CARTHAGENENSIS Sacc.

Mich. **2**:144.

On indefinite whitish spots on the branches; pycnidia aggregated, lenticular, at first covered, ostiolate, 100μ in diameter; sporules greenish, oblong, obtuse at both ends, uniseptate, constricted at septum, 7–9 x 3–3½μ. On branches of *Manihot carthagenensis.*

11 ASCOCHYTA CASSANDRAE Pk.

38th Report, p. 94.

Spots suborbicular or irregular, reddish-brown or grayish with a reddish-brown margin; pycnidia epiphyllous, minute, erumpent, blackish; sporules hyaline, oblong-fusiform, acute at each end, uniseptate, 10–16 x 3–4μ. On leaves of *Chamaedaphne calyculata.*

12 ASCOCHYTA CEPHALANTHI Ell. & Evht.

Sacc. Syll. Fung. **3**: 392.

Spots orbicular, brownish white with a narrow somewhat raised margin, 3–6 mm. in diameter; pycnidia epiphyllous-

innate, irregularly scattered, depressed-hemispherical, 60–75μ in diameter; sporules from narrow-elliptical to ovate-oblong, brownish, uniseptate, 7–9 x 2½–3μ. On leaves of *Cephalanthus occidentalis.*

13 ASCOCHYTA CHEIRANTHI Bres.

Hedwigia **39**: 326.

Spots scattered, round to oblong, alutaceous to brownish with dark margin; pycnidia epiphyllous, arranged in a circle, pale, 100–140μ; sporules hyaline, oblong to subcylindrical, occasionally somewhat curved, uniseptate, 7–9 x 2½–3½μ. On leaves of *Cheiranthus cheiri.*

14 ASCOCHYTA CHRYSANTHEMI Stevens.

Bot. Gaz. **44**: 246.

Pycnidia "few, immersed, early erumpent, single or scattered, round, hemispherical, amber colored, 100–200 mostly about 150μ, ostiolum central, small, dark bordered, often raised by a short neck, surface reticulate; pycnidia on agar media irregular, often with two ostioles and varying much in size, black in color; mycelium abundant, innate, also superficial, aerial, floccose, richly septate;" sporules "oblong, straight or irregular, 10–20 x 3–6.2μ mostly 6.2 x 10μ, ends obtuse or acute, septum usually one, often obscure, rarely 2 or 3, usually without constriction until germination, protoplasm vacuolate, hyaline or light pink in mass." On *Chrysanthemum indicum* (cult.).

15 ASCOCHYTA CITRULLINA C. O. Smith.

Del. Ag'l Exp. Station Bull. 70.

Diplodina citrullina (C. O. Sm.) Grossenbacher. N. Y. Ag'l Exp. Station, Tech. Bull. **9**:226.

On whitened areas on the stems; pycnidia numerous, depressed-globose, pale brown, ostiolate, with thin-cellular walls, 90–150μ in diameter; sporules hyaline, oblong to obovate, ends rounded, uniseptate, becoming constricted at the septum, 14 x 4–5μ. On stems of *Citrullus vulgaris* (cult.).

16 ASCOCHYTA CLEMATIDINA Thuem.

Pilzfl. Sibir. no. 619.

Spots suborbicular to irregular, brown becoming cinereous with a blackish brown border; pycnidia epiphyllous, prominent,

hemispherical to globose, succineous to light brown, 100–125μ in diameter; sporules oblong, hyaline, 2–4 guttulate, becoming 1–3 septate, 10–18 x 3–7μ. Wrinkling of the cuticle sometimes gives the spots the appearance of bearing radiating whitish fibrils. On leaves of *Clematis*.

16*a* Var. THALICTRI Davis.
Trans. Wis. Acad. **16**: 757.
Pycnidia smaller; sporules 8–10 x 2–3μ. On *Thalictrum dioicum*.

17 ASCOCHYTA COMPOSITARUM Davis.
Trans. Wis. Acad. **19**: 700.
Spots definite, subcircular to irregular, brown, 1–5 cm. long on large indefinite brown areas; pycnidia few, innate, succineous to brown, depressed-globose, ostiolate above, about 100μ in diameter; sporules hyaline, oblong to cylindrical, ends rounded, 4–guttulate, becoming uniseptate, more or less constricted, 14–24 x 4–6μ. On leaves of *Eupatorium urticaefolium*, *Aster Drummondii* and *Helianthus strumosus*. On the thinner leaves of *Eupatorium* the affected areas are lighter colored and less determinate.

17*a* var. PARVA Davis.
Trans. Wis. Acad. **19**: 701.
Sporules 10–15 x 2½–3½μ. On leaves of *Helianthus strumosus*.

18 ASCOCHYTA CONFUSA Ell. & Evht.
Journ. Mycol. **10**: 168.
Spots amphigenous, round or irregular, white, thin, almost transparent with a narrow dark brown raised border, 2–5 mm. in diameter; sporules ovate or elliptical, smoky-hyaline, 7–12 x 3½–4½μ. On leaves of *Smilax hispida* and *sp. indet.*

19 ASCOCHYTA CORNICOLA Sacc.
Mich. **1**:169.
Spots irregular, white with a red border; pycnidia punctiform, lenticular, ostiolate, 80μ in diameter; sporules olive tinted, oblong-elliptical, uniseptate, 7–15 x 3½–6μ. On leaves of *Cornus*.

20 ASCOCHYTA CYCADINA Scalia.

Fungi Sicil. orient. ser. III, p. 12 (1902).

Spots subcircular, white with a red border; pycnidia epiphyllous, black, punctiform, globose-depressed, walls parenchymatous, composed of small polygonal olive-brown cells , ostiolate, up to 300μ in diameter; sporules yellowish, oblong, ends rounded or subacute at base, uniseptate, little or not at all constricted, 10–13 x 3–4μ, borne on filiform basidia of about equal length. On leaves of *Cycas revoluta.*

21 ASCOCHYTA DIANTHI (A. & S.) Lib.

Sphaeria (Depazea) dianthi Alb. & Schw. Lus. tab. VI, fig. 2.

Spots indefinite, pale; pycnidia amphigenous, small, dark brown, ostiolate; sporules hyaline, long fusiform to clavate-fusiform, rounded at ends, with a small appendage, uniseptate, constricted, 14–16 x 3–4½μ. On *Dianthus.*

22 ASCOCHYTA DIAPENSIAE Rostr.

Oest. Groenl. Svampe, p. 28.

On white areas involving the entire leaves; pycnidia epiphyllous, globose, minute; sporules cylindrical, thickened at the end, often uniseptate, 12–15 x 3–5μ. On leaves of *Diapensia lapponica.*

23 ASCOCHYTA FRAGARIAE Sacc.

Mich. 1: 169.

Spots subcircular, becoming white with a reddish black margin; pycnidia globose-lenticular with thin parenchymatous subochraceous walls conspicuously thickened about the broad ostiole, 100μ in diameter; sporules olive-tinted, oblong-fusoid, straight, uniseptate, not constricted, 12–15 x 3–4μ. On leaves of *Fragaria.*

24 ASCOCHYTA FREMONTIAE Hark.

Fungi Pacif. 5: 439.

Pycnidia hypophyllous, scattered, small; sporules brown tinted, subcylindrical, narrower at the ends, flexuous, 1– (1–3) septate, 30–40 x 6–12μ. On leaves of *Fremontia californica.*

25 ASCOCHYTA GRAMINICOLA Sacc.
Mich. **1**: 127.

Spots definite, sordid white, purple bordered, oblong to oval, 5–8 mm. long, or none; pycnidia aggregated, punctiform, lenticular, with distinct parenchymatous, fuligenous walls, ostiolate, 100–120μ in diameter; sporules hyaline, fusoid to ovate-fusoid, 10–20 x 2½–4μ. On leaves of grasses. There is apparently some confusion of this with *Darluca filum* (Biv.) Cast.

26 ASCOCHYTA HANSENI Ell. & Evht.
Bull. Torr. Bot. Club **24**: 464.

Spots amphigenous, irregular, definite, livid purple above, paler and sub-rufous below, 2–10 mm. in diameter; sporules brown tinted, oblong-cylindrical, obtuse, slightly curved, 1– (1–2) septate, not constricted, 15–20 x 6μ. On leaves of *Arbutus Menziesii.*

27 ASCOCHYTA GARRETTIANA Syd.
Ann. Mycol. **3**: 185.

Immaculate; pycnidia on leaves, rarely on stems also, black, globose, about 175–250μ in diameter; sporules hyaline, granular, cylindrical, rounded at the ends, usually straight, uniseptate, 11–20 x 2½–3½μ. On *Orthocarpus Tolmiei.*

28 ASCOCHYTA IMPERFECTA Pk.
Report for 1911 pp. 21 and 106. (March, 1912).

Spots amphigenous, variably orbicular, semicircular or subtriangular, the larger ones usually terminal or marginal, pale brown or smoky brown, not sharply defined; pycnidia amphigenous, few, depressed, brown or blackish brown, 300–600μ in diameter; sporules variable, hyaline, oblong or subcylindrical, obtuse, continuous or pseudo-uniseptate, 6–15 x 2½–4μ. On leaves of *Medicago sativa.*—"It may be separated from *Ascochyta medicaginis* Bres. by its habitat and smaller perithecia and spores."

29 ASCOCHYTA INFUSCANS Ell. & Evht.
Journ. Mycol. **5**: 148.

Spots indefinite, brown, sometimes faintly zonate or on large indefinite brown areas on leaves, petioles, branches and stems;

pycnidia innate but often pushing up the epidermis, ostiolate, 120–180μ; sporules hyaline, oblong, obtuse, constricted, guttulate, cytoplasm 1–3 divided, 10–18 x 2½–6μ. On *Ranunculus abortivus.*

30 ASCOCHYTA LEDI Rostr.
Fung. Groenl. p. 570.

Pycnidia sphaeroid-lenticular, black, 200–300μ in diameter; sporules oblong, obtuse, uniseptate, 12–13 x 3μ. On branches of *Ledum groenlandicum.*

31 ASCOCHYTA LEONURI Ell. & Dearn.
Proc. Canad. Inst. 1897, p. 92.

Spots numerous, round to angular, thin, arid, 1–1½ mm. in diameter, sometimes confluent; pycnidia visible on both sides, 150–170μ; sporules pale, oblong-cylindrical, uniseptate, 14–17 x 3½μ. On leaves of *Leonurus cardiaca.*

Specimens on *Lycopus americanus* having sporules 8–10 x 4–6μ, uniseptate, constricted, have been referred to this species.

32 ASCOCHYTA LOPHANTHI Davis.
Trans. Wis. Acad. **14**: 95.

Spots definite, blackish brown, round to oval, margin often repand, 5–20 mm.; sporules short-cylindrical, ends rounded, uniseptate, constricted, 20–30 x 10–12μ. On leaves and sometimes branches of *Agastache scrophulariaefolia.*

32*a* Var. OSMOPHILA Davis.
Ibid. **19**: 700.

Sporules 12–21 x 3–5μ. On leaves of *Agastache Foeniculum.*

33 ASCOCHYTA LYCOPERSICI Brun.
Champ. Saint. 1887, p. 430.

Spots large, suborbicular to irregular, red to brownish; pycnidia scattered, black, small; sporules hyaline, oblong, uniseptate, constricted, 8–10 x 2½μ. On leaves of *Lycopersicon esculentum.*

34 ASCOCHYTA MALI Ell. & Evht.
Bull. Torr. Bot. Club, **27**: 56.

"Spots circular, ½–1 cm. in diameter, concave, of a pale brick red color, with the margin narrowly free, sometimes becoming

much larger, extending for 2 cm. and nearly surrounding the limb. These spots appear to be formed from the altered substance of the bark which is changed in color and cracks away, around the margin, from the surrounding bark which remains in its normal condition; perithecia at first solitary, a single one erumpent in the center of the circular disk, finally 2–4 or more scattered on the same disk; sporules oblong or oblong-elliptical, smoky-hyaline, uniseptate, 6–8 x 2½–3½μ." On living limbs of *Pyrus malus*.

35 ASCOCHYTA MEDICAGINIS Bres.

Hedwigia **39**: 326.

(Ascochyta medicaginis Fckl. was referred to *Phyllosticta* by Saccardo.)

Spots amphigenous, small, pale, angular, clustered; pycnidia somewhat flattened at base, apex prominent, pale straw color drying black, walls parenchymatous, 200 x 160μ; sporules hyaline, cylindrical, straight or somewhat curved, becoming uniseptate, 16–26 x 3½–5μ ("12–20 x 3–6"). On leaves of *Medicago lupulina*.

36 ASCOCHYTA MELILOTI (Trel.)

Gloeosporium (Marsonia) meliloti Trel. Trans. Wis. Acad. **6**: 120 (16); *Ascochyta caulicola* Laubert; *Ascochyta lethalis* Ell. & Barth. F. Col. *1808*.

Spots orbicular to elliptical, sordid with a dark purple or brown margin, 2–5 mm. in diameter, often confluent; pycnidia globose, prominent, brown, darker about the ostiole, 100–180μ; sporules hyaline, oblong, uniseptate, constricted, straight or curved, 10–18 x 3½–5½μ. On stems and sometimes leaves of *Melilotus alba*.

37 ASCOCHYTA MARGINATA Davis.

Trans. Wis. Acad. **18**: 263.

Spots circular to subcircular, at first green becoming brown with a paler central portion and a darker periphery and a distinct narrow margin, 5–15 mm. in diameter; pycnidia epiphyllous, scattered, pale brown, irregularly globose with a thin cellular wall and a dark round ring around the ostiole, about 100μ; sporules hyaline, ovoid to oblong with rounded ends, some of them uniseptate, 6–12 x 2–3½μ. On leaves of *Aralia nudicaulis*.

38 ASCOCHYTA MENZIESII Ell. & Evht. "n. sp. in litt."

On leaves of *Arbutus Menziesii.* San Gabriel Mts. McClatchie, Flora of Pasadena.

I have seen no description or specimen of this. A description, apparently, was never published.

39 ASCOCHYTA MENYANTHIS Oud.

Contrib. Fl. Mycol. Pays Bas. **17**: 262.

Spots irregularly scattered, brown, variable in size; pycnidia amphigenous but more abundant below; sporules hyaline, cylindrical, ends rounded, 2–4 guttulate, uniseptate, 14–19 x 2–3½ μ. On leaves of *Menyanthes trifoliata.*

40 ASCOCHYTA OXYBAPHI Trel.

Trans. Wis. Acad. **6**: 121 (17).

Spots rounded, dark brown, 1–2 mm.; pycnidia epiphyllous, brown, blackened about the ostiole, small; sporules hyaline, uniseptate, sometimes constricted 10–17 x 4μ. On leaves of *Oxybaphus nyctagineus.*

41 ASCOCHYTA OXYTROPIDIS Schroet.

Pilz. Labrad. :19.

Immaculate; pycnidia irregularly scattered, black, about 250μ; sporules hyaline, long-ellipsoid, nearly bacillary, often arcuate, ends rounded, uniseptate, 9–11 x 2½–3μ. On dead leaves and petioles of *Oxytropis.*

42 ASCOCHYTA PARASITICA Fautr.

Rev. Mycol. 1891 p. 79.

Spots epiphyllous, white; sporules 6–9 x 3½–4μ. On leaves of *Althaea rosea.*

43 ASCOCHYTA PAULOWNIAE Sacc. & Brun.

Fungi Gall. no. 2241.

Spots epiphyllous, various, brownish grey; pycnidia lenticular, ostiolate, 90μ; sporules olive tinted, fusoid, 4–guttulate, uniseptate, scarcely constricted, 15–18 x 3μ. On leaves of *Paulownia.*

44 ASCOCHYTA PETUNIAE Speg.

Nov. Add. no. 156.

Spots circular becoming angular, fuligenous, zonate; pycnidia black, 100–130μ; sporules hyaline, cylindric-elliptical, uniseptate, little or not at all constricted, 5–8 x 2μ. On leaves of *Petunia.*

45 ASCOCHYTA PHLOGIS Vogl.

Ann. R. Acc. Agr. Torin: 51.

Spots oblong to irregular, sordid to white, sometimes with a brown border; pycnidia gregarious, somewhat prominent, conical, black; sporules hyaline, elliptical, becoming uniseptate, slightly constricted, 10–3μ. On leaves of *Phlox Drummondii* (cult.). Fairman described "subspecies *phlogina*" from American material as follows: "Spots white, irregular or rounded, girt by a brown area of discolored leaf tissue; pycnidia minute, punctiform, generally clustered in the center of the white spots, black; spores uniseptate, hyaline, 10–14 x 3μ." (Ann. Mycol. **8**:323).

46 ASCOCHYTA PIRINA Pegl.

Contr. Micol. Avell. p. 23.

Pycnidia black, 300μ; sporules hyaline, uniseptate, slightly constricted, 12–14 x 4–5μ. On fruit and leaves of *Pyrus communis*.

Spots alutaceous, at length abscissed and falling away; pycnidia with parenchymatous walls thickened about the ostiole, cylindrical, hyaline or pale honey color, uniseptate, pale fuligenous, 150–180μ, ostiole 20μ in diameter; sporules hyaline, uniseptate, not constricted, 12–16 x 4μ borne on very short, somewhat conical, basidia. On *Pyrus arbutifolia* (Saccardo, *N. Giorn. Bot. Ital.* **23**:195).

47 ASCOCHYTA PISI Lib.

Exs. No. 12. Saccardo, F. Herb. Brux. no. 35.

Spots definite, circular, yellowish-brown with a darker margin, sometimes white sometimes dark in the center; pycnidia gregarious, somewhat prominent, depressed-globose, light brown, ostiolate, 100–200μ; sporules hyaline, oblong, ends rounded, straight or somewhat curved, 1– (1–3) septate, somewhat constricted, 14–16 x 4–6μ. On leaves, stems and pods of *Pisum* and *Vicia* and leaves of *Lupinus perennis*.

48 ASCOCHYTA PRIMULAE Trail.

Scot. Nat. 1887, p. 88.

Spots amphigenous, large, white, becoming arid, often with a yellowish border; pycnidia epiphyllous, scattered, pale brown, depressed-globose, ostiolate, 100–110μ; sporules hyaline, cylindrical, obtuse, uniseptate, 5–6 x 2–2½μ. On leaves of *Primula*.

49 ASCOCHYTA QUERCUS Sacc. & Speg.
Mich. **I**:162.

Spots various, becoming whitish; pycnidia punctiform, sublenticular, 80–90μ; sporules hyaline, oblong-ellipsoid, obtuse, uniseptate, more or less constricted, 12 x 3–4½μ. On leaves of *Quercus*._ Perhaps the fungus which Trelease reported under this name as occurring on oak leaves in Wisconsin is not distinct from *Marssonina martini* (Sacc. & Ell.) Magn.

50 ASCOCHYTA QUERCUUM (Cke.) Sacc.
(*Sphaerellopsis quercuum* Cke., Grevillea **12**:23)

Pycnidia hypophyllous, scattered, dark brown, subsuperficial, subglobose, 150μ; sporules hyaline, lanceolate, uniseptate, 16 x 4μ. On leaves of *Quercus virens*.

51 ASCOCHYTA RHEI Ell. & Evht.
Proc. Acad. Nat. Sci. Phila., 1893, p. 160.
(*Phyllosticta rhei* Ell. & Evht. Journ. Mycol. **5**:145.)

Spots mostly marginal, subconfluent, rusty brown, concentrically zoned, either with or without a definite, slightly darker limiting line surrounded by a broad border of light yellow, 1–2 cm. in diameter; pycnidia few, visible on both surfaces of the leaf, slightly prominent, up to 150μ; sporules hyaline, oblong-elliptical, ends rounded, becoming uniseptate and mostly constricted, 5–12 x 2½–4μ. On leaves of *Rheum*.

52 ASCOCHYTA RHYNCHOSIAE (Thuem.) Sacc.
Sacc. Syll. Fung. **3**: 398.

Spots irregular, dark brown with a darker margin; pycnidia epiphyllous, scattered, immersed, black, globose; sporules hyaline, fusiform, acute at each end, uniseptate, 9 x 3μ. On *Rhynchosia simplicifolia*.

53 ASCOCHYTA RUBI Lasch.
Bot. Zeit. (1848) p. 294.

Spots pale; pycnidia subglobose, blackish brown; sporules exuded in white cirri. On *Rubus*.

54 ASCOCHYTA SAMBUCI Sacc.
Mich. **I**: 168.

Spots indefinite, becoming whitish and arid; pycnidia few, punctiform, ostiolate; sporules olivaceous, fusoid, uniseptate, not

constricted, 15–18 x 3–3½μ. On leaves of *Sambucus nigra aurea.*

55 ASCOCHYTA SILENES Ell. & Evht.
Journ. Mycol. **5**:148.

Spots pale yellowish, the entire leaf finally assuming the same color, the spots, which are then hardly discernible becoming paler; pycnidia not confined to the spots but scattered over the entire leaf, erumpent, discoid, broadly ostiolate, 120–150μ; sporules hyaline, oblong, rounded at the ends, becoming 1– (1–2) septate, 10–14 x 2½–3μ. On leaves and stems of *Silene antirrhina.*

56 ASCOCHYTA SISYMBRII Ell. & Kell.
Journ. Mycol. **5**:142.

Immaculate; pycnidia amphigenous, scattered, innate, black, depressed-globose, 200–285μ in diameter, 100–195μ high, ostiole 20–25μ in diameter; sporules subhyaline, vermiform-cylindrical, mostly uniseptate, 18–45 x 3½–6μ, mostly 25–38 x 4–5μ. On leaves and petioles of *Sisymbrium canescens.* "Not to be confounded with *Septoria sisymbrii* Ell. which is on spots and has smaller spores."

57 ASCOCHYTA SMILACIS Ell. & Evht.
Journ. Mycol. **8**: 12. Not *A. smilacis* E. & M. which is *Stagonospora smilacis* (E. & M.) Sacc.

"Spots small (1–4 mm.) of irregular shape, dirty white with a brown border or large brown areas 1–2 cm. in diameter; pycnidia scattered, epiphyllous but mostly visible from below, punctiform, black; sporules elliptical, obtuse, smoky hyaline, uniseptate, not constricted, 6–8 x 4μ." On *Smilax hispida.*

58 ASCOCHYTA SOLANI-NIGRI Died.
Hedwigia **42**: Beiblatt (166).

Spots scattered, orbicular or oval, arid, whitish with a dark margin; pycnidia globose, brown, thin walled, ostiolate, about 80μ; sporules cylindrical with rounded ends, straight or a little curved, uniseptate, not constricted, 6–8 x 3μ. On leaves of *Solanum Melongena.*

59 ASCOCHYTA SPARTINAE Trel.

Trans. Wis. Acad. **6**:121 (17).

Spots small, rounded, pale yellow; sporules hyaline, flesh color in mass, straight or slightly curved, usually a little narrower at one end, 1– (1–3) septate, averaging about 35 x 3μ. On leaves of *Spartina Michauxiana.*

60 ASCOCHYTA SYMPHORICARPOPHILA Fairman.

Ann. Mycol. **8**:323.

Spots irregular, brown, mostly marginal; pycnidia epiphyllous, black, minute; sporules hyaline, elliptical, ends rounded, uniseptate, not constricted, 6–9 x 3–4μ. On leaves of *Symphoricarpos racemosus.* Said to differ from *A. symphoriae* Br. & Har. in the somewhat shorter sporules not being constricted.

61 ASCOCHYTA TERETIUSCULA Sacc. & Roum. (?)

Mich. **2**:621.

Immaculate; pycnidia innate, punctiform, ostiolate, 100–110μ; sporules hyaline, cylindrical, ends rounded, uniseptate, scarcely constricted, 10–14 x 2½μ. On leaves of *Cyperus.*

62 ASCOCHYTA THASPII Ell. & Evht.

Journ. Mycol. **5**:148.

Spots amphigenous, suborbicular, dirty brown, with a definite margin and surrounded by a narrow yellow border, about 1½ cm. in diameter; pycnidia entirely buried in the substance of the leaf and scarcely visible, pale, 100–120μ in diameter; sporules cylindrical, ends rounded, 3–4 guttulate, uniseptate, 18–30 x 4–8μ. On leaves of *Thaspium barbinode* and *Zizia aurea.*

62*a* var. SANICULAE (Davis).

(*Ascochyta saniculae* Davis. Trans. Wis. Acad. **18**[1]:105.)

On indefinite, discolored, more or less mottled areas which may include the entire leaf; pycnidia scattered, innate, globose to lenticular, light reddish brown with a dark ring around the ostiole, 100–170μ; sporules hyaline, cylindrical, usually straight, quadriguttulate, uniseptate, 20–30 x 4–6μ. The pycnidia are very inconspicuous. They are most readily seen by transmitted light when they show as translucent points. On *Sanicula marilandica.*

63 ASCOCHYTA TRELEASEI Sacc. & Vogl.

Ascochyta sp. Trelease. Trans. Wis. Acad. **6**:121 (17).

Spots circular, brown, about 3 mm. in diameter; pycnidia

epiphyllous, brown, blackened about the ostiole, 100–200μ; sporules hyaline, ovoid, oblong, or reniform, 2–4 guttulate, becoming uniseptate, frequently constricted, 7–14 x 5–7μ. On leaves of *Silphium integrifolium* and *Vernonia noveboracensis.*

64 ASCOCHYTA VERATRINA Ell. & Evht.

Proc. Acad. Nat. Sci. Phila. 1894 p. 364.

Pycnidia scattered, sunk in the substance of the leaf with the apex and conic-papilliform ostiolum erumpent, about ⅓ mm. in diameter; sporules hyaline, cylindrical, obtuse, 3–4 guttulate, becoming uniseptate, about 12 x 2⅓–3μ. On dead leaves and petioles of *Veratrum californicum.*

"Differs from *A. Veratri,* Cavarra (Fungi Langobardiae No. 98) in its larger ostiolate perithecia, not on any spots and in its smaller, straight sporules."

65 ASCOCHYTA VIOLAE Sacc. & Speg.

Mich. **1**:163.

Spots various, becoming whitened; pycnidia gregarious, globose-lenticular, walls parenchymatous, blackened about the ostiole, 180–200μ; sporules hyaline, short-fusoid, uniseptate, not constricted, 15–18 x 3½–4μ. On leaves of *Viola.*

66 ASCOCHYTA WISCONSINA Davis.

Trans. Wis. Acad. **18**[1]:101.

Spots orbicular to elliptical, grey with a narrow black border and frequently zonate above, brown to olivaceous with a less distinct border below, 1–3 cm. long; pycnidia epiphyllous, scattered, brown, prominent, globose to sublenticular, 85–110μ; sporules hyaline, ovoid to oblong, 4–8 x 2½–3½μ. Some of the longer sporules have a medium septum. On leaves of *Sambucus canadensis* and *S. racemosa.*

67 ASCOCHYTA ZEICOLA Ell. & Evht.

Hedwigia **42**, Beiblatt (166).

On slightly darker, irregular or sub-elongated areas; pycnidia gregarious, suberumpent, ostiolate, 100–150μ; sporules hyaline, yellowish in mass, oblong-cylindrical, obtuse, uniseptate, not constricted, 6–8 x 1½–2μ. On old stalks of *Zea Mays.* "Very different from *A. Zeina* Sacc. which is on the leaves and has sporules 18 x 7μ."

INDEX TO HOSTS

NOTES ON PARASITIC FUNGI IN WISCONSIN—IV.

J. J. DAVIS.

A provisional list of parasitic fungi in Wisconsin was published in the Transactions of the Wisconsin Academy of Science, Arts & Letters, **17**[2]:846–984. Supplementary notes bearing the title above were issued in the same publication, **18**[1]:78–92 (I) 93–109 (II) and 251–271 (III).

Parasitic fungi were less abundant in Wisconsin than usual in 1915. This may be attributed to a reversal of season in the spring, a warm April having been followed by a cold May.

In the first number of these notes there was mention of the occurrence of *Plasmopara humuli* Miyabe & Takahashi on *Humulus Lupulus* at Racine in southeastern Wisconsin. This seems to be the first, and as yet the only, American locality from which this Japanese mildew has been reported. In September, 1915 it was collected on the same host near Lynxville on the Mississippi and at Gays Mills and Petersburg in the Kickapoo valley in western Wisconsin. At Lynxville the Japanese host *Humulus japonicus* was abundant on vacant lots and it was also observed at Gays Mills in cultivation and as an escape but the mildew was not found on this species.

Dimerosporium collinsii (Schw.) Thuem. is referred to a new genus, *Apiosporina*, by von Hoehnel. *(Fragm. zur Mykol.* no. 506).

For the "black knot" fungus recorded under the name *Plowrightia morbosa* (Schw.) Sacc. the new genus *Dibotryon* is proposed by Theissen and Sydow. *(Ann. Mycol.* **13**: 663.)

The fungus recorded in the provisional list under the name *Dothidella ulmea* (Schw.) Ell. & Evht. and referred to in

"Notes" III:258, as *Euryachora ulmea* (Schw.) Rehm is referred to *Gnomonia* by Theissen and Sydow. Von Thuemen referred it to this genus in *Flora,* 1878, p. 178, and *Gnomonia ulmea* (Schw.) Thuem. was given in Saccardo's *Sylloge Fungorum* **1**: 570 in the section *Dubiae.* Klebahn has shown that *Phleospora ulmi* (Fr.) Wallr. which Fuckel thought to be a conidial state of this fungus is really connected with *Mycosphaerella.*

A form of *Phyllactinia corylea* (Pers.) Karst. occurs at Madison and in Buffalo county near Arcadia on *Quercus velutina* in which a profuse superficial mycelium is developed.

According to Theissen and Sydow *Physalospora ambrosiae* Ell. & Evht. as given in the provisional list is *Phyllachora ambrosiae* (B. & C.) Sacc. (*Ann. Mycol.* **13**: 556).

Gnomonia caryae F. A. Wolf has been collected at Madison on leaves of *Carya ovata* that had borne *Gloeosporium caryae* Ell. & Dearn. the previous year. It occurred both on leaves lying on the ground and those wintered in a wire cage. We find the ascospores about 2μ thick.

Montagnella heliopsidis (Schw.) Sacc. is referred to the genus *Rosenscheldia* by Theissen and Sydow (*Ann. Mycol.* **13**:649).

Phyllachora junci (Fr.) Fckl. is referred to their genus *Endodothella* by Theissen and Sydow (*Ann. Mycol.* **13**:586). On the following page they refer to a collection of *Endodothella strelitziae* (Cke.) Theiss. & Syd. on *Strelitzia angusta* made at Madison by Trelease.

The record of *Exoascus cerasi* (Fckl.) Sacc. in "Notes" II, p. 97 seems to have been due to an error. No Wisconsin specimen of this species is in the herbarium.

The name *Stagonospora smilacis* (E. & M.) Sacc. was used in the provisional list to designate the fungus that causes orbicular, sordid-arid, purple or brown bordered spots on leaves of *Smilax.* As usually collected the pycnidia contain continuous sporules varying in different specimens from oblong-fusoid and up to 21μ long to broad oval or subglobose. This is usually distributed as *Phyllosticta smilacis* Ell. & Mart. which it doubtless is. As the leaf tissue included in the spot usually disintegrates I assume that the sporules seldom reach maturity on the host and

as septate sporules are now and then found, that septation comes with maturity. A collection on *Smilax rotundifolia* from Lynxville bears ovoid or ovate more deeply tinted smaller sporules and is perhaps *Ascochyta confusa* Ell. & Evht. [See Dearness. *Mycologia* **9**: 359–60.]

Septoria canabina West. of the provisional list should be *Septoria cannabis* (Lasch.) Sacc. In a specimen from Lynxville the sporules are 25–45 (mostly 30–36) x 2–2½μ.

The host given as *Rumex altissimus* in the provisional list is probably *R. mexicanus*.

R. E. Stone finds *Septoria ribis* Desm. to be genetically connected with *Mycosphaerella grossulariae* (Fr.) Auersw. (*Phytopathology* **6**:109).

The fungus recorded in the provisional list under the name *Cylindrosporium ribis* Davis is evidently conspecific with Brenckle's *Fungi Dakotenses 320* which was determined by Saccardo as *Septoria sibirica* Thuem. Saccardo gives a description in *Annales Mycologici* **13**:122. This seems quite different from European material distributed under this name.

For the fungus described by Trelease under the name *Ascochyta salicifoliae* and referred to *Septoria* by Berlese & Voglino and by Ellis & Everhart I am using the name *Cylindrosporium salicifoliae* (Trel.) as better expressing the acervular character of the spore body as I find it.

Dr. E. A. Burt of the Missouri Botanical Garden has kindly examined the type of *Gloeosporium (Marsonia) meliloti* Trel. and sent mounted sections thereof. It proves to be the *Ascochyta caulicola* of Laubert and *A. lethalis* Ell. & Barth. R. E. Stone has connected it with an ascigerous form to which he gave the name *Mycosphaerella lethalis* Stone. In the description Trelease designated the spore bodies "Perithecia" which was changed to "acervuli" in the *Sylloge Fungorum* doubtless to conform to the character of the genus to which it was referred. The word pycnidium was not then in use.

Examination of type material of *Gloeosporium populinum* Pk. received from Dr. H. D. House shows it to be the same as

Marssonina rhabdospora (Ell. & Evht.) Magn. Both specific names were published in 1893 but Peck's description probably was issued later in the year than Ellis & Everhart's.

As I see it the fungus known as *Gloeosporium trifolii* Pk. develops, when perfectly formed, which often it is not, a definite pycnidial wall and the sporules, when mature, have a median septum. Occasional sporules develop 2–3 septa as is so frequently the case in *Ascochyta.* I have not had the opportunity to bring them to germination to see if they then become triseptate as is the case in *Stagonospora dearnessii* Sacc. on *Trifolium repens.* What appears to be a state of this, probably immature, has been collected with sporules but about 8 x 2½μ, continuous and what is possibly a spermogonial or microconidial state occurs frequently with sporules 4–8 x 1–1½μ, continuous. In this form the distal portion of the pycnidium is imperfect and it is much like the fungus on *Medicago* known as *Sporonema phacidioides* Desm.

Specimens of *Ramularia ionophila* Davis collected at Long Lake in 1915 show that the spots become light yellowish brown with the death of the included leaf tissue and that the conidia are often catenulate. The spots are usually 2–5 mm. in diameter and the limiting veinlets sometimes give the appearance of a narrow colored margin. It was confined here, as in the type locality, to the single species of host, *Viola canadensis.*

When well developed *Ramularia nemopanthis* Pk. is of the *Ovularia* type, the conidia being continuous, catenulate, 7–15 x 3–6μ.

In "Notes" I: 89–90 it was noted that *Ovularia asperifolii* Sacc. var. *lappulae* Davis seems quite similar to var. *symphytituberosi* Allesch. Jaap has raised the latter to specific rank and referred it to *Ramularia* because of occasional septate conidia *(Ann. Mycol.* **14**: 41). When conidia are borne in chains the proximal members are usually longer than the distal and sometimes septate. I take it that the septum is due to a failure of the abstriction process which becomes less active toward the base of the chain. In such forms the distinction between the genera is difficult to hold. I am inclined to think that it would be better to include in *Ovularia* only species that bear ovoid conidia singly.

The fungus referred to *Fusicladium radiosum* (Lib.) Lind var. *microsporum* (Sacc.) Allesch. in "Notes" III: 256, is perhaps not distinct from *Cladosporium subsessile* Ell. & Barth. The conidia are 12–15 x 4μ, continuous. This has since been collected at Whitehall.

A specimen on *Aster puniceus* was collected in Oconto County, Wisconsin, July 19, 1909, and placed in my herbarium with *Cercosporella cana* Sacc. and *Aster puniceus* was given as a host of this species in the provisional list. Inside the packet I find the following description: On angular or indefinite areas that finally become brown; conidiophores hypophyllous, fasciculate, cylindrical or tapering upward, denticulate, sometimes branched, 20–35 x 5μ; conidia hyaline, obclavate, pluriseptate, straight, or curved, 60–130 x 3μ. In the absence of definite knowledge of the relationship of this to *Cercosporella cana* Sacc. on *Erigeron* and to *C. reticulata* Pk. *C. nivea* Ell. & Barth., *C. ontariensis* Sacc. and *C. dearnessii* Bubak & Sacc. on *Solidago,* I am designating it *Cercosporella cana* Sacc. var. GRACILIS n. var.

Specimens of *Cercospora corni* Davis collected at Gays Mills in September show some of the conidia darker, thicker walled and strongly constricted at the septa, suggesting ultimate division into separate globose cells which might perhaps retain vitality through the winter.

To the original description of *Cercospora ageratoides* Ell. & Evht. *(Journ. Mycol.* **5**: 71) is appended a reference to a form on *Eupatorium album* having shorter (40μ) conidiophores and longer (70–80μ) and narrower (3μ) conidia. In a collection on *Eupatorium urticaefolium* from Lynxville the conidiophores are 20–40 x 3–6μ, and the conidia up to 100 x 3–4½μ, effused over indefinite areas.

Cercospora zebrina Pass. is referred to *C. helvola* Sacc. as a variety by Ferraris *(Fl. Ital. Crypt.* **1**:**8**:423.).

Urocystis waldsteiniae Pk. was inadvertently omitted from the provisional list. It has been collected but once in Wisconsin but it was then abundant at the station which was at Planting Ground lake near Three Lakes.

A. A. Potter reports that the smut given in the provisional list as *Sphacelotheca sorghi* (Lk.) Clinton is *S. cruenta* (Kuehn) Potter. (*Phytopathology* **5**:152–3.)

Puccinia caricis-solidaginis Arth., *P. caricis-asteris* Arth., *P. caricis-erigerontis,* Arth., and *P. dulichii* Syd. are now included in *P. extensicola* Plowr. by Arthur (*Mycologia* **7**:70 and 80–81.)

I am informed by Dr. Arthur that the rust on *Melica striata* that was recorded in "Notes" II under the name *Puccinia melicae* (Erikss.) Syd. is *P. erikssonii* Bubak. It has since been collected at Solon Springs on the same host.

The rust on *Agropyron repens* given in the provisional list under *Puccinia rubigo-vera* (DC.) Wint. is now believed to be *P. agropyri* Ell. & Evht. developing its aecia on Ranunculaceous hosts. *P. tomipara* Trel. probably belongs here also. (*Mycologia* **7**: 73–5.) It has been collected on *Agropyron tenerum* also at Solon Springs where "*Aecidium ranunculacearum*" occurred on *Anemone quinquefolia.*

Cultures made by Dr. Arthur have shown that *Aecidium nesaeae* Ger. is the aecial stage of *Puccinia minutissima* Arth. (*Mycologia* **7**:86).

For the rust of which *Caeoma abietis-canadensis* Farl. is the aecial form Ludwig makes the new combination *Melampsora abietis-canadensis (Phytopath.* **5**: 279). There is objection by some mycologists to the extension of aecial specific names to apply to telial states and the objection is especially cogent when the name is derived from that of the aecial host of a heteroecious species. Many rust names are derived from that of the telial host and it is confusing to have introduced among them an occasional one taken from that of the aecial host. In the present case, as in others, the aecial host bears also telia referred to another species and that is the one that the name would suggest. To the present day uredinologist this is a matter of little importance but when one considers the generations of botanists to come it seems well worth while to remove these obstacles from a path that is none too smooth. I am using in the herbarium *Melampsora populi-tsugae* nom. nov. referring to it specimens on *Populus grandidentata* from Gaslyn (II) and Racine (II, III), and on *Populus tremuloides* from Wausaukee.

For want of another I used in the provisional list the name *Puccinia impatientis* Arth. for a species forming uredinia and telia on *Elymus*. For this I suggest the designation *Puccinia elymi-impatientis* nom. nov. For the rust given in the provisional list as *Puccinia albiperidia* Arth. I am now using the name *Puccinia pringsheimiana* Kleb. as there seems to be no reason for considering the American rust as distinct from that of Europe.

ADDITIONAL HOSTS

Albugo candida (Pers.) Kuntze. On *Dentaria diphylla*. Laona. Oospores only; in leaves.

Basidiophora entospora Roze & Cornu. On *Erigeron canadense*. Long Lake. Monstrous conidia, up to 63 x 30μ, with suppression of conidiophores were found in this collection (Cfr. Farlow, *Botanical Gazette* **7**: 311).

Peronospora potentillae D By. On *Agrimonia mollis*. Lynxville.

Peronospora trifoliorum D By. Collected in small quantity on *Lupinus perennis* at Millston.

In "Notes" I, p. 85 mention was made of the collection of *Synchytrium* at Athelstane on *Rubus hispidus* and on no other host. The station was visited again in July, 1915, but the organism was not found on *Rubus*. It was found however, in small quantity, on *Viola conspersa* and on a single leaf of *Clintonia borealis*. In August collections were made at Solon Springs on *Viola conspersa, Halenia deflexa,* and in small quantity on *Rubus triflorus*. It may be that these represent more than one species but the effects of stage of development, host and environment have not been worked out.

Sphaerotheca humuli fuliginea (Schl.) Salm. On *Bidens cernua*. Madison.

Microsphaera alni (Wallr.) Wint. On *Ostrya virginiana*. Lynxville. *Juglans cinerea,* Madison. *Lonicera hirsuta,* Solon Springs.

Microsphaera diffusa C. & P. On *Symphoricarpos orbiculatus* (cult.) Madison. (Denniston and Trelease.)

Erysiphe graminis DC. Conidia on *Poa triflora.* Solon Springs. Perithecia on *Hordeum vulgare* (cult.) Madison (C. S. Reddy).

Erysiphe cichoracearum DC. On *Napaea dioica.* Gays Mills.

Epichloe typhina (Pers.) Tul. On *Glyceria nervata.* Athelstane.

Exoascus communis Sadeb. "On fruit of wild plum" Madison. (A. B. Seymour, Econ. Fungi *31*) and Racine.

In the preliminary list of parasitic Fungi of Wisconsin Trelease recorded *Exoascus pruni* Fckl. "On the fruit of *Prunus,*" causing "plum pockets" or "bladder plums." This may have been, in part at least, what is now known as *Exoascus communis* Sadeb. on native plums. Atkinson, however, referred to specimens on *Prunus domestica* from Wisconsin (*Cornell University Ag'l Exp. Station Bulletin* **73**: 329).

Taphrina coerulescens (Desm. & Mont.) Tul. On *Quercus ellipsoidalis.* Athelstane and Solon Springs.

Taphrina potentillae (Farl.) Johans. On *Potentilla canadensis.* Merrimack.

Phyllosticta cruenta (Fr.) Kickx. On *Polygonatum biflorum.* Marquette State Park, Grant County. Red border of spots 1 mm. or less wide; sporules very large, 18–24 x 6–9μ.

Phyllosticta minima (B. & C.) E. & E. On *Acer saccharinum.* Wisconsin river bottoms opposite Bridgeport. On dark brown spots which become alutaceous except the peripheral portion.

In specimens of what appears to be *Phyllosticta decidua* Ell. & Kell. on leaves of *Agrimonia striata* collected at Long Lake the older sporules (7–10 x 3½–5μ) are distinctly brown. In another collection on the same host, same locality and same day the sporules (4–7x3μ) have a fuligineous coloration.

Septoria epilobii West. On *Epilobium adenocaulon.* Ladysmith. This is the fungus described under this name by Ellis & Everhart in *Journal of Mycology,* **3**:81.

Septoria erigerontis Pk. On *Erigeron canadense,* Long Lake. There is much diversity in *Septoria* on *Erigeron.* In this collection the pycnidia are scattered through indefinite, somewhat paler areas which become confluent and mottled with small (2–4 mm.) indefinite, dead spots before the death of the entire leaf. The sporules are subarcuate, 21–38x1½–2μ and appear rigid. At the other extreme is *Fungi Columbiani 1680* on the same host species with definite small (1 mm.) white-arid, conspicuously bordered spots bearing each one or two pycnidia containing sporules that are usually narrow (1–1½μ) lax and thread-like. I have labeled the Long Lake collection var. EFFUSA n. var.

Phleospora aceris (Lib.) Sacc. On *Acer saccharinum.* Wisconsin river bottoms opposite Bridgeport.

Gloeosporium nervisequum (Fckl.) Sacc. On *Platanus occidentalis.* From a tree on the university campus. (H. R. Rosen.)

Marssonina castagnei (Desm. & Mont.) Magn. On *Populus balsamifera.* Laona.

Cylindrosporium saccharinum Ell. & Evht. On *Acer spicatum.* Athelstane. Sporules 30–40 x 2μ crescentic, 3–septate, borne in imperfect pycnidia. Doubtfully distinct from *Phleospora aceris* (Lib.) Sacc.

The fungus that was reported in Notes II under the name *Cylindrosporium vermiforme* Davis has been collected at Millston on *Corylus americana.* The larger sporules are 6μ in diameter.

Ramularia uredinis (Voss) Sacc. On *Populus deltoides.* Madison. On *Salix cordata.* Lynxville. Parasitic, together with *Darluca filum* (Biv.) Cast., on *Melampsora.*

Ramularia multiplex Pk. On *Vaccinium Oxycoccus.* Solon Springs.

Septocylindrium concomitans (Ell. & Hals.) Hals. On *Bidens vulgata.* Ladysmith.

Ramularia virgaureae Thuem. On *Solidago altissima.* Lynxville. Well developed conidia are obclavate and sometimes attain a length of 100μ. This fungus varies from an *Ovularia* to a *Cercosporella* type according to the activity of the abstriction process.

Entyloma compositarum Farl. On *Aster macrophyllus.* Laona.

Entyloma polysporum (Pk.) Farl. On *Rudbeckia hirta.* Athelstane.

Puccinia eatoniae Arth. A specimen of *Sphenopholis obtusata* in the herbarium bears this rust. It was collected by Lapham at Milwaukee.

Puccinia patruelis Arth. Aecia on *Lactuca spicata* collected at Laona are referred to this species.

Puccinia minuta Diet. On *Carex (trichocarpa?),* Madison.

Puccinia obscura Schroet. Uredinia on old leaves of *Luzula saltuensis* in April. Merrimack.

Puccinia pruni-spinosae Pers. On *Prunus cuneata.* Millston.

Melampsora arctica Rostr. The uredinial stage has been collected on *Salix* at Princeton by M. W. Gardner and determined by J. C. Arthur. Uredinia and telia have also been collected on *Salix pedicellaris* and *S. discolor* at Solon Springs.

ADDITIONAL SPECIES

Not hitherto recorded as occurring in Wisconsin.

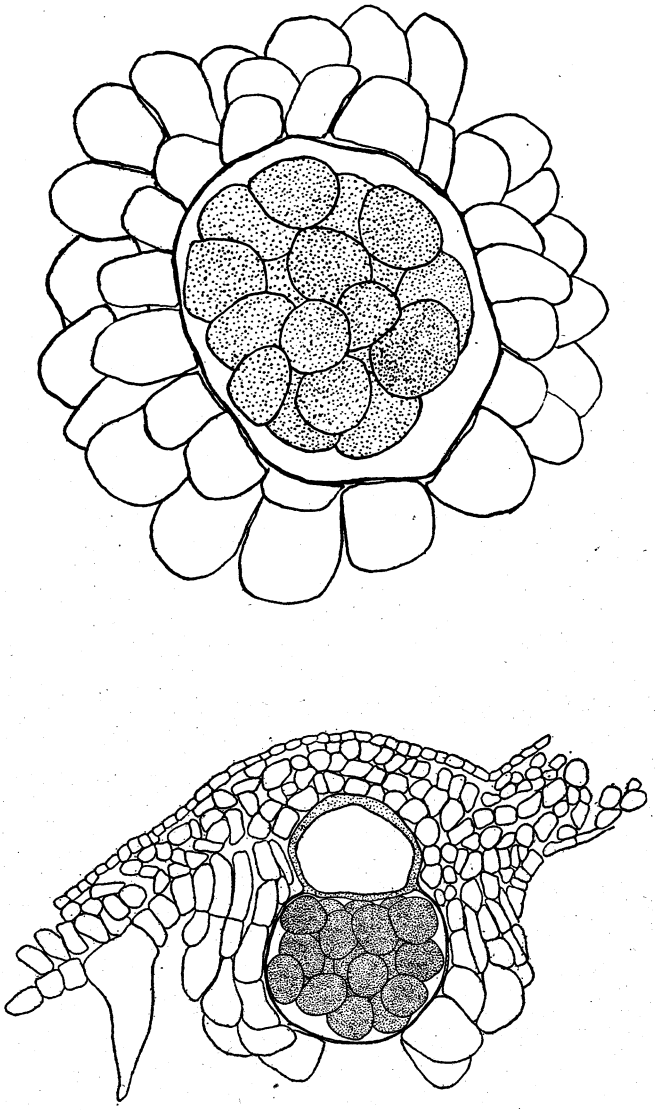
SYNCHYTRIUM CELLULARE n. sp.

Galls of summer sporangia 130–240μ wide x 110–150μ high, consisting of a central cell surrounded on the sides by smaller (30–40μ) thin walled, superhemispherical cells which form an investment about two cells thick; central cell often divided by a horizontal septum into an empty basal cell and a larger upper cell which contains the summer sporangia; sporangia 30 or more, yellow, spherical to elliptical, 18–26 x 15–22μ; resting spores globose to elliptical, brown, 50–90 x 40–80μ, single in simple galls but little larger than the spores, and often at the base of the summer galls. Amphigenous on the leaves and on the petioles of *Boehmeria cylindrica*. This has been found only at the bottom of a kettle hole in the glacial drift at Devils Lake. It was first observed in 1913 on the few plants at the bottom of the kettle hole. In 1914 the quantity was less and in 1915 but a single infected leaf was seen which was not disturbed, but in 1916 no trace of the organism was found and careful search has not revealed it elsewhere. This recalls the career of *Doassansia ranunculina* Davis which was first collected in the early 90's. It increased in abundance and range year by year until it could be found wherever the host occurred in any direction within a radius of five miles or more from the city of Racine. In about ten years it suddenly disappeared and has not been seen since nor has it ever been reported from any other locality. I suspect that its extermination was due to freezing weather in late spring that killed the infected leaves.

Aphanomyces phycophila D By.
On *Spirogyra spp. indet.* Madison (E. M. Gilbert).

What is probably *Sphaeria solidaginis* Schw. has been collected on *Solidago altissima* at Petersburg and Gays Mills. The character of this production was discussed by Farlow in the *Bibliographical Index*, 270–1.

Phyllachora Wittrockii (Erikss.) Sacc. was collected in an immature condition on *Linnaea borealis americana* at Solon



Horizontal optical section of summer sorus of *Synchytrium cellulare* n. sp. (above) and vertical section of same (below). Magnified 775 diameters; lower figure reduced.
Drawn by Mabel M. Brown with the aid of camera lucida.

Springs. Good specimens were collected on Isle Royale, Michigan, by Stuntz and Allen in 1901.

Exoascus mirabilis Atk. On *Prunus americana* (cult). Mountain. Collected also by Prof. L. R. Jones on wild plum trees at Albion and Edgerton. Some of the galls bear *Monilia* with conidia mostly about 15 x 7–9μ.

Sporonema phacidioides Desm. *(Phyllosticta medicaginis* (Fckl.) Sacc.). On *Medicago sativa* (cult.) as well as the ascigerous stage, *Pyrenopeziza medicaginis* Fckl. Madison, (F. R. Jones.)

Phyllosticta ivaecola Ell. & Evht. On *Iva xanthifolia.* Dresser Junction.

Asteroma tiliae Rud.

On *Tilia americana.* Bell Center.

Asteroma ribicolum Ell. & Evht. On *Ribes americanum.* Lake Mills, Madison and Gays Mills. It has also been observed in Kenosha county but always sterile whether on living leaves or on fallen leaves in the spring. Material wintered outdoors in a wire cage showed no further development in June.

Perhaps this is not distinct from the European *Asteroma umbonatum* Desm.

Stagonospora atriplicis (West.) Lind.

Of a collection on leaves of *Chenopodium (Blitum) capitatum* made at Laona July 14, 1915, the following characters were noted: Spots light brown, subcircular to irregular, 3–10 mm. in diameter; pycnidia epiphyllous, scattered, having a thin cellular wall which is hyaline below and black above especially about the large ostiole, 120–150μ in diameter; sporules hyaline, oblong, straight, or sometimes curved, 15–21 x 7–9μ, 1–3 septate. This I have referred to *Ascochyta chenopodii* (Karst.) Rostr.

A collection on the same species of host made at Sturgeon Bay by R. E. Vaughan, August 21, 1913, has smaller, paler spots, more uniformly colored pycnidia and sporules but 8–15 x 2½–3μ with one or occasionally two or three septa. This I have referred to *Septoria chenopodii* West. Ellis & Everhart *N. A. F.* 2nd series *3076* issued under the name *Septogloeum atriplicis* Desm. with *Phyllosticta atriplicis* given as a synonym is the same fungus. It seems not improbable that these are forms of a single species. [Since this was written I have observed that

Lind unites these, with other forms, under the name *Stagonospora atriplicis* (West.) Lind. *(Danish Fungi* 2387 p. 444.] Grove uses *Septovia chenopodii* West: for the group. (*Journ. of Bot.* **55**: 348)

Stagonospora typhoidearum (Desm.) Sacc. On leaves of *Typha latifolia.* Mountain. Sporules 15–20 x 4–5μ about 4-guttulate. Cytoplasm 1–3 times divided when treated with iodine. *Hendersonia typhae* Oud. which has been collected on *Typha* at Madison is perhaps parasitic.

Stagonospora dearnessii Sacc. On *Trifolium repens.* Madison and Athelstane. In these collections the sporules are 1-septate with occasional 2–3 septate ones. The young sporules contain 6–8 small guttulae which are larger and 4 in number when the septum is formed and probably disappear at maturity. I find the sporules to be uniformly triseptate when brought to germination. In the original description *(Stagonospora trifolii* Ell. & Dearn. Phila. Acad. Sci., 1891, p. 82) the sporules are given as 2–4 nucleate but nothing is said of septa. Since this was written a collection has been made on *Trifolium hybridum* (Hixton, July 7–1916) with sporules 9–14 x 3μ, uniseptate, rarely biseptate. They are somewhat fusoid while those that I have seen on *T. repens* are cylindrical or even somewhat narrowed in the middle. Of what I take to be a state of this fungus the following notes were made: Spots lethal brown, immarginate, elliptical to oblong or triangular, sometimes confluent, 3–10 mm. long, the long axis being parallel to the veins; pycnidia hypophyllous, scattered, succineous, widely open, about 100μ in diameter; sporules mostly bacillary, 3–6 x 1–1½μ but occasionally ovoid, 2–3 x 1½μ. Oconto Co., June 25, 1915. It may be that *Phyllosticta trifolii* Rich. and *Phyllosticta Trifoliorum* Barbarine were founded upon something like this which I take to be a spermogonial condition very similar to *Sporonema phacidioides* Desm. on *Medicago.*

Gloeosporium trifolii Pk. (See p. 674) and *Stagonospora dearnessii Sacc.* I take to be congeneric if indeed they are not more closely related as they may be to the following forms on clover that have been described: *Phleospora trifolii* Cav. with sporules 16–18 x 4–5μ, continuous or with 1–3 indistinct septa and var. *recedens* C. Massal. 16–24 x 5–5½μ 1–3 septate; *Ascochyta trifolii* Siemaschko, 18–20 x 5–6μ with one or rarely 2–3 septa; *Ascochyta trifolii* Boud. & Triouss. and *Ascochyta confusa* Bubak said by Jaczewski to be probably conspecific with the foregoing; *Stagonospora trifolii* Fautrey, 16–22 x 3–4μ,

3–septate; *Stagonospora compta* (Sacc.) Died. *(Septoria compta* Sacc.) 15–25 x 4–5μ, pluriguttulate or 4–5 septate. The economic importance of the clovers is such that we may anticipate that intensive work will be given to their diseases and the nomenclature may perhaps be allowed to rest until that is done.

Septoria sigmoidea Ell. & Evht. On *Panicum virgatum.* Lynxville. In these specimens the pycnidia are borne in long dead leaf areas. The sporules have a fuligenous tint and are little more than 3μ thick.

Septoria glumarum Pass. On dead, rusted leaves of *Triticum vulgare* (cult.) Athelstane. Sporules 15–36 x 2½–3μ, 1–3 septate as shown by staining. In a collection made at Independence July 29, 1916, the pycnidia are sometimes accompanied by perithecia that seem referable to *Sphaerulina.*

SEPTORIA NEMATOSPORA n. sp. Spots amphigenous, indeterminate, pale yellow becoming brown, 3–6 mm. long and of the width of the leaf, often confluent; pycnidia hypophyllous, intervenular, dark brown, ostiolate, globose to elliptical, 75–150 x 75–100μ; sporules filiform, somewhat curved, lax, continuous, eguttulate, 37–55 x ½–1μ. On leaves of *Carex pennsylvanica.* Ladysmith, Wisconsin, July 31, 1915. This was referred to in "Notes" III, p. 246. While examining the Ladysmith collection a pycnidium was observed which contained sporules 18–20 x 3–4μ, 3–septate like those noted in the same reference.

Septoria anemones Desm. On *Anemone quinquefolia.* Racine. On longitudinal blackish brown areas associated with an immature Sphaeriaceous fungus.

On looking over some unidentified collections I find one on cultivated *Chrysanthemum* made at Racine May, 1894, by F. L. Stevens which I refer to *Septoria rostrupii* Sacc. & Syd. The spots are mostly green, the black, epiphyllous pycnidia about 90μ in diameter and the sporules 42–48 x 1–2μ, subflexuose. This is given as a synonym of *Septoria chrysanthemella* Cav. on the label of Vestergren's *Micromycetes rariores selecti 1646.*

Specimens from an elm in a city lot at Platteville bear *Sacidium ulmi-gallae* Kell. & Sw. They were sent by Mr. S. E. Livingston. The sporules are mostly oval to ovate, 6–9 x 4μ. The host is *Ulmus fulva* and the accompanying galls are those caused by *Schizoneura americana* as I am informed.

MYRIOCONIUM COMITATUM n. sp.

On dead areas that often include the entire leaf. From a thin, pale, discoid stroma arise erect, simple, hyaline, crowded basidia, 10–15 x 1–1½μ on which are borne apical, globose, hyaline sporules 2–3μ in diameter. These sporules exude in droplet-like masses which dry on the surface of the leaf. The acervuli are usually nervisequent and variable in size sometimes exceeding 1 mm. in length. On *Populus tremuloides.* Mountain, Long Lake, Wausaukee and Athelstane. On *Salix discolor,* Athelstane. On *Salix longifolia,* Suring. On *Populus* the acervuli are hypophyllous, on *Salix* epiphyllous. On *Populus tremuloides* this fungus was invariably associated with *Sclerotium bifrons* Ell. & Evht. The midribs of the attacked leaves of *Salix longifolia* are black. I am separating the form on *Salix* as var. SALICARIUM n. var.

What I take to be a microconidial state of *Marssonina castagnei* (D. & M.) Sacc. occurs also on *Populus tremuloides* in scattered groups having sporules about 4 x 1μ.

There were collected on leaves of *Astragalus canadensis* at St. Croix Falls August 27th, 1914, specimens that have been filed in the herbarium under the name *Gloeosporium astragali* ad interim. The following notes were made: Spots circular, alutaceous, often zonate, about ½ cm. in diameter; acervuli epiphyllous, yellowish, 150μ in diameter; sporules ovoid to oblong, hyaline, 4–8 x 3μ. The sporules are much like those of *Gloeosporium davisii* Ell. & Evht. on fruit of *Vicia americana.* It was not abundant. No *Gloeosporium* was collected on any other host in the vicinity. I think it best to consider this as merely a herbarium name for the present.

Colletotrichum nigrum Ell. & Hals. On *Capsicum* (cult.) Milwaukee (R. E. Vaughan).

COLLETOTRICHUM SILPHII n. sp.

Spots definite, orbicular, light brown becoming cinereous, margin darker above, ½–1 cm. in diameter, sometimes confluent; acervuli epiphyllous, scattered, little or not at all prominent, about 75μ wide; sporules hyaline, continuous, arcuate, acute at both ends, 22–27 x 2½–3μ; setae brown black, sometimes subflexuose, occasionally bent, sometimes 1–2 septate, 36–75 x 4μ. On leaves of *Silphium perfoliatum.* Lynxville, Wisconsin, Sept. 9, 1915.

CYLINDROSPORIUM EMINENS n. sp.

Spots suborbicular, brown usually with more or less of a reddish halo above, dark grey below, 1–2 mm. in diameter; acervuli epiphyllous, more or less prominent, 75–100μ wide; sporules hyaline, straight or curved, becoming pluriseptate, 25–75 x 2–3μ. On leaves of *Helianthemum canadense*. Solon Springs, Wisconsin, Sept. 7, 1914.

Septocylindrium caricinum Sacc. On *Carex grisea*. Blue Mounds.

Ramularia aromatica (Sacc.) Hoehn. (*Septocylindrium aromaticum* Sacc.). On *Acorus Calamus* in the experimental drug garden at Madison.

RAMULARIA LUCIDAE n. sp.

Spots orbicular to elliptical to angular, castaneous with a darker periphery and a raised margin, paler and more livid below, 3–6 mm. in diameter; conidiophores amphigenous but mostly hypophyllous, densely fasciculate, straight, hyaline, 20–40 x 2–3μ; conidia cylindrical to fusoid-cylindrical, usually straight, hyaline, guttulate, occasionally showing a median division of the cytoplasm, 23–42 x 2½–3μ. On leaves of *Salix lucida*. Laona, Wisconsin, July 12, 1915. This differs from *Ramularia rosea* (Fckl.) Sacc. in the fewer and more definite spots and the longer conidia.

Heterosporium gracile (Wallr.) Sacc. On *Iris* (cult.) Madison (H. W. Browning).

Cercospora nasturtii Pass. was collected on *Radicula Nasturtium-aquaticum* in the Fox river above Burlington in 1908.

CERCOSPORA SANICULAE n. sp.

Spots angular, limited by the veinlets, at first light sordid brown, 1–2 mm. in diameter, becoming confluent and blackish brown; conidiophores hypophyllous, scattered or in small fascicles of 2–4, straight, simple, continuous, or rarely with 1 or 2 septa, denticulate or subtorulose near the apex, brown, 15–45 x 3½–6μ; conidia narrow obclavate, tapering from near the base, subolivaceous, indistinctly guttulate, straight or somewhat curved, 50–110 x 3½–4½μ. On *Sanicula gregaria*. Gays Mills, Wisconsin, Sept. 15th, 1915.

RAMULARIA VARIATA n. sp.

Spots amphigenous, angular, limited in part by the veins, yellowish brown becoming black, ½–1½ cm. in diameter; conidiophores hypophyllous, fasciculate, hyaline, simple, straight or apical portion oblique, continuous or indistinctly septate, denticulate, 25–45 x 2½–3μ; conidia subapical, catenulate, hyaline, ovoid to fusoid to cylindrical, continuous or the longest 1–septate, 10 x 5–30 x 3μ. On *Monarda fistulosa.* Lynxville, Wisconsin, Sept. 3, 1915. This is very similar to *Ramularia lamiicola* C. Massal. but in the absence of knowledge as to the cause of the resemblance I am considering the American form on *Monarda* as specifically distinct.

In Farlow's *Host Index Mentha canadensis* is given as a host of *Ramularia menthicola* Sacc. and in the provisional list collections on this host were recorded under that name. The Wisconsin specimens however as well as those collected in Montana by E. T. and E. Bartholomew and issued in *Fungi Columbiani 4380* I am now referring to the species described above. They differ from *R. menthicola* Sacc. as described, in the character of the spots and in the shorter conidiophores. In the Montana specimens the spots apparently do not become black as they do in the Wisconsin ones. It may be that this is not distinct from *Ramularia lycopi* Hollos which I have not seen. A word as to the *Monarda* host: as it occurs in Wisconsin the under surface of the leaves bears very short (30–40μ), conical, erect hairs that form a pile that is somewhat velvety to the touch. With these are much longer white, pilose hairs that are usually few but in some specimens more abundant.

Cercospora depazeoides Sacc. On *Sambucus canadensis.* Grant County opposite Bridgeport.

Cercospora gentianicola Ell. & Evht. On *Halenia deflexa.* Solon Springs. The following notes were made from this collection: Spots dark, indefinite, becoming confluent; conidiophores hypophyllous or epiphyllous, fasciculate from small black stromatic tubercles, fuligenous to dark brown, straight or more often more or less flexuose, continuous, entire or denticulate, 10–40x3–4μ; conidia hyaline, obclavate-cylindrical, straight or curved, becoming tri-septate, 40–72 x 3–5μ. I take *Cercospora gentianae* Pk. to be a synonym.

Cercospora crassa Sacc. On *Datura Stramonium* and *Datura Metel* and other species in the experimental drug garden at Madison. The type of *Cercospora daturae* Pk. was collected in June and appears to be a somewhat immature condition of the same fungus. In the Madison material zonation of the spots is conspicuous and vertical septa in the conidia are well developed and the fungus should be referred to *Alternaria* [This is *Alternaria crassa* (Sacc.) Rands. *Phytopathology* 7:337].

Macrosporium saponariae Pk. which occurs in Wisconsin on leaves of *Saponaria officinalis* has not been recorded in any of the state lists of parasitic fungi.

Ustilago violacea (Pers.) Fckl. was reported in the 4th supplementary list but was unintentionally omitted from the provisional list. It has been collected at Racine, Madison, and in Kenosha county in the anthers of *Arenaria lateriflora.*

Puccinia uniporula Orton. On *Carex gracillima.* Racine.

Puccinia karelica Tranz. On *Carex paupercula irrigua.* Price and Sawyer counties.

The above species on *Carex* were determined by Dr. J. C. Arthur.

SCLEROTIUM DECIDUUM n. sp.

Mycelium hypophyllous, white, branched, continuous (?) 3–4μ in diameter, at first effused but soon aggregated into rounded masses 0.1–3 mm. in diameter. The larger of the mycelial masses become compacted into grey, globose to elliptical sclerotia about 2 mm. in diameter which usually fall away before mature. The affected leaf areas become pale and dead and usually studded with brown dots that mark the location of the mycelial ganglia. This was referred to in the supplementary list of parasitic fungi of Wisconsin, No. 495, as occurring on "*Silphium, Helianthus,* etc." at Racine. The following hosts are represented by specimens in our herbaria: *Adiantum pedatum, Pteris aquilina, Aralia nudicaulis, Mitella diphylla, Diervilla Lonicera, Steironema ciliatum, Solidago "canadensis," Silphium terebinthinaceum.* The paucity of specimens is because of falling away of the sclerotia.

UNIVERSITY OF WISCONSIN HERBARIUM,
MADISON, WISCONSIN, APRIL, 1916.

NOTES ON PARASITIC FUNGI IN WISCONSIN—V.

J. J. DAVIS.

Plasmopara humuli Miyabe & Takahashi which has been reported as occurring in Racine and Crawford counties was found in 1916 in Monroe County also. It appears to be indigenous to Wisconsin.

R. E. Stone has described *Mycosphaerella aurea* n. sp. as the ascogenous stage of *Septoria aurea* Ell. & Evht. (*Phytopath.* **6**: 424.)

Hendersonia typhae Oud. is referred to *Scolecosporium* by von Hoehnel (*Fragm. zur Mykol.* no. 268).

Septoria salicina Pk., as I understand it, appears first as small scattered round or subangular black spots which increase in size (2–5 mm.) and more or less of the central portion becomes grey and arid. In this central portion the few hypophyllous pycnidia appear. The deeply lying ones are globose but those that impinge upon the unyielding epidermis of the host are flattened thereby so that sometimes they resemble acervuli. The sporules are arcuately curved, acute, 25–52 (mostly 30–45) x 2–3μ. They have usually a single median septum but some of the longer ones have 2 or 3 or even 4. I have seen this in Wisconsin on *Salix lucida* only and the herbarium specimens are on this host or on *Salix Fendleriana* except *North American Fungi,* 2nd series *3064* which is labeled *Salix cordata. Fungi Columbiani 3872* bears much larger zonate spots due perhaps to the unusual thinness of the leaves of the host. *Gloeosporium boreale* Ell. & Evht. *(N. Am. Fungi 3279)* appears to be a small spored form of the same fungus. *N. Am. Fungi* 2nd series *3472* issued as *Septogloeum salicinum* (Pk.) Sacc. does not seem to differ from *Septoria albaniensis* Thuem.

which, in the provisional list was included in *Septoria salicina* Pk. as a short spored form. *Fungi Columbiani 3872* issued as *Septogloeum salicinum* (Pk.) Sacc. I would refer to *Septoria salicina* Pk. although as noted above the spots are much larger. *N. Am. Fungi* 2nd series *3064, F. Col. 3779 & 4387* I would refer also to this species. They were issued as *Septoria salicis* West. which I have not seen but with the description of which the American specimens do not agree in any respect. None of the specimens that I have examined bear sporules as long as is indicated in the description unless they are measured along the curve. In type material the longest sporule that I saw was 52μ in a straight line connecting the extremities.

[See Dearness, *Mycologia* **9**:359].

Gloeosporium cylindrospermum (Bon.) Sacc. which was recorded as occurring on *Alnus incana* in Wisconsin in "Notes" II is referred to *Leptothyrium alneum* (Lev.) Sacc. by Diedicke (*Krypt. fl. M. Brandenburg, Pilze* **7**: 707–8). Klebahn has shown its connection with *Gnomoniella tubiformis* (Tode) Sacc.

A fungus on *Acer Negundo* collected at Whitehall on samaras and leaves and referred to *Gloeosporium apocryptrum* Ell. & Evht. bears sporules 12–15 x 5–6μ.

Pestalozzia kriegeriana Bres. is placed in the genus *Hyaloceras* by Diedicke (*Krypt. fl. M. Brand.: Pilze* **7**: 877).

In the provisional list *Ramularia modesta* Sacc. was recorded as occurring in Wisconsin on *Fragaria virginiana*. The reference to this species was because of the small size of the conidia. In July, 1916, a collection was made on *Fragaria virginiana* at Whitehall from examination of which the following notes were made: Spots suborbicular to angular, brown, paler below, immarginate, 5–8 mm., sometimes confluent; conidiophores hypophyllous, fasciculate from a black stromatic base, straight, simple, septate, tapering upward, olivaceous-brown, 45–75 x 3μ; conidia hyaline, catenulate, 5–11 x 1½ x 2½μ. The entry in the provisional list was based on specimens collected at Spooner and on referring to them I found the following notes in one of the packets: "Hyphae hypophyllous, olivaceous-brown, rigid, 40–70 x 4μ; conidia hyaline, obtuse, catenulate, 6–12 x 2–3μ. *Cercospora vexans* C. Massal?" The reference to that species

seems warranted by the description and I have so labeled the specimens. *Ramularia modesta* Sacc. should therefore be elided from the list.

The fungus on *Aster* given in the provisional list as *Ramularia asteris* (Trel.) Barth. appears to be conspecific with *Fusidium* (?) *Asteris* Phil. & Plowr. (*Grevillea* **6**:23) which has been referred to *Ramularia* by Bubak (*Ann. Mycol.* **6**:27). The name should therefore be written *Ramularia asteris* (Phil. & Plowr.) Bubak, as is done by Vestergren in *Micromycetes rariores selecti 1094* except that he followed Saccardo and Bubak in transposing the names of the authors of the specific name.

By some oversight *Urocystis anemones* (Pers.) Schroet. was omitted from the provisional list. I am indebted to Professor J. G. Sanders for calling my attention to the omission. The smut is common on *Hepatica triloba, H. acutiloba* and *Anemone quinquefolia* and was reported by Trelease in the preliminary list as occurring on *Anemone canadensis.*

Aecia of *Uromyces* were collected on *Trifolium pratense* and *T. hybridum* at Madison by W. H. Davis. They have also been collected on the latter host at Melvina.

Puccinia bartholomaei Diet. was included in the provisional list because of an *Aecidium* on *Asclepias syriaca* which occurs in the state which proves however to be connected with *Puccinia seymouriana* Arth. instead. (Arthur, *Mycologia,* **8**:134). My observations both at Racine and Madison lead me to believe that this rust may overwinter on *Spartina* probably as mycelium.

Specimens of the *Roestelia* stage of *Gymnosporangium globosum* Farl. on *Crataegus* collected at Maiden Rock and St. Croix Falls in 1916 have peridial cells but 40–60μ long. Perhaps the dwarfing was due to the unusually hot weather.

Peridermium comptoniae (Arth.) Orton & Adams was observed in June 1916 at Millston in Jackson County. Its presence is most easily detected after a rain when the fresh sori appear. I find it usually on the east side of the trunk near the base. In this region rain is usually preceded by easterly winds hence the fresh sporidia are most likely to be lodged on the east

side of the trunk when moisture conditions are favorable for infection. After a rain the basal portion of the trunk remains moist long after the higher portions have dried off. This has since been collected in Adams and Juneau counties and its range in Wisconsin probably approximates that of the host *Pinus Banksiana.*

Peridermium pyriforme Pk. was collected during the same month at Melvina, Monroe County, and at Millston. As usual, in my experience in collecting this rust, but a single specimen was found in each locality.

ADDITIONAL HOSTS

A very scanty development of *Bremia lactucae* Regel was observed at Arcadia on *Krigia amplexicaulis.*

Plasmopara halstedii (Farl.) Berl. & De Toni on *Artemisia ludoviciana.* Taylor.

Peronospora potentillae D By. On *Agrimonia striata.* Arcadia.

Peronospora rubi Rabh. On *Rubus hispidus.* Millston. But little of the mildew was seen on this host.

Uncinula macrospora Pk. On *Ulmus racemosa.* St. Croix Falls.

Taphrina coerulescens (Desm. & Mont.) Tul. On *Quercus macrocarpa.* Granville. (I. A. Lapham, 1867.)

Phyllosticta decidua Ell. & Kell. On *Agrimonia gryposepala.* Arcadia. Sporules 4–5 x 2½–4μ, fuligenous tinted.

Ascochyta wisconsina Davis. On *Sambucus racemosa.* Lynxville.

Diplodia uvulariae Davis. On *Uvularia grandiflora.* Maiden Rock. This collection bears mostly pycnidia containing sporules 4–5 x 1μ which I take to be a spermogonial state. The *Diplodia* is immature, the sporules being still hyaline and but few of them septate.

Septoria graminum Desm. On leaves of *Bromus altissimus.* Maiden Rock.

Septoria polygonorum Desm. On *Polygonum pennsylvanicum.* Lynxville. Sporules up to 60μ long.

Septoria lepidiicola Ell. & Mart. On leaves of *Lepidium apetalum.* Sparta.

Septoria solidaginicola Pk. On *Aster azureus.* Danbury.

In "Notes" III p. 259, reference was made under *Gloeosporium caryae* Ell & Dearn., to an epiphyllous form on *Carya cordiformis* and I find a collection of the same kind made at Richland Center on *Carya alba* by R. A. Harper & G. M. Reed. This is *Phyllosticta caryae* Pk. but in both the Wisconsin and New York material the sporules arise from a subcuticular stroma.

Colletotrichum lagenarium (Pass.) Ell. & Hals. On *Cucurbita Melo* (cult.) Madison. On *Cucumis sativus* (cult.) Princeton (M. W. Gardner.)

Cylindrosporium vermiforme Davis. On *Corylus rostrata.* Cameron. A collection of this fungus from Danbury shows globose swellings up to 20μ in diameter in the continuity of the conidia. These vesicles are rich in cytoplasm and suggest chlamydospore formation.

Ramularia desmodii Cke. On *Desmodium paniculatum.* Maiden Rock.

Ramularia lysimachiae Thuem. On *Steironema lanceolatum.* Lynxville.

Cercospora circumscissa Sacc. On *Prunus pennsylvanica.* Neopit and Blair. On *Prunus serotina.* Athelstane and Alma.

Entyloma australe Speg. On *Physalis pubescens* (cult.) Waupaca. (R. D. Rands).

Entyloma polysporum (Pk.) Farl. On *Ambrosia trifida.* Maiden Rock. Forming definite, orbicular, yellow, somewhat thickened spots about 5 mm. in diameter.

Uromyces proeminens DC. On *Euphorbia dentata.* Lynxville. This is *U. poinsettiae* Tranz.

Puccinia coronata Cda. On *Cinna arundinacea.* Luck.

Puccinia graminis Pers. On *Bromus secalinus.* Independence.

Puccinia koeleriae Arth. Aecia *(Aecidium liatridis* Ell. & And.) on *Liatris scariosa.* Solon Springs, Millston and Hixton and in small quantity on *Liatris cylindracea* at Millston. Uredinia and telia on *Koeleria cristata* Millston. In the description the aecial host was given as *Mahonia* but Bethel has established the connection here given.

Puccinia patruelis Arth. Uredinia and telia on *Carex siccata.* Black River Falls. Field observation indicated that the aecia connected with this collection were borne on *Krigia amplexicaulis.*

ADDITIONAL SPECIES

Plasmopara acalyphae G. W. Wilson.

Because of the discovery of a trace of this mildew *Acalypha virginica* was interrogatively given as a host of *Peronospora euphorbiae* Fckl. in the provisional list. As stated in "Notes" II (1914) but a single additional conidiophore had been found up to that time. In 1915 however, enough was secured to show that it is an undescribed species of *Plasmopara* and it was sent to Prof. Guy West Wilson, who has given special attention to the *Peronosporales,* for description and publication. In 1916 still further material was secured. [See *Mycologia* **10**:169.]

In "Notes" III, p. 252, mention was made of a *Lophodermium* on *Pinus Banksiana* differing from *L. pinastri* (Schrad.) Chev. A number of collections of this were made in 1916 which show that it is constant and distinct, differing from *Hypodermella,* as characterized by Lagerberg, only in the broad perithecia.

LOPHODERMIUM AMPLUM n. sp.

On sordid spots or terminal leaf areas; perithecia amphigenous, prominent, black, elliptical, ½–1 mm. long; asci cylindrical to clavate-cylindrical, narrowed at the apex, sometimes

curved, 90–165 x 18–30μ; spores embedded in mucus, overlapping, hyaline, continuous, attenuate at base, clavate-cylindrical, rarely fusoid-cylindrical, 30–72 x 3–6μ; paraphyses numerous, filiform, a little longer than the asci. On leaves of *Pinus Banksiana.* Muscoda, Sparta, Millston, Black River Falls, Taylor, Gordon. May to July. *Lophodermium pinastri* (Schrad.) Chev. occurs on this host in Wisconsin, on dead, fallen leaves, while *L. amplum* develops on leaves that are, in part, living or that are *in situ.* What relation this bears to the *Hypoderma desmazieri* Duby reported by Peck as occurring on leaves of *Pinus rigida* in New York I do not know.

Lophodermium lineare Pk. On *Pinus Strobus.* Pembine.

COCCOCHORA RUBI sp. nov. Stromata epiphyllous, scattered, black, shining, prominent, suborbicular, subcuticular, ¼–1 mm. in diameter; loculi one to several, 45–60μ high, 60–90μ wide, opening at the apex; asci cylindrical, more or less curved, 45–50 x 7–9μ, octosporous; spores brown, clavate-oblong, with a single septum which is more or less submedian, not constricted, 11–15 x 4–6μ; paraphyses filiform, inconspicuous. On leaves of *Rubus hispidus,* Millston, Wisconsin, August 19, 1915, and July 19, 1916. Small stromata containing but a single locule are subhemispherical, the larger compound ones, which are sometimes circinate, are tuberculate. The adnate clypeus is large and merges into the normal cuticle at the edge. This fungus suggests *Asterina rubicola* Ell. & Evht. when seen in the field.

In the provisional list hosts were not enumerated under *Phyllachora graminis* (Pers.) Fckl. In *Annales Mycologici* **13**:436 *et seq.* Theissen and Sydow have divided this into a large number of species. More knowledge of their biological relations is needed for a satisfactory classification of the North American forms. It may be of service to give some notes of measurements of asci and spores taken from Wisconsin specimens.

Elymus: Asci 66–75 x 6–7μ; spores 8–9 x 4½–6μ. Theissen & Sydow take a specimen on this host genus as the type of *Phyllachora graminis* (Pers.) Fckl. with characters with which these measurements agree except that the asci of the type are thicker (8–10μ).

Hystrix patula: Asci 66–79 x 6–9μ; spores 8–12 x 5–6μ. This

seems to be so like the form on *Elymus* as to indicate specific identity. There is a *Phyllachora asprellae* Roum. & Fautr. in France the asci and spores of which are described as being larger.

Panicum latifolium: Asci 65–80 x 9μ; spores 8–9 x 4–5μ. A later collection: Asci 60–70 x 8–12μ; spores 9–11 x 5–6μ.

Panicum huachucae: Asci 50–60 x 6–9μ; spores 8–9 x 4–5μ.

Panicum sp. indet.: Asci 57–63 x 9μ; spores 7–9 x 5μ. The *Panicum* specimens appear conspecific and are what has been distributed in this country as *Phyllachora graminis* var. *panici* (Schw.) Shear. If they belong with any of the species described by Theissen & Sydow as occurring on *Panicum* it is probably the South American *Phyllachora panici* (Rehm).

Muhlenbergia: Asci 50–67 x 5–6μ; spores 6–8 x 4μ. This is probably *Phyllachora vulgata* Theiss. & Syd. (*loc. cit.* 450).

Agropyron repens: Asci 60–80 x 6–8μ; spores 8–12 x 4–5μ.

Calamagrostis canadensis: Asci 51–72 x 5–6μ; spores 7–9 x 5–6μ. *Phyllachora* has been observed in northern Wisconsin on *Oryzopsis asperifolia* but no mature specimens have been preserved. This is presumed to be *Phyllachora oryzopsidis* Theiss. & Syd. (*loc. cit.* 451).

Taphrina coryli Nishida. On leaves of *Corylus americana.* McFarland, Madison, Sparta, Melvina, Hixton, Taylor, Blair, Whitehall. In 1916 this was found scattered about through the woods in western Wisconsin in a way that left no room for doubt as to its being indigenous. The appearance in the field suggests *Microsphaera.*

Of a collection on leaves of *Echinocystis lobata* made at Whitehall, July 28, 1916, the following notes were made: "Spots suborbicular, immarginate, pale brown, ½–1 cm. in diameter; pycnidia scattered, lenticular, succineous, ostiolate, 75–100μ; sporules hyaline, oval to oblong, 4–8 x 1½–3μ. Accompanying *Plasmopara australis* (Speg.) Swingle and perhaps secondary." I have referred it to *Phyllosticta orbicularis* Ell. & Evht.

Sphaeropsis betulae Cke. var. FOLIICOLA n. var. On large, light brown dead leaf areas; pycnidia mostly epiphyllous, scattered or aggregated, blackish brown, depressed-globose, blackened about the ostiole, 100–150μ; sporules oblong with rounded

ends, usually straight, continuous, fuligenous to brown, 18–24 x 9μ. On leaves of *Betula alba papyrifera.* Maiden Rock, Wisconsin, August 5th, 1916.

Ascochyta graminicola Sacc. On leaves of *Calamagrostis canadensis.* Maiden Rock. Of this collection the following notes were made: Spots definite, sordid white, purple bordered, oval to oblong, 5–8 mm. long, sometimes confluent; pycnidia numerous, depressed-globose, wall thin, parenchymatous, ostiole surrounded by a black ring, about 120μ in diameter; sporules hyaline, fusiform, acute, at both ends, uniseptate, 15–20 x 2½–3μ. The sporules resemble those of *Darluca filum* (Biv.) Cast.

Specimens on *Actaea rubra* collected at Blair, July 17, 1916, have the following characters: On indefinite, blackened, dying, areas of the leaves the cuticle on the upper surface of which is sometimes wrinkled in dendritic lines; pycnidia mostly epiphyllous, scattered, amber colored, globose, about 100μ in diameter; sporules hyaline, cylindrical, uniseptate, 17–24 x 5–6μ. The pycnidial wall is at first hyphal but at maturity consists of a single layer of flat polygonal cells and is not thickened around the ostiole. This appears to be *Actinonema actaeae* (Allesch.) Died. *Stagonosporopsis actaeae* (Allesch.) Died. and probably *Marsonia actaeae* Bres. which is *Marssonina actaeae* (Bres.) Magn. It differs from *Ascochyta clematidina* Thuem. in the size of the sporules as the latter does from the form on *Thalictrum* that I have called var. *thalictri* (Trans. Wis. Acad. **16**: 557). I have labeled the specimen *Ascochyta actaeae* (Bres.) n. comb. These three forms are so similar that it seems to me that it would be proper to indicate the fact by grouping them in a single species. On *Thalictrum* the sporules are 8–10 x 2–3μ, on *Clematis* 10–15 x 3μ, on *Actaea,* 17–24 x 5–6μ. Such series on the same or on related hosts seem to be not uncommon, but there appears to be no way in the present state of taxonomy to indicate the relationships by grouping them, especially as increased spore length often brings increased septation and thereby passes generic limits as now understood.

Ascochyta imperfecta Pk. On *Medicago sativa* (cult.) Madison. Sporules 8–11 x 3–3½μ.

Ascochyta cucumis Fautr. & Roum. On *Cucumis sativus* (cult.) Platteville. (E. Carsner, com. M. W. Gardner.)

There are in Wisconsin a number of foliicolous *Sphaerioidaceae* that constitute a definite group as seen in the field. They cause blackish brown spots of subcircular form but irregular outline with more or less black crustaceous thickening of the upper surface. The pycnidia are innate, inconspicuous, few, scattered, pale, thin walled with the ostiole directed toward the upper surface of the leaf, 100–150μ in diameter and can often be distinguished with a strong hand lens and good transmitted light, especially if the leaf is wet. The sporules are cylindrical with rounded ends and septate. It is in the size and septation of the sporules that variation occurs. What the relation of these forms to each other may be is for the future to disclose through field observation and artificial infection. In the meantime some means of designation is needed and I have tentatively arranged them as follows:

Stagonospora apocyni (Pk.?) n. comb. Spots definite, immarginate, subcircular, reddish brown, somewhat paler below, 1–2 cm. in diameter; pycnidia few, scattered, epiphyllous-innate, globose, succineous, thin walled, becoming more or less thickened and blackened about the ostiole; sporules hyaline, fusoid-cylindrical, 3–7 septate with a large droplet in each cell, 33–50 x 6μ. On leaves of *Apocynum androsaemifolium.* This is the fungus recorded under the name *Septogloeum apocyni* Pk. in the provisional list. The material at hand of that species, on *Apocynum cannabinum* does not enable me to determine whether that is also a *Stagonospora.* Certainly the sporules are similar.

Next comes a form on *Cirsium.*

STAGONOSPORA CIRSII n. sp.

On circular brown or cinereous spots, often with a whitened center ½–1 cm. in diameter or on large brown areas; pycnidia few, scattered, innate, depressed-globose, brown, ostiolate, 125–150μ in diameter; sporules cylindrical, ends rounded, straight or slightly curved, hyaline, 2–5 septate, not constricted, 20–32 x 5–6μ. On *Cirsium altissimum.* Maiden Rock, Wisconsin, August 7th and 16th, 1916.

Next is *Ascochyta lophanthi* Davis (Trans. Wis. Acad. **14**:95) on *Agastache scrophulariaefolia* with uniseptate sporules 20–30 x 10–12μ.

var. OSMOPHILA n. var.

Spots like those of the type; sporules uniseptate 12–21 x 3–5μ. On *Agastache Foeniculum.* Danbury.

var. LYCOPINA n. var.

Spots suborbicular to angular, blackish brown above, lighter below, immarginate, 3–10 mm. in diameter; pycnidia few, scattered, innate, ostiole directed toward the upper surface of the leaf, very inconspicuous; sporules hyaline or smoky tinged, cylindrical with rounded ends, uniseptate, 16–24 x 7–8μ. On *Lycopus uniflorus.* Shiocton, Wisconsin, August.

Collections on *Sanicula marilandica* having uniseptate sporules 20–30 x 4–6μ were described in Trans. Wis. Acad. 18[1]:105, under the name *Ascochyta saniculae* n. sp. The affected leaf areas are usually larger and less definite in the form on this host. This has also been collected on *Zizia aurea* at Melvina. On this host definite orbicular to elliptical olivaceous spots ½–1 cm. long occur. Both of these probably should be referred to *Ascochyta thaspii* Ell. & Evht. the sporules of which were described as being 25–30 x 6–8μ. In the Wisconsin specimens on *Zizia* the sporules are 18–25 x 4–6μ and are perhaps immature.

Next comes a form that may be designated

ASCOCHYTA COMPOSITARUM n. sp. Forming large indefinite brown areas and also smaller more definite spots about 1 cm. in diameter; pycnidia as in the previously mentioned forms; sporules hyaline, uniseptate, 15–22 x 4–6μ. On *Eupatorium urticaefolium, Helianthus strumosus* and *Aster Drummondii.* Of one specimen on the former host it was noted "sporules not well developed, 12–16 x 3–4μ."

Var. PARVA n. var.

Character of the species except that the sporules are but about 10–15 x 2½–3½μ, uniseptate. On *Helianthus strumosus.* Maiden Rock.

It is probable that this species occurs upon other Composites and that *A. thaspii* E. & E. will be found on other *Umbelliferae.* Indeed I have seen the latter on *Cicuta maculata* but did not secure enough for a specimen.

Ascochyta treleasei Sacc. & Vogl. the types of which were collected in Wisconsin on *Silphium* and *Vernonia* I have not

seen but the small spots and proportionately broader sporules described have deterred me from referring these collections to that species.

STAGONOSPORA ZONATA n. sp. Spots orbicular, clay colored with concentric dark lines, ½–2 cm. in diameter; pycnidia few, epiphyllous-immersed, depressed-globose, honey color, ostiolate, 120–180μ in diameter; sporules oblong to cylindrical, hyaline, 4–guttulate becoming 3–septate, not constricted, 12–25 x 3½–6μ. On living leaves of *Asclepias syriaca.* Independence, Wisconsin, July 29, 1916; Arcadia, Wisconsin, July 31st, 1916. Perhaps this is a better developed state of *Ascochyta asclepiadis* Ell. & Evht.

Septoria mitellae Ell. & Evht. On "*Mitella* or *Tiarella.*" Merrimack. Name of collector not given.

Septoria stachydis Rob. & Desm. On *Stachys tenuifolia.* Melvina.

Septoria krigiae Dearn. & House. Spots definite, suborbicular, reddish brown, paler in the center, 3–8 mm.; pycnidia epiphyllous, scattered, black, prominent, 50–75μ; sporules hyaline, straight or sometimes curved, 18–27 x ¾–1μ. On leaves of *Krigia amplexicaulis.* Arcadia.

CYTODIPLOSPORA ELYMINA n. sp. Pycnidia in the loculi of *Phyllachora,* spherical to elliptical, 100–135μ; sporules oblong, hyaline, often 4–guttulate, becoming uniseptate, 7–10 x 2½–3μ. On leaves of *Elymus virginicus.* Madison. This is doubtless a spermogonial state of the *Phyllachora.*

Gloeosporium leptospermum Pk. On *Pteris aquilina;* accompanying *Phyllachora.* Whitehall. The longest sporules attain 30μ and the thickest 5μ.

Colletotrichum circinans (Berk.) Vogl. Reported as occurring on onion bulbs in Wisconsin. (J. C. Walker)

Marssonina rubiginosa Ell. & Evht. On *Salix sp. indet.* Madison.

Monilia corni Reade. On petioles, midribs and peduncles of *Cornus paniculata.* Melvina. The fungus is apparently locally abundant on this species of host but I found conidia but once.

Ovularia rigidula Delacr. On *Polygonum erectum.* Black River Falls and Sechlerville.

In the Wisconsin collection I find the conidiophores 24–70 x 3–4μ. *Ovularia avicularis* Pk. does not seem to be separable. I find amphigenous conidiophores on European material (Jaap, *F. selecti exsic.* 291).

RAMULARIA DISPAR n. sp. On small indeterminate leaf areas which become yellowish and finally dead and brown; conidiophores hyaline, lax, mycelioid, often ascending the trichomes; conidia lateral in branched chains, hyaline, cylindrical, subacute, becoming 1–3 septate, 18–33 x 2½–3½μ. On leaves of *Eupatorium purpureum.* Danbury, Wisconsin, August 30, 1916. This is of the aberrant character of *Cercosporella trichophila* Davis and suggests *Sporotrichum* in habit.

CLADOSPORIUM HUMILE n. sp. Spots dark reddish brown above, plumbeous below, suborbicular to polygonal, 2–10 mm. in diameter, sometimes confluent; conidiophores epiphyllous, most arising from small black pseudostromata of loose texture, erect or assurgent, dark brown, usually septate and often constricted at the septa, straight, flexuous or geniculate, 10–35 x 3–5μ; conidia fuligenous, catenulate, oblong to fusoid-cylindrical, usually straight, becoming, in part at least, uniseptate, 15–37 x 4μ. On leaves of *Acer rubrum.* Luck, Wisconsin, August 25, 1916. The spots appear to be made up of small black intervenular areas some of which are apparent at the periphery and others altogether detached. It may be that this fungus is secondary. [This has since been collected on *Acer saccharinum* at Plover and Arcadia].

Cercospora velutina Ell. & Kell. On *Baptisia leucantha.* Lynxville. In these specimens the conidiophores are borne on the lower surface of orbicular spots 3–10 mm. in diameter which are sometimes confluent and the tubercles are scarcely present. The conidiophores are 30–60 x 2–3μ, fasciculate from a stromatic base and usually somewhat curved.

Cercospora longispora Pk. On *Lupinus perennis,* Millston.

Cercospora polytaeniae Ell. & Kell. On *Polytaenia Nuttallii.* Sparta. The conidiophores of this species were described as "very short." In this collection I find them 30–60 x 5μ, flexuous

or geniculate, becoming denticulate and developing one or two septa.

Alternaria sonchi Davis. Parasitic on leaves of *Sonchus asper*. Madison. The description was published by John A. Elliott in the Botanical Gazette, **62**: 416 (1916).

Uromyces murrillii Ricker. The aecial stage, *Aecidium houstoniatum* Schw., has been collected on *Houstonia longifolia* at Solon Springs and Millston but the further stages on *Sisyrinchium* have not yet been detected in Wisconsin. This is *Uromyces houstoniatus* J. L. Sheldon, a name that violates the rule that I am following.

Uromyces striatus Schroet. Uredinia on *Medicago sativa* (cult.) Weirgor. (F. R. Jones).

Puccinia eriophori Thuem. Following field observations by Dr. House it has been shown by Dr. Arthur by means of inoculation that the rust on *Eriophorum* is distinct from that on species of *Scirpus* and known as *Puccinia angustata* Pk. and that it developes aecia on *Senecio*. Aecia on *Senecio aureus* have been collected in widely separated localities in Wisconsin and Dr. Arthur reports a collection of the rust on *Eriophorum virginicum* at Elm Grove by Dr. C. L. Shear.

Cronartium ribicola Fisch. de Waldh.. The dreaded white pine blister rust has been found on *Pinus Strobus* in Polk County. Specimens of the aecial stage were collected by Moody, Sanders & Pierce in May, 1916, and Professor J. G. Sanders kindly furnished specimens of uredinia on *Ribes cynosbati* collected in June.

Aecidium uvulariae Schw. On *Oakesia sessilifolia*. Melvina and Hixton. I suspect that this is not distinct from *Aecidium majanthae* Schum. and that it is connected with *Puccinia sessilis* Schneid.

In the 3rd supplementary list (Trans. Wis. Acad. **14**: 92) reference was made to the occurrence of a *Doassansia* on *Sagittaria heterophylla* that was referred to *Doassansia sagittariae* (West.) Fisch as forma *confluens* with the statement that it appeared to be physiologically distinct which opinion has been supported by subsequent observation. Morphologically, however,

I am not able to distinguish it from forms on *Sagittaria latifolia, S. arifolia* and *S. sagittifolia* with crowded and distorted sori. In the summer of 1916 collections were made of what proved to be a quite different type on *Sagittaria heterophylla.*

DOASSANSIA (DOASSANSIOPSIS) FURVA n. sp.

Spore balls in the leaf blades, loosely clustered, discrete, brown-black, spherical to oval, 100–150μ long; spores in a single layer surrounding the sterile parenchymatous central portion, rounded-cuboidal, 8–10μ long; cortical cells inconspicuous, plano-convex to flattened, 6–9μ wide by 1–3μ high. In leaves of *Sagittaria heterophylla.* Arcadia, Wisconsin, July 31st and August 2nd, 1916. This differs from *Doassansia martianoffiana* (Thuem.) Schroet. in the darker color, habitat and probably in the absence of conidia; from *D. deformans* Setch. in the much darker sori, part of the host attacked and in not causing hypertrophy. In color and to some extent in structure it recalls *Doassansia zizaniae* Davis (Bot. Gaz. **26**:353) which was referred to *Sclerotium* in the provisional list and suggests that that is also a member of this group.

UNIVERSITY OF WISCONSIN HERBARIUM,
MADISON, WISCONSIN, APRIL, 1917.

NOTES IN PARASITIC FUNGI 1N WISCONSIN--VI

J. J. Davis.

A provisional list of parasitic fungi in Wisconsin was published in the Transactions of the Wisconsin Academy of Sciences Arts and Letters **17**[2]: 846–984 [1914]. Notes bearing a supplementary relation thereto were issued through the same medium: **18**[1]: 78–109 and 251–271 [1915]: **19**:

In the provisional list *Urophlyctis major* Schroet. was given as occurring on *Rumex verticillatus*. All of the Wisconsin specimens appear to be on *R. Britannica*.

Peck should be cited as the author of the binomial *Phyllosticta hamamelidis*, not Cooke as given in the provisional list.

The merging of *Marssonina castagnei* (Desm. & Mont.) Magn. with *M. populi* (Lib.) Magn. by Lind (*Danish Fungi, 2761*) did not surprise me and I am now using the latter name, instead of the former.

The classification of *Septoria* on *Solidago, Aster* and *Erigeron* is in an unsatisfactory state and probably will remain so until aided by the results of investigation by inoculation methods. A collection on *Solidago serotina* (Adams, June 21, 1917) that I have referred to *Septoria davisii* Sacc. bears sporules 42–75 x 1½–2μ.

The sporules of *Septoria bacilligera* Wint. as it occurs in Wisconsin are not typical. I find them to vary from 10–50 x 1–2μ.

Haymaker who has made studies of *Monilia* at the University of Wisconsin considers the forms on *Prunus serotina* and *P. vir-*

giniana in the vicinity of Madison identical, conforming to the description of *Monilia angustior* (Sacc.) Reade.

The *Monilia* on plum fruit given as *M. fructigena* Pers. in the provisional list is now referred to *M. cinerea* Bon. It is sometimes abundant on "plum pockets" on *Prunus americana* and *P. nigra.*

The Mucedine on *Ranunculus abortivus* recorded as *Septocylindrium ranunculi* Pk. in the provisional list I am now referring to *Ramularia aequivoca* (Ces.) Sacc. together with specimens on *Ranunculus septentrionalis* from Madison and St. Croix Falls. On the latter host the conidiophores are in larger fascicles from a stromatic base as in the type of *Septocylindrium ranunculi* Pk. and *Ramularia acris* Lindr. Both of these are on *Ranunculus acris* and they seem to be identical. Ferraris (*Fl. Ital. Crypt.: Hyphales:* 800) distinguishes *R. acris* Lindr. from *R. aequivoca* (Ces.) Sacc. by the longer (30–60μ) conidiophores but the distinction does not hold in *Mycotheca Germanica, 1286.* I have seen no specimen of *Ramularia sceleratа* Cke.

In the description of *Cercosporella filiformis* Davis (*Trans. Wis. Acad.* **18**[1]: 266) the maximum length of the conidia should be increased to 100μ as shown by specimens collected at Hixton in July 1916.

Instead of *Cercospora leptosperma* Pk. or *Cylindrosporium leptospermum* Pk. I am now using *Cercosporella leptosperma* (Pk.).

Fusarium heterosporum Nees is referred to *F. graminum* Cda. by Ferraris (*Fl. Ital. Crypt.: Hyphales:* 90).

Puccinia claytoniata (Schw.) Syd. (*P. mariae-wilsoni* Clint.) seems also to have been omitted from the provisional list. Aecia and telia occur in Wisconsin on *Claytonia virginica* as was stated in the supplementary list.

ADDITIONAL HOSTS.

Albugo candida (Pers.) O. Kuntze. On *Arabis canadensis.* Friendship.

Plasmopara halstedii (Farl.) Berl. & DeToni. On *Ambrosia psilostachya.* Adams.

Peronospora trifoliorum D By. On *Lupinus perennis.* Millston.

Lophodermium pinastri (Schrad.) Chev. On *Pinus resinosa.* Black River Falls.

Pseudopeziza autumnalis (Fckl.) Sacc. On *Galium Claytoni.* Shiocton. This fungus was erroneously listed as *Ps. repanda* in the provisional list.

Exoascus communis Sadeb. On fruit of *Prunus pumila.* Two Rivers.

Phyllosticta cruenta (Fr.) Kickx. On *Polygonatum commutatum.* Darwin. In this collection the sporules are 13–21 x 4–6μ. The spots appear to have been caused by leaf miners and have a very narrow border.

Phyllosticta decidua Ell. & Kell. On *Galeopsis Tetrahit* accompanying *Septoria galeopsidis.* Kewaunee. That this is distinct from species that have been described as occurring on *Labiatæ* in Europe seems open to question. In this collection the sporules are 6–9 x 2½–3μ often contain 2 or 3 guttulae and not infrequently there is a median division of the cytoplasm. On *Humulus Lupulus.* Black River Falls.

Ascochyta thaspii. E. & E. var. *lycopina.**

Spots suborbicular to angular, blackish brown above, lighter below, becoming paler in the center, immarginate, 3–10 mm. in diameter; pycnidia few, scattered, usually innate, ostiole directed toward the upper surface of the leaf, very inconspicuous; sporules hyaline or smoky tinged, cylindrical with rounded ends, uniseptate, not constricted, 16–24 x 7–9μ. On leaves of *Lycopus uniflorus.* Two Rivers, July, Shiocton, August, 1917.

Darluca filum (Biv.) Cast. In telia of *Kuehneola uredinis* on *Rubus hispidus,* Millston and those of *Puccinia curtipes* Howe on *Heuchera hispida.* Hixton. These are the only exceptions

* Duplication of v. 700.

that I have seen to the rule that this parasite is confined to uredinia.

Stagonospora smilacis (Ell. & Mart.) Sacc. On *Smilax ecirhata.* Shiocton. Immature. Sporules continuous, 10–12 x 5–6μ.

Specimens on leaves of *Triticum vulgare* (cult.) collected at Madison seem to me to be referable to *Septoria agropyri* Ell. & Evht. The pycnidia are globose to oval, 75–100 x 60–90μ; sporules 15–40 x 1–1½μ.

A *Septoria* on leaves of *Secale cereale* (cult.) collected at Lyndon Station I have referred to *S. passerinii* Sacc. The pycnidia are scattered over more or less elongated, light yellow areas, are black and about 100μ in diameter; sporules straight or curved, acute, continuous, 30–42 x 1½μ. The distinctness of this and the preceding seems to be questionable.

Septoria caricinella Sacc. & Roum. On *Carex cephalophora.* Seymour.

Phleospora aceris (Lib.) Sacc. On *Acer saccharinum.* Grant county and Shiocton.

Leptothyrium dryinum Sacc. On *Quercus ellipsoidalis.* Lyndon Station. Sporules about 17 x 9μ.

Gloeosporium confluens Ell. & Dearn. On *Sagittaria arifolia.* Shiocton.

Colletotrichum graminicolum (Ces.) Wilson. On *Calamagrostis canadensis.* Spooner. *Bromus altissimus.* Plover.

Septogloeum salicinum (Pk.) Sacc. On *Salix rostrata.* Danbury. *Salix discolor.* Shiocton. What I take to be a microconidial state, bearing sporules 4–8 x 1μ, has been collected at Danbury.

Monilia has been collected at Madison on *Amelanchier oblongifolia* with conidia 8–15 x 6–12μ. Its relationships are not clear.

Ramularia alismatis Fautr. On *Sagittaria heterophylla.* Arcadia. While this mucedine is not uncommon on *Alisma* and *Alisma* and *Sagittaria* frequently grow together I have seen the parasite on *Sagittaria* but once and then not in abundance.

Cercospora dubia (Riess) Wint. On *Chenopodium capitatum.* Shiocton. I do not find that the distinctions drawn by Bubak between this species and *C. chenopodii* Fresen. (*Ann. Mycol.*

6: 28) hold in Wisconsin. The collection on this host is more of the *C. chenopodii* type.

Cercospora absinthii (Pk.) Sacc. On *Artemisia Absinthium.* Casco.

Doassansia sagittariae (West.) Fisch. On *Sagittaria arifolia* Shiocton and Racine.

Gymnoconia peckiana (Howe) Trotter. Caeoma on *Rubus hispidus.* Necedah.

Cronartium quercus (Brondeau) Schroet. Uredinia on *Quercus bicolor* and *Q. ellipsoidalis.* Necedah. Telia on *Q. macrocarpa, Q. rubra* and *Q. ellipsoidalis.* The latter is the most common host of the telia in Wisconsin.

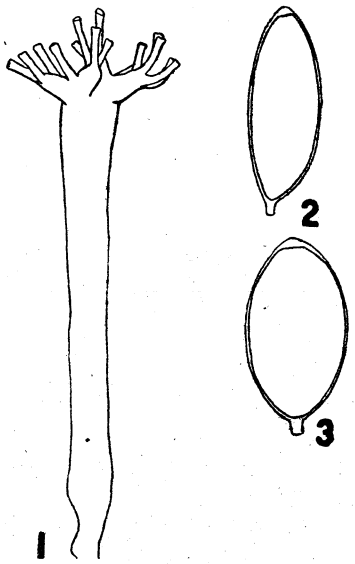
Peridermium comptoniae (Arth.) Orton & Adams. On *Pinus austriaca* in the state nursery on Trout lake in Vilas county.

ADDITIONAL SPECIES.

Physoderma vagans Schroet. To this species I am provisionally referring specimens on leaflets of *Sium cicutaefolium* collected in August, 1917 at Shiocton. The galls are round to elliptical, prominent, 1–2 mm. long; the resting spores 1–8 in each cell, globose to elliptical, 18–21 x 14–18μ with walls about 3μ thick. The host cells become inflated and rounded (up to 70 x 50μ) the walls thin, fuscous and having a chitinoid appearance.

PLASMOPARA CEPHALOPHORA n. sp. Conidiophores hypophyllous, effused, stout, straight, often clavate, 150–270 x 6–14μ, simple to the apex which is divided into a few short (6–15μ) stout branches which are irregularly divided into the ultimate branchlets which are terete, straight, truncate sometimes swollen at the apex; conidia hyaline, elliptical to oblong-fusoid, more or less acute at each end, flattened on one side, stipitate, furnished with an apical papilla, 45–70 x 20–33μ. On leaves of *Physostegia parviflora.* Shiocton, Plover and Dexterville, August, 1917. The conidia are imbricated in a compact head, bending of the pedicel (about 3μ long) allowing adjustment of position under pressure. The flattening of the conidia is perhaps the result of crowding; at least it facilitates close packing.

This mildew is near *Basidiophora* which it resembles in the position of the conidia and the character of the basidia but differs in the apical portion of the conidiophore not being abruptly swollen and rounded, but branched or cleft. In the Dexterville collection immature oöspores of the ordinary *Plasmopara* type were found.



Plasmopara cephalophora n. sp. 1. Conidiophore. 2 and 3 side and face views of conidium. Magnified 775 diameters and reduced one-half. Drawn by Mabel M. Brown with aid of camera lucida.

Peronospora silenes Wilson. On stems, leaves and fructification of *Silene antirhina.* Necedah. Oospores especially abundant in the capsules.

Peronospora linariae Fckl. On leaves and stems of *Linaria canadensis.* Lyndon Station. With oöspores.

Of a collection on living leaves of *Urtica* the following notes were made: Spots immarginate, round to oval, blackish brown becoming cinereous above, olivaceous becoming brownish below, 1–2½ cm. long, often confluent; perithecia hypophyllous, scattered, prominent, about 120μ in diameter; asci clavate-cylindrical, incurved, octosporous, 40–60 x 7–9μ; ascospores hyaline, fusoid, obtuse, becoming triseptate, 16–21 x 4–5μ; paraphyses filiform, very slender. On living leaves of *Urtica gracilis.*

Whitehall, Wisconsin, July 27, 1916. I have referred this, with some doubt, to *Metasphaeria chaetostoma* Sacc. var. *urticae* Rehm.

Keithia tsugae Farl. occurs on *Tsuga canadensis* in Wisconsin but has not been included in these lists because of doubt as to its parasitism. It has been found only on dead leaves attached to dead twigs. More favorable opportunities for observation however lead me to surmise that the death of the twigs is caused by the organism that sporulates on the leaves. The appearance of an infected tree reminds one of fire blight. It was especially abundant at Two Rivers in 1917.

Lophodermium juniperinum (Fr.) De Not. On *Juniperus communis depressa.* Two Rivers.

PHYLLOSTICTA BOEHMERIICOLA n. sp. Spots suborbicular, olivaceous with a darker margin and pale, sordid, central portion, 3–10 mm. in diameter; pycnidia epiphyllous, scattered, lenticular, succineous, ostiolate, 100–150μ; sporules oval to oblong, fuligenous tinted, 4–7 x 2–3μ. On leaves of *Boehmeria cylindrica.* Shiocton. August 1917.

Phyllosticta minutissima Ell. & Evht. On *Acer saccharinum;* accompanied by *Phloeospora aceris.* Shiocton.

Phyllosticta ulmicola Sacc. Under this name I am recording the occurrence of a fungus having the following characters: Spots definite, immarginate, orbicular, light brown becoming cinereous above and lacerate, finally falling away in fragments, 3–7 mm. in diameter, sometimes confluent; pycnidia epiphyllous, scattered, black, globose to depressed-globose, 60–80μ; sporules globose to elliptical, olivaceous-hyaline, continuous, 3–6 x 2–3μ. On *Ulmus americana.* Tisch Mills August 3, 1917. *Ulmus racemosa* August 5, 1917. This is probably a member of a group to forms of which various names have been applied in Europe and America.

Phyllosticta mitellae Pk. On *Mitella diphylla.* Melvina. In the collection that I am provisionally referring to this species the pycnidia are light brown and the sporules oblong to elliptical, 4–6 x 2–3μ. Occasional sporules have a median septum.

ASCOCHYTA NEPETAE n. sp. ad interim. Spots orbicular to elliptical, olivaceous usually with a narrow darker margin, 4–10

mm. long; pycnidia epiphyllous, few, scattered, depressed-globose, succineous with a dark ostiolar ring; sporules hyaline, oblong, uniseptate, not constricted, 10–14 x 3μ. On leaves of *Nepeta cataria.* Shiocton, August 1917.

Septoria sedi West. On *Sedum purpureum.* Plover. This is the fungus issued under this name in *Fungi Columbiani* 3081 and described by Peck as *Septoria sedicola* n. sp. (Report 1909 p. 29). I have not seen European material.

Septoria chamaecisti Vestergr. On leaves of *Lechea intermedia.* Plover.

Septoria acerina Pk. On *Acer spicatum.* Casco. This appears to be a member of the *Phloeospora aceris* group referred to in "Notes" I, pp. 80–81 and the spore body as I have seen it is an acervulus. Inoculation work seems to be required to define the relationship of the members of this group.

Septoria purpurascens Ell. & Mart. On *Potentilla arguta.* Lyndon Station. This is the form with epiphyllous pycnidia distributed in *Fungi Columbiani* 3487 and *F. Exot. Exsicc.* 143. I have not seen *S. potentillica* Thuem. the description of which suggests similarity.

Septoria delphinella Sacc. Spots orbicular to linear, brown to umber, 3–12 mm. long; pycnidia amphigenous but more numerous above, globose, with dark brown wall about 3μ thick, 60–90μ in diameter; sporules acicular, straight or curved, 35–50 x 1μ. On leaves of *Delphinium Penardi.* Hixton, July, 1916. This often causes the death of the portion of the narrow leaf lobe which is distal to the spot. The fungus is allied to *Septoria hepaticae* Desm. and *S. aquilegiae* Ell. & Kell.

Septoria araliae Ell. & Evht. On *Panax trifolia.* Millston.

Septoria menthicola Sacc. & Let. On *Mentha arvensis.* Madison. Pycnidia globose, about 60μ in diameter; sporules curved, 18–33 x 1–1½μ.

Septoria lupincola Dearn. On *Lupinus perennis.* Black River Falls.

Typical specimens of *Septoria paupera* Ellis have been collected in Wisconsin but I have not been able to divide the specimens on *Helianthus* satisfactorily.

Specimens on *Lactuca Scariola integrata* collected at Madison September 30, 1916 bear orbicular, cinereous spots with a pronounced dark border; the pycnidia are innate and the sporules 24–30 x 1–1½μ. This seems to be *Septoria unicolor* Wint. A collection on *Lactuca Scariola* made at the same station October 6, 1916 has the spots somewhat angular, definite but not margined, sometimes confluent and sporules about 30 x 1μ.

Colletotrichum salmonicolor O'Gara (*Mycologia*, **7**: 40) Of a collection that seems referable to this species the following notes were made.

Spots numerous, scattered, subcircular, black, about 1 mm. in diameter, sometimes confluent; acervuli hypophyllous and caulicolous, flat, 60–120μ wide, usually solitary in the center of the spot, soon exposed, the spore masses bright salmon color; sporules hyaline, as seen singly, with thin walls and granular contents, cylindrical, usually straight, 18–27 x 3½–6μ borne on similar but smaller (10–15 x 3μ) basidia. On leaves of *Asclepias syriaca.* Arcadia, Wisconsin, September, 1917. On the midribs and stems the spots are longer and acute at each end. Black setae occur in the acervuli occasionally. This differs from *Hainesia* only in the thick sporophore and occasional setae. It was abundant at the station where observed. It may be that this is not distinct from the fungus described by Saccardo as *Gloeosporium mollerianum* Thuem. var. *folliculosum* which occurred on follicles of *Asclepias verticillata* in a botanical garden in Portugal. (*Fl. Myc. Lusit.* **11**: 13 [1903], *Syll. Fung.* **18**: 458) of which I have not seen specimens.

In July 1916 *Gaylussacia baccata* bushes were noticed at Hixton that had the appearance of having been attacked by *Monilia* and on examination a few broad-limoniform conidia were found which measured 24–27 x 18–20μ. The material hardly warrants a determination but has been filed under the label *Monilia peckiana* Sacc. & Vogl.

Rhynchosporium secalis (Oud.) n. comb. (*Marsonia secalis* Oud. *Rhynchosporium graminicola* .Heinsen). On *Hordeum vulgare* (cult.) Madison.

SEPTOCYLINDRIUM ACUTUM n. sp. Spots definite, elliptical, brown becoming grey, often confluent, 1–8 mm. long; conidia amphigenous but more abundant above, hyaline, lance-fusoid,

catenulate, becoming uniseptate, straight or somewhat curved, 15–39 x 3μ. On leaves of *Agrostis alba.* Black River Falls, June 30, 1916. The septa are thin and not conspicuous.

Ovularia pulchella (Ces.) Sacc. var. AGROPYRI n. var.

Spots linear to oblong, dark brown becoming paler in the center, usually surrounded by a yellowish discoloration of the leaf, 2–5 mm. long, sometimes confluent; conidiophores amphigenous in small tufts or scattered, hyaline, straight or geniculate, 40–65 x 2–3μ; conidia acro-pleurogenous, spherical to oval, hyaline, 9–12 x 6–9μ. On leaves of *Agropyron tenerum.* Hixton, July 7, 1916.

In the supplementary list, p. 173, a *Ramularia* on *Fagopyrum* was noted under *Ramularia rufomaculans* Pk. but it was not included in the provisional list. It has been described as *Ramularia anomala* n. sp. by Peck in the Report of the State Botanist for 1912, p. 47.

RAMULARIA UMBRINA n. sp.

Spots orbicular to elliptical to angular, umber colored with a narrow, dark, raised margin and surrounded by more or less purple discoloration of the upper surface of the leaf, 2–5 mm. in diameter; conidiophores mostly hypopyllous in small tufts, subulate to terete, hyaline, straight, simple, continuous, often denticulate near the apex, 9–17 x 2–3μ; conidia hyaline, straight, catenulate, fusiform to cylindrical, sometimes uniseptate, 5–16 x 1½–2μ. On leaves of *Diervilla Lonicera.* Millston, Wisconsin, June 27, 1916. Hixton, Wisconsin, July 5, 1916.

Cercospora violae Sacc. On *Viola sp. indet.* Monroe (Copeland, 1901).

CERCOSPORA PANICI n. sp.

Spots fusoid, ferruginous, central portion sordid white, 2–4 x 1–2 mm. Conidiophores amphigenous, caespitose, fuliginous, straight or more or less flexuose and nodulose, 30–40 x 3μ; conidia hyaline, cylindrical, straight or curved, catenulate (?), 30–40 x 2–3μ. On leaves of *Panicum latifolium.* Shiocton, Wisconsin, August 15, 1917.

Fusarium sphaeriae Fckl. var. ROBUSTUM n. var. Conidia 7–11 septate, 60–75 x 5–6μ. On perithecia of *Apiosporina collinsii.* Hixton, July 4, 1916.

CERCOSPORA CICHORII n. sp.

Spots suborbicular, light brown to alutaceous to cinereous, more or less marked by concentric lines, 2–6 mm. in diameter, sometimes confluent; conidiophores mostly epiphyllous in small spreading tufts, brown, straight, curved or somewhat flexuose, terete or torulose and denticulate, continuous or septate, 20–75 x 3–6μ; conidia hyaline, obelavate-cylindrical, straight, septate, 90–150 x 4–6μ. On leaves of *Cichorium Intybus*. Madison, Wisconsin. September and October. If no Cercospora occurs on chicory in Europe one would suspect that this is an American species that has passed over from some related host but if so I do not know what it is.

ENTYLOMA PARVUM n. sp.

Sori on the upper portion of the culm, black, linear, about ½ mm. long; spores aggregated, compacted, fuligenous, subglobose or sometimes oval or ovate, smooth, 7–10μ long. On *Eleocharis acicularis*. Plover, Wisconsin, August 1917, Madison, Wisconsin, August 1892, (Cheney) Cambridge, Mass. 1906. (Grossenbacher, com. Farlow). This is most nearly allied to *Entyloma lineatum* (Cke.) Davis.

All collections of *Caeoma nitens* were tested as to spore germination in 1917. Of them one, from blackberry in the horticultural garden, developed promycelia and sporidia. This was the earliest collection of the season. All the others formed germ tubes. Arthur proposed the genus *Kunkelia* for the short cycled form in which the spores germinate as teliospores. (*Bot. Gaz.* **63**: 4 [1917].

University of Wisconsin Herbarium.
Madison, Wisconsin, April 1918.

INDEX TO HOSTS

IN "NOTES" IV, V, and VI.

INDEX TO FUNGI

Referred to in "Notes" I-VI

CONTRIBUTION TO THE CHEMISTRY OF AMERICAN CONIFERS.

BY A. W. SCHORGER.

The chemistry of conifers is deserving of particular study both on account of its scientific interest and economic importance. Pinene, the chief terpene occurring in the volatile oils of the *Coniferae,* derived its name from the genus *Pinus* in which it is found so abundantly. Through the study of pinene and its derivatives was laid the foundation of the chemistry of the terpenes which forms one of the most interesting chapters developed in organic chemistry during the past twenty-five years; and yet in spite of a vast amount of research, pinene is still one of the few terpenes whose constitution is not known with absolute certainty.

From the economic standpoint the conifers are the most important of the forest trees. The annual cut of timber from this class in the United States exceeds that of all other woods by fourfold, while the value of the various oils and resins obtained as by-products amounts to approximately $40,000,000. The conifers also supply the bulk of the raw material used in the pulp and paper industry. In addition, the development of terpene chemistry has pointed out the way for new uses of forest products. Camphor can be successfully synthesized from pinene, and turpentine can be broken down into isoprene which can in turn be readily polymerized into rubber. Success in the latter direction depends upon improvement in the yields of isoprene.

The present survey of the composition of the conifers has been made for the purpose of determining their constituents *per se* with a view to their utilization in the arts, and as a preliminary step in obtaining more fundamental knowledge of the constituents themselves. The naval stores industry of the South is de-

pendent upon the longleaf pine and Cuban pine and the exhaustion of these species is in sight. It has been found, however, that the western yellow pine will be capable of furnishing naval stores satisfactory both as to quality and quantity after the species in present use are exhausted.

There are approximately 92 species of conifers in the United States. From each species there is a possibility of obtaining four distinct oils from as many different portions of the tree, namely, the needles, bark, oleoresin, and wood, making in all 368 separate oils. This large field for investigation has been practically untouched. The author has examined 25 different volatile oils and oleoresins while approximately 26 others have received close investigation by various chemists.

Previous Work

The literature has been carefully examined in order to obtain references to the composition of the oils and oleoresins of American species. In some cases the information is very meager being limited to the yield of oil, saponification number or other constants. Certain species have been excluded from the following table since the articles relating to them gave no definite information in regard to the constituents present.

Needle Oils

Red spruce (*Picea rubens* Sarg.)[1]
Black spruce (*Picea mariana* Mill.)[2]
White spruce (*Picea canadensis* Mill.)[1]
Hemlock (*Tsuga canadensis* Linn.)[3]
Balsam fir (*Abies balsamea* Linn.)[4]
Tamarack (*Larix americana* Mich.)[2]
White cedar (*Thuja occidentalis* Linn.)[5]

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[1] Hanson and Babcock, Jour. Am. Chem. Soc. 28 (1906) 1198.

[2] Kremers, Pharm. Rund. 13 (1895) 135; Hanson and Babcock, Jour. Am. Chem. Soc. 28 (1906) 1198; Schimmel & Co., Ber. Oct. (1897) 25.

[3] Hunkel, Pharm. Rev. 14 (1896) 34; Bertram and Walbaum, Arch. d. Pharm. 231 (1893) 290; Power, "Descriptive Catalogue of Essential Oils," p. 74; Hanson and Babcock, loc. cit.; Pancoast and Graham. Proc. Pa. Pharm. Ass. (1905) 184; Schimmel and Co. Ber. Oct. (1894) 21, Oct. (1897) 25.

[4] Hunkel, Am. Jour. Pharm. 67 (1895) 9.

[5] Wallach, Annalen 272 (1892) 99; Jahns, Arch. d. Pharm. 221 (1883) 748; Ayer, Oil, Paint and Drug Rep. June 25, 1906, p. 17.

Western red cedar (*Thuja plicata* Don.)[6]
Red juniper (*Juniperus virginia* Linn.)[7]
Douglas fir (*Pseudotsuga taxifolia* Britt.)[8]

OLORESINS

Amabilis fir (*Abies amabilis* Forb.)[9]
Balsam fir (*Abies balsamea* Mill.)[10]
Douglas fir (*Pseudotsuga taxifolia* Britt.)[11]
Norway pine (*Pinus resinosa* Ait.)[12]
Cuban pine (*Pinus heterophylla* Sud.)[13]
Longleaf pine (*Pinus palustris* Mill.)[14]
Pond pine (*Pinus serotina* Mich.)[15]
Shortleaf pine (*Pinus echinata* Mill.)[16]
Loblolly pine (*Pinus taeda* Linn.)[16]
Digger pine (*Pinus sabiniana* Dougl.)[17]
Jeffrey pine (*Pinus Jeffreyi*).[18]

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[6] Rose and Livingston, Jour. Am. Chem. Soc. 34 (1912) 201; Blasdale, Jour. Am. Chem. Soc. 29 (1907) 539; Brandel and Dewey, Pharm. Rev. 26 (1908) 248; Schimmel & Co. Report, April (1909) 89.

[7] Schimmel & Co., Ber. April (1894) 56; April (1898) 13; Hanson & Babcock, loc. cit.

[8] Brandel and Sweet, Pharm. Rev. 26 (1908) 326.

[9] Rabak, Pharm. Rev. 46 (1905) 23.

[10] Tschirch and Brüning, (*Abies canadansis*). Arch. d. Pharm. 238 (1900) 487; Emmerich, Am. Jour. Pharm. 67 (1895) 135.

[11] Blasdale, Jour. Am. Chem. Soc. 23 (1901) 162; Frankforter, Jour. Am. Chem. Soc. 28 (1906) 1467; Rabak, Pharm. Rev. 22 (1904) 293.

[12] Frankforter, Jour. Am. Chem. Soc. 28 (1906) 1467; Ibid. 31 (1909) 561.

[13] Herty, Jour. Am. Chem. Soc. 30 (1908) 863; Kremers, Pharm. Rundsch, 13 (1895) 135; Long, J. Anal. and Appl. Chem. 6 (1891) 1; 7 (1893) 99.

[14] Kremers, Pharm. Rund. 13 (1895) 135; Pharm. Rev. 15 (1897) 7; Long, Jour. Am. Chem. Soc. 16 (1894) 844; 21 (1899) 637; Jour. Anal. and Appl. Chem. 6 (1891) 1; Semmler, Ber. 33 (1900) 1455; Ahlström and Aschan, Ber. 39 (1906) 1441; Barbier and Hilt, Compt. Rend. 108 (1889) 519; Herty, Jour. Am. Chem. Soc. 30 (1908) 863; Herty and Dickson, Jour. Ind. Eng. Chem. 4 (1912) 495; Tschirch and Koritschoner, Arch. d. Pharm., 240 (1902) 568.

[15] Herty and Dickson, Jour. Am. Chem. Soc. (1908) 872.

[16] Herty and Stern, Science, 27 (1908) 327.

[17] Wenzell, Am. Jour. Pharm. 44 (1872) 97; Pharm. Rev. 22 (1904) 408; Thorpe, Jour. Chem. Soc. 35 (1879) 296; Samuels, Pharm. Rec. 8 (1888) 39; Blasdale, Jour. Am. Chem. Soc. 23 (1901) 162; Kremers Pharm. Rev. 18 (1900) 165; Rabak, Pharm. Rev. 25 (1907) 212; for additional references see Bull. 119, U. S. Forest Service, p. 21.

[18] Wenzell, Pharm. Rev. 22 (1904) 408; Blasdale, Jour. Am. Chem. Soc. 23 (1901) 162; Leuchtenberger, Arch. d. Pharm. 245 (1907) 701.

Wood Oils

Red juniper (*Juniperus virginiana* L.)[1]
Longleaf pine (*Pinus palustris* Mill.)[2]
Western yellow pine (*Pinus ponderosa* Laws.)[3]
Singleleaf pine (*Pinus monophylla Torr.*)[3]
Jeffrey pine (*Pinus Jeffreyi*).[3]

Examination of Woods

The literature on the analysis of American woods is very meager. Usually only one or two determinations were made on each species. De Chalmot[4] determined the yields of furfural from a large number of woods, and Dean and Tower[5] give the cellulose content of a few species.

Scope of Present Work

The present investigation covers the examination of 25 oils and oleoresins, and seven species of wood. Only three of these oils had been previously examined by other investigators, the remaining 22 being new to chemical literature. The following tables give the products analyzed:

Needle Oils

Longleaf pine (*Pinus palustris* Mill.)
Cuban pine (*Pinus heterophylla* Sud.)
Western yellow pine (*Pinus ponderosa* Laws.)
Sugar pine (*Pinus lambertiana* Dougl.)
Digger pine (*Pinus sabiniana* Dougl.)
Lodgepole pine (*Pinus contorta* Loud.)
Red fir (*Abies magnifica* Murr.)
White fir (*Abies concolor* Parry.)
Douglas fir (*Pseudotsuga taxifolia* Britton.)
Incense cedar (*Libocedrus decurrens* Torr.)

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[1] Walter, Ann. Chim. Phys. 1 (1841) 501; 8 (1843) 354; Chapmann and Burgess, Proc. Chem. Soc. 168 (1896) 140; Semmler and Hoffmann, Ber. 40 (1907) 3521.

[2] Teeple, Jour. Am. Chem. Soc. 30 (1908) 412; Kremers, Pharm. Rev. 22 (1904) 150; Schimmel and Co., Ber. April (1910) 109; Toch, Jour. Ind. Eng. Chem. 6 (1914) 720.

[3] Adams, Jour. Ind. Eng. Chem. 7 (1915) 957.

[4] Am. Chem. J. 16 (1894) 224, 589.

[5] Jour. Am. Chem. Soc. 29 (1907) 1119.

Cone Oils

Western yellow pine (*Pinus ponderosa* Laws.)
Sugar pine (*Pinus lambertiana* Dougl.)
Longleaf pine (*Pinus palustris* Mill.)

Oleoresins

Western yellow pine (*Pinus ponderosa* Laws.)
Western yellow pine (*Pinus ponderosa scopulorum* Engelm.)
Sugar pine (*Pinus lambertiana* Dougl.)
Lodgepole pine (*Pinus contorta* Loud.)
Digger pine (*Pinus sabiniana* Dougl.)
Piñon pine (*Pinus edulis* Engelm).
Sand pine (*Pinus clausa* Sarg.)
Singleleaf pine (*Pinus monophylla* Torr. and Frem.)
Jeffrey pine (*Pinus Jeffreyi*).

Bark Oils

Incense cedar (*Libocedrus decurrens* Torr.)
White fir (*Abies concolor* Parry.)

Wood Oils

Port Orford cedar (*Chamaecyparis lawsoniana* Parlatore.)

Examination of Woods

Woods from four conifers and three hardwoods were analyzed the species being:

White spruce(*Picea canadensis* B. S. P.)
Douglas fir (*Pseudotsuga taxifolia* Britton.)
Longleaf pine (*Pinus palustris* Mill.)
Western larch (*Larix occidentalis* Nutt.)
Sugar maple (*Acer saccharum* Marsh.)
Yellow birch (*Betula lutea* Michx.)
Basswood (*Tilia americana* Linn.)

The three hardwoods are added for comparison with the conifers.

The analysis of each wood covered the following determinations: (1) moisture; (2) ash; (3) ether extract; (4) cold water extract; (5) hot water extract; (6) solubility in alkali; (7) pen-

tosan and methylpentosan; (8) cellulose; (9) acids formed by hydrolysis; (10) methoxy groups; and (11) the ash, pentosan, and methylpentosan content of the cellulose. A great amount of experimentation was necessary in order to work out methods giving accurate results.

Resume of Results

The composition of some of the volatile oils is particularly interesting. From the oil of Port Orford cedar wood was obtained a very pure d-α-pinene having a higher specific rotation than had been previously reported for this terpene. The leaf oil of incense cedar contained a new sesquiterpene that was named "labocedrene". Previous to the present work β-pinene had been detected in quantity in only one oil while in 12 of the oils from American conifers β-pinene was found to be the major constituent. It was possible to obtain apparently pure fractions of β-pinene from some of the conifers, and the constants for the natural terpene were found to be considerably higher than those recorded by Wallach for his synthetic β-pinene.

Turpentine oils usually consist only of terpenes, α-pinene, β-pinene, and camphene being the usual constituents. Phellandrene had not been previously recorded as occurring in oils of this class but it was found that the turpentine oil of *P. contorta* consisted almost entirely of this terpene. The same holds true with respect to sesquiterpenes. Cadinene was identified in the turpentine oils of *P. edulis* and *P. monophylla,* and an unidentified sesquiterpene occurs in *P. ponderosa.* So far as known the only turpentine previously mentioned as containing a sesquiterpene is *P. longifolia* of India.[1]

The composition of the oils has brought out several points of phytochemical and botanical interest. The oil obtained from the oleoresin of *P. sabiniana* consists almost entirely of n-heptane while that from the needles consists of terpenes. The source of the small amount of heptane, 3 per cent, present in the needle oil may be safely attributed to the small twigs, since they were not removed from the needles before distillation. The phytochemical processes occurring in the wood and in the needles are accordingly entirely different.

[1] Schimmel and Company.

Recently the composition of certain oils has been applied to determining species.[1] On the Pacific coast there occur two species of pines, *P. ponderosa* and *P. Jeffreyi,* that appear to gradually merge into each other, the intermediate forms being known as "cross variety" or "bastard" pine. Identification in the field and in the laboratory even by the trained botanist was very uncertain. Analyses of the oleoresins from five typical trees of each type showed clearly that there was no intergradation between *P. ponderosa* and *P. Jeffreyi* and that the "cross variety" pines should be referred to *P. ponderosa.* Heptane was found only in typical *P. Jeffreyi.*

The eastern form of *P. ponderosa* occurring in the Rocky Mountain region is known as *P. ponderosa scopulorum.* Some botanists maintain a distinction between the two while others class them under a single species. It was found, however, that the turpentine oils were distinctly different. The oils from the *P. ponderosa* were laevo-rotatory and consisted very largely of β-pinene, while those from *P. ponderosa scopulorum* were d-rotatory and contained about 65 per cent α-pinene. The well-defined differences between the volatile oils shows that a distinction between the species and its variety should be maintained.

The cellulose content of the woods was found to be nearly constant both for the conifers and hardwoods especially if the percentage content is based on the wood free from water soluble and ether soluble constituents. The quantity of pentosans present in the hardwoods is considerably greater than in the conifers. This distinction is also maintained in the celluloses. It is a striking fact that the pentosan content of the isolated celluloses is practically the same as that of the original wood, pointing to distinctly different kinds of cellulose. The quantities of methoxy groups and hydrolytic acid obtained from the conifers are also smaller than from the hardwoods.

The wood of the western larch (*Larix occidentalis*) was found to contain about 10 per cent of a galactan[2] that had not been previously described in the literature. This galactan yielded only galactose on hydrolysis. Further investigation showed that

[1] Schorger—"Chemistry as an Aid in the Identification of Species", Proc. Soc. Am. Foresters, 11 (1916) 33–39.

[2] Schorger and Smith, "The Galacton of *Larix Occidentalis*", Jour. Ind. Eng. Chem. 8 (1916).

galactans were characteristic of the conifers as they were detected in five additional species. The significance, if any, of their occurrence remains to be determined.

Examination of Oils and Oleoresins

Oil of Port Orford Cedar [*Chamaecyparis lawsoniana* (Murr.) *Parlatore*].[1]

This species is limited in its distribution to southwestern Oregon and northern California. Selected pieces of resinous wood when distilled with steam gave 10 per cent of oil having the constants: $d_{15}°$ 891; $n_{D15}°$ 1.477. After standing in a tightly stoppered amber-colored bottle for four years, the oil had the constants: $d_{15}°$ 0.9061; $n_{D15}°$ 1.4806. On rectification by shaking with sodium carbonate solution and distillation with steam the oil lost 16.4 per cent by volume. The rectified oil had nearly the same properties as the original oil as shown by the following; $d_{15}°$ 0.8905; $n_{D15}°$ 1.4758; $a_{D25}°$ +39.60; acid No. 0.30; ester No. 32.8; ester No. after acetylation 71.57.

A very pure d-α-pinene was obtained by repeated fractionation, the constants of which were as follows: b. p. 156.0 – 156.1° (760 mm.); $d_{15}°$ 0.8631; $n_{D15}°$ 1.4684; specific rotation $[a]_D$ + 51.52°; molecular refraction, M = 43.88; calculated for $C_{10}H_{16}$ *f*, 43.54. This is the highest previously recorded rotation for α-pinene. Vezes[2] had found for d-α-pinene from Grecian turpentine oil the rotation $[a]_D$+48.4°, and for l-α-pinene from eucalyptus oil *(E. laevopinea)* Smith[3] had found $[a]_{D19}°$–48.63°. In conformity with its high rotation it was found impossible to obtain a nitrosochloride from the purified pinene; oxidation with alkaline $K_2Mn_2O_8$ gave d-pinonic acid ($[a]_D$+92.69°) m. p. 68–69°, the semicarbazone of which melted at 203–205°.

Dipentene was detected by means of the tetrabromide m. p. 124°. Saponification of the ester fractions gave an oil containing d-borneol as shown by formation of d-camphor on oxidation; the semicarbazone melted at 236–237°. Cadinene, m. p. of dihydrochloride 117–8°, occurred in the high boiling fractions.

Analysis of the silver salts of the combined acids showed them to consist of silver acetate and silver caprinate: Ag in silver

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[1] Jour. Ind. Eng. Chem., 6, 631 (1914).
[2] Bull. Soc. Chim. (4) 5, 932 (1909).
[3] Jour. and Proc. Roy. Soc. N. S. W. 32, 195 (1898).

caprinate, $C_9H_{19}COO$ Ag, calculated 38.66%—found 38.45%; Ag in silver acetate, CH_3COO Ag—calculated 64.64%—found 64.42%. Acetic, caproic and formic acids were also found free. in the old oil.

The rectified oil had approximately the following composition: d-α-pinene 60–61%; dipentene 6–7%; free d-borneol 11%; bornyl acetate 11.5%; cadinene 6–7%; losses 5%.

Leaf Oil of Douglas Fir (*Pseudotsuga taxifolia* Britt)[1]

The oil of this species was examined by Brandel and Sweet[2] who claimed to have found free borneol, bornyl acetate and considerable camphene; pinene and limonene were thought to be present but were not identified. The results obtaind by the author were very different. Camphene could not be detected and β-pinene was found to be the principal constituent.

A series of six samples were examined with the following results: $d_{15}°$ 0.8727–0.8759; $n_{D_{15}}°$ 1.4758–1.4780; $a_{D_{20}}°$–17.02° to –22.17; acid No. 0.65–1.10; ester No. 11.13–24.25; ester No. after acetylation 27.50–51.78.

Furfural was detected in the first fraction of the oil by the deep rose-color obtained on treating the aqueous extract with aniline-hydrochloric acid solution. From a fraction b.p. 156–157°, $d_{15}°$ 0.8682, $a_{D_{22}}°$–11.94°, α-pinene, m. p. of nitrosochloride 103°, was obtained; its nitrolpiperidine melted at 118°. Examination of the fraction b. p. 160–162° for camphene gave negative results.

A fraction b. p. 170–172°, $d_{15}o$ 0.8628, $a_{D_{25}}o$–28.12° gave a dihydrochloride m. p. 50°. The next fraction b. p. 172–178.2°, $d_{15}°$ 0.8616, $a_{D_{25}}$–26.24°, gave with difficulty a tetrabromide m. p. 117–119° after two crystallizations from ethyl acetate, and after a third crystallization, at 121–2°. The high rotation of the fractions combined with the melting point of the tetrabromide indicate the presence of liminene in addition to dipentene.

Borneol was isolated from a fraction b. p. 208–213, $a_{D_{26}}°$–19.42°, as the pthalic ester. The liberated alcohol on oxidation gave camphor melting at 174°. The borneol was in part combined with acetic acid since the silver content of the salt isolated

[1] Jour. Am. Chem. Soc. 35, 1895 (1913).
[2] Pharm. Rev. 26, 326 (1908).

contained 64.26 per cent Ag; Ag calculated for CH_3COOAg is 64.64 per cent.

The highest boiling fractions contained "green oil" of which no definite derivatives were obtainable.

The major portion of the oil boiled between 164–167° and consisted of β-pinene. A fraction, b. p. 164–166°, $d_{15}°$ 0.8720, $a_{D22}°$ –17.19° gave a large yield of sodium nopinate on oxidation with alkaline $K_2Mn_2O_8$. The free nopinic acid melted at 126°.

The approximate composition of the oil was the following: l-α-pinene 25%; l-β-pinene 48%; i-(and l-) limonene 6%; bornyl-acetate 6%; free alcohol as borneol 6.5%; "green oil" 3%; furfural, trace.

Oleoresin of Sand Pine (*Pinus clausa* Sarg.)[1]

The sand pine is a small tree practically confined in its range to the state of Florida. The oleoresin contained 18.93% volatile oil, 72.30% rosin, the remainder consisting of water and foreign matter. Two samples of the volatile oil had the following properties: $d_{15}°$ 0.8725–0.8723; $n_{D15}°$ 1.4768–1.4767; $a_{D20}°$ –22.49 to –22.80°.

The first fraction of the oil b. p. 157–160°, $d_{15}°$ 0.8656, $a_{D20}°$ –20.17°, cantained α-pinene the nitrolpiperidine of which melted at 119°. Camphene was found in the fraction b. p. 160–162°, $d_{15}°$ 0.8671, $a_{D20}°$ –29.31°, by conversion into isoborneol melting at 207–9°.

β-pinene was found to constitute about 75% of the oil. The sodium nopinate obtained was oxidized to nopinene whose semicarbazone melted at 189°. Since the oil consisted so largely of β-pinene an attempt was made to isolate it in a pure state. After ten fractionations over metallic sodium, two fractions of fairly constant boiling point were obtained. The properties of these fractions and of a synthetic β-pinene prepared by Wallach were the following.

Fraction	B. P.	$n_{D20°}$	$d_{\frac{20°}{20°}}$	$[a]D$	M found	M Calculated for $C_{10}H_{16}$
1	164–165°	1.4772	0.8700	–25.00°	44.19	43.54
2	165–166°	1.4784	0.8709	–23.73°	44.23	43.54

[1] Jour. Ind. Eng. Chem. 7, 321 (1915).

Wallach's[1] synthetic β-pinene:

B. P.	$^{n}D22^{\circ}$	$^{d}22^{\circ}$	^{a}D	M found	Calculated for $C_{10}H_{16}$
163–164°	1.4724	0.8660	−22°20′	44.13	43.54

Fraction 2 was about four times as large as fraction 1 and the differences in the constants suggests the presence of a second terpene. All the constants have higher values than those given by Wallach but this has been the author's general experience in the examination of those volatile oils of which β-pinene was the chief constituent. On the possibility that the increased values might be due to the presence of camphene, fraction 1 was examined for this terpene but with negative results.

β-pinene is widely distributed in nature, but it may be of interest to mention that previous to the examination of the present series of volatile oils, this terpene had not been deteced in quantity except in hyssop oil.[2] The author has found β-pinene to be the principal constituent of the following oils: the needle oils of longleaf pine, Cuban pine, Douglas fir, white fir, western yellow pine, sugar pine and lodgepole pine; the cone oils of western yellow pine and sugar pine; the bark oil of white fir; and the turpentine oils of western yellow pine and sand pine.

The rosin from the sand pine crystallized readily from acetone. The abietic acid crystals began to melt at 150–151° and were completely liquid at 157°. When the rosin was crystallized from alcohol containing 10% of concentrated hydrochloric acid the crystals began to melt at 157–158° and were not completely liquid until 167°. The resin acids occurring in the oleoresin evidently undergo rearrangement with heat, and in presence of acids and other reagents. The abietic acid had the rotation $[a]_D$–85.46°. Analysis of the silver salt follows:

0.4885 g. silver salt gave 0.1234 g. Ag.	=26.34% Ag.
Calculated for silver abietate Ag. ($C_{20}H_{29}O_2$),	26.37% Ag.

The turpentine oil of the sand pine has the following composition: l-α-pinene, 10%; l-camphene, 10%; l-β-pinene, 75%. The rosin consists mainly of abietic acid.

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[1] Ann. 363, 1 (1908).

[2] Schimmel & Company, April Report, 1908, p. 58.

THE OLEORESIN OF JEFFREY PINE *(Pinus Jeffreyi.)*[1]

Five samples of oleoresin were examined in all. The yield of oil varied from 8.81–11.25%, the average being 9.96%. The oils had the following properties: $d_{15}°$ 0.6951–0.7110; $n_{D_{15}°}$ 1.3927–1.4060. On fractionation 92.45% of this oil distilled between 98.2–113.0° principally between 99 and 102°. Redistillation using a Hempel column gave an oil whose properties (b. p. 98.4° and $d_{15}°$ 0.6881) showed it to consist of n-heptane.

The residue left above the temperature 113° distilled principally between 200–215°. A portion of the oil formed a flocculent precipitate with sodium bisulphite and showed other properties characteristic of an aldehyde. A semicarbazone melting at 91–92° was prepared. Owing to lack of material no further derivatives could be prepared. Judging from the lemon-like odor and the m. p. of the semi-carbazone it was thought that citronellal[2] was probably present, the racemic form of citronallal semicarbazone melting at 96°.

The colophony had an acid No. of 147.6, a saponification No. 178.1, and contained 12.5 per cent of resene. Using acetone as the solvent, resin acid crystals melting at 137–8° were obtained, while when crystallized from acetone containing hydrochloric acid they melted at 145–6°. The colophony of this species crystallizes readily differing in this respect from the colophony of *P. sabiniana* which also yields heptane.

The resin crystals obtained from the crude oleoresin melted at 170–171°. The silver salt was analyzed as follows: 0.3926 g. of silver salt gave 0.1027 g. Ag = 26.16% Ag. Silver abietate, Ag ($C_{20}H_{29}O_2$), requires 26.37% Ag.

Leuchtenberger[3] extracted the colophony of Jeffery pine with ammonium carbonate and sodium carbonate solutions obtaining the following acids: α-jeffropinic acid $C_{10}H_{14}O_2$, m. p. 160–161°; β-jeffropinic acid, $C_{12}H_{18}O_2$, m. p. 81–82°; α-jeffropinolic acid, $C_{14}H_{20}O_2$ or $C_{14}H_{22}O_2$, m. p. 117–118° and β-jeffropinolic acid having the latter formula, m. p. 77–78°. None of these acids agree in melting point with those obtained by the author. The

[1] Jour. Ind. Eng. Chem. 5 (1913) 971.

[2] Schimmel & Co. (Report, Oct. 1914–April 1915, p. 45) working with a considerable quantity of material showed that the non-heptane constituents consisted of n-decylic aldehyde, linalool, and methylchavicol.

[3] Arch. d. Pharm. 245 (1907) 701.

acid obtained from the oleoresin melted at 170–171°, and its silver salt contained 26.16% of Ag agreeing with the formula of abietic acid, $C_{20}H_{30}O_2$. α-jeffropinic acid requires 39.51% Ag and α-jeffropinolic acid requires 32.98% Ag. To obtain acids of these formulae it would be necessary for the original resin acids to undergo profound alteration in heating to 145°C. which is contrary to experience with resin acids.

THE OLEORESIN OF SINGLELEAF PINE (*Pinus monophylla* Torr.)[1]

The oleoresin contained 19.00% of volatile oil having the following properties; $d_{15}°$ 0.8721–0.8733; $n_{D15}°$ 1.4732–1.4733; $a_{D15}°$ + 14.41° to + 17.26°.

The oil distilled mainly between 156–160° and consisted largely of d-α-pinene; m. p. of nitrolpiperidine 118°. β-pinene was not detected. The fraction b. p. 170–180°, $a_{D18}°$–1.18°, gave readily dipentene dihydrochloride, m. p. 49°. The highest boiling fractions contained cadinene. The fraction, b. p. 250–280°, $a_{D25}°$ +10.58, gave l-rotatory cadinene dihydrochloride, whose crystals melted at 117–118°.

The acid No. and saponification No. of the colophony were 155.9 and 163.3, respectively. The resin crystals from the colophony melted at 119–120° and were completely liquid at 129°. The oleoresin contained large resin acid crystals that melted at 129–130° after six crystallizations from acetone. The resid acid evidently has the formula, $C_{20}H_{30}O_2$, since the silver salt contained 26.65% Ag; Ag ($C_{20}H_{29}O_2$) requires 26.37% Ag.

The composition of the volatile oil is approximately as follows: 85% d-α-pinene; 4–5% i- or l-limonene; and 4–6% cadinene.

THE LEAF AND TWIG OIL OF CUBAN PINE (*Pinus heterophylla* Ell.)[2]

Four samples of leaf and twig oil had the following range of properties: $d_{15}°$ 0.8877–0.8894; $n_{D15}°$ 1.4845–1.4869; $a_{D28}°$–32.09 to –35.67°; acid No. 0.65–0.75; ester No. 9.73–10.54; ester No. after acetylation 46.26–53.81; average yield of oil 0.271%.

A sample of oil distilled from needles free from twigs had the constants: $d_{15}°$ 0.8895; $n_{D15}°$ 1.4880; $a_{D28}°$–36.54; acid No. 0.78; ester No. 8.75; ester No. after acetylation 43.46; yield of oil 0.193%.

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[1] Jour. Ind. Eng. Chem. 5 (1913) 971.
[2] Journ. Ind. Eng. Chem. 6 (1914) 723.

Furfural was qualitatively determined in the pinene fractions by the colorimetric method with aniline salts. The presence of l-α-pinene was shown by means of the nitrolpiperidine, m. p. 117–8°. The l-rotatory fractions b. p. 160–162° contained camphene which was converted into isoborneol, m. p. 209–210°, and then into camphor; m. p. of semicarbazone, 235–236°.

The principal terpene present in the oil was β-pinene a fraction having the constants, b. p. 164–166°, $d_{15}°$ 0.8704, $a_{D_{25}}°$–24.12° gave one third of its weight of sodium nopinate. The nopinic acid, m. p. 125°, was further oxidized to nopinone; m. p. of semicarbazone 185–6°. Dipentene was detected by means of the dihydrochloride m. p. 49°, and tetrabromide, m. p. 115–7°. Phellandrene was absent.

The ester fraction contained l-rotatory borneol, m. p. 201–202°, isolated by means of the pthalic ester. The combined acids appeared to be caprylic and caproic acids from analysis of their silver salts. The fraction b. p. 270–280°, $d_{21}°$ 0.9190, $a_{D_{21}}°$–14.76°, gave crystals of cadinene dihydrochloride, m. p. 118°. An 11.13% solution of the crystals had the rotation $a_{D_{21}}°$–3.51°.

The leaf and twig oil of Cuban pine has the following composition: furfural trace; l-pinene 4%; l-camphene 10%; l-β-pinene 35–36%; dipentene 8%; bornyl ester (as acetate) 3.5%; free borneol 11.4%; d-cadinene 18–19%. The combined acids were apaprently caproic and caprylic acids.

LEAF AND TWIG OIL OF LONGLEAF PINE (*Pinus palustris* Mill.)[1]

A series of four oils had the following constants: $d_{15}°$ 0.8829–0.8849; $n_{D_{15}}°$ 1.4818–1.4825; $a_{D_{28}}°$–26.78 to –30.49°; acid No. 0.55–0.73; ester No. 6.05–7.22; ester No. after acetylation 36.53–46.37; average yield of oil 0.401%.

It was thought that the yield of oil from the various species might be correlated with the number and size of the oil ducts. Microphotographs of cross sections of the needles brought out this relation in a striking manner. The leaf of the long-leaf pine containing five large ducts gave 0.401% of oil, the Cuban pine needles containing ten small oil ducts gave 0.271% of oil while the lodgepole pine needles containing only two oil ducts gave but 0.112% of oil.

[1] Jour. Ind. Eng. Chem. 6 (1914) 723.

In harmony with most of the needle oils, small amounts of furfural were present. The first fraction of the oil contained l-α-pinene, m. p. of nitrolpiperidine 119°. Camphene was also present in the fractions boiling between 160–162° as shown by obtaining isobornoel, m. p. 207–210°, by hydration with acetic acid-sulphuric acid mixture. β-pinene was present in large amounts; m. p. of nopinic acid 126–7°; m. p. of nopinone semicarbazone 187°. Dipentene was detected through its tetrabromide, m. p. 124°. Phellandrene and sylvestrene were apparently absent.

The ester fraction after saponification yielded an l-rotatory oil, $a_{D_{28}}$°–37.17°, having the boiling point of borneol. By means of the pthalic ester method borneol, m. p. 201–202°, was obtained which was further identified by oxidation to camphor; m. p. of semicarbazone 231–3°. The highest boiling fractions contained cadinene whose dihydrochloride melted at 117–118°.

The approximate composition of the oil is the following: furfural trace; l-α-pinene 8–9%; l-camphene 13–14%; l-β-pinene 44%; dipentene 5%; bornyl ester (as acetate) 2.4%; free alcohol 10.0%; d-cadinene 10–11%.

LEAF OIL OF LONGLEAF PINE (*Pinus palustris* Mill.)

An oil obtained by distilling needles from which all the woody twigs had been removed by hand had the following properties: d_{15}° 0.8841; $n_{D_{15}}$° 1.4834; $a_{D_{28}}$–32.50; acid No. 0.67; ester No. 5.91; ester No. after acetylation 40.46; yield of oil 0.417%.

Analysis allowed the same constituents to be present as in the leaf and twig oil. The oil in the wood of this species consists mainly of α-pinene. It was anticipated that the leaf oil would accordingly contain less α-pinene and have a higher alcohol and ester content than the leaf and twig oil. Less α-pinene was actually found but the ester content was slightly lowered rather than increased. The same result was obtained in the oils of the Cuban pine.

The turpentine oils of the Cuban and longleaf pines are very similar. It is interesting to note that the leaf and twig oils of the two species contain the same constituents in practically the same proportion.

The composition of the leaf oil is the following: furfural trace: l-α-pinene 2%; l-camphene 12–13%; l-β-pinene 50%; dipentene

5%; bornyl ester 2%; free borneol 9.8%; d-cadinene 11%; the combined acids consist of caprylic acid, with probably heptoic and caproic acids.

THE CONE OIL OF LONGLEAF PINE *(Pinus palustris* Mill.)[1]

The green cones distilled in June gave 0.363% of oil having the following constants; $d_{15}°$ 0.8756; $n_{D15}°$ 1.4760; $a_{D28}°$ –9.22°; acid No. 0.42; ester No. 3.95; ester No. after acetylation 31.07.

The fraction, b. p. 156–158°, $d_{15}°$ 0.8637, $a_{D24}°$ +6.82°, gave pinene nitrolpiperidine melting at 118–9°. In the cone oil accordingly d-α-pinene is present while in the leaf and twig oil of this species the α-pinene is decidedly l-rotatory. Camphene was present in the weakly l-rotatory fractions b. p. 160–162° as shown by conversion into isoborneol m. p. 208–210°. β-pinene was identified by oxidation to nopinic acid; m. p. 125–126°. Dipentene was present, m. p. of tetrabromide 123–4°, but phellandrene was not found.

The higher boiling fractions contained borneol, m. p. 202–203°, and cadinene, m. p. of dihydrochloride 116–7°.

The cone oil has the following composition: furfural; d-α-pinene 39–40%; l-camphene 12%; l-β-pinene 25%; dipentene 6–7%; bornyl ester 1.4%; free borneol 7.6%; d-cadinene 1–2%.

THE LEAF AND TWIG OF WHITE FIR *(Abies concolor* Parry.)[2]

A series of seven oils had the following range of constants: $d_{15}°$ 0.8720–0.8777; $n_{D15}°$ 1.4781–1.4796; $a_{D25}°$ –20.11° to –27.94°; acid No. 1.01–1.81; ester No. 14.48–27.34; ester No. after acetylation 47.84–55.51; average yield of oil 0.128%. The oil distilled from leaves taken from the top showed a slightly higher total alcohol content than the oil from leaves at the base of the same tree.

Furfural was detected in the first fraction. L-α-pinene was present in small quantity; m. p. of nitrolpiperidine 118.5°. From the fraction b. p. 160–162°, $d_{15}°$ 0.8695, $a_{D23}°$ –27.39°, isoborneol, m. p. 209–210°, was obtained showing the presence of camphene. β-pinene was the chief constituent of the oil. The fraction b. p. 164–166.5° $d_{15}°$ 0.8715, $a_{D23}°$ –23.66°, gave on oxidation large quantities of sodium nopinate.

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[1] Jour. Ind. Eng. Chem. 6 (1914) 723.
[2] Jour. Ind. Eng. Chem. 6 (1914) 809.

The free nopinic acid melted at 126.6–127°. The oil boiling between 170–180° gave a mass of crystals of phellandrene nitrite melting at 102°. Limonene was not detected as ether the dihydrochloride or tetrabromide.

Borneol was shown to be present by oxidation to camphor the semicarbazone of which melted at 236–7°. The bornyl ester is mainly the acetate since the silver salt of the acid obtained by saponification contained 64.56% Ag. The fraction b. p. 265–285° was emerald green in color and had the constants: $d_{19.5°}$ 0.925; $n_{D15°}$ 1.4936; $a_{D20°}$–0.49° for a 37.83% solution in ether. No solid derivatives of this oil was obtained.

The leaf and twig oil had the following composition: furfural trace; α-pinene 12%; l-camphene 8%; l-β-pinene 42%; l-phellandrene 15%; bornyl acetate 6.5%; free borneol 9.5%; "green oil" 3%.

THE BARK OIL OF WHITE FIR (*Abies concolor* Parry.)[1]

Two oils, distilled from the bark from small trees peeled for poles had the following constants: $d_{15°}$ 0.8767–0.8702; $n_{D15°}$ 1.4833–1.4809; $a_{D20°}$–20.95° to 20.15°; acid No. 1.22–0.87; ester No. 6.88–6.43; ester No. after acetylation 23.34–20.45; average yield of oil 0.095%.

About 80% of the oil distilled between 162.5° and 180°. Furfural was present as usual. About 9.0% of the oil consisted of l-α-pinene, the nitrolpiperidene of which melted at 118°. The fractions boiling between 163–170° gave an oxidation nopinic acid, m. p. 126–127°; further oxidation gave the ketone nopinone whose semicarbazone melted at 188°. The presence of β-pinene is accordingly assured. The l-rotatory fraction boiling between 170–180° gave dipentene dihydrochloride but no solid derivative was obtained on bromination.

The fraction, b. p. 192–250°, containing the esters amounted to only 4.5 g. and was not further examined. The ester and free alcohol are calculated as bornyl acetate and borneol. The highest boiling fraction consisted of "green oil".

The bark oil has the following composition: furfural; l-α-pinene 9.0%; 1-β-pinene 60%; dipentene 12–13%; ester as bornyl acetate 2.5%; free broneol 4.5%; "green oil" 5%.

[1] Jour. Ind. Eng. Chem. 6 (1914) 809.

THE LEAF AND TWIG OIL OF WESTERN YELLOW PINE (*Pinus ponderosa* Laws.)[1]

A series of 10 oils distilled from the leaves only had the following range of properties: $d_{15}°$ 0.8718–0.8849; $n_{D_{15}}°$ 1.4789–1.4815; $a_{D_{220}}°$–15.73 to –19.59°; acid No. 0.85–2.36; ester No. 3.88–7.83; ester No. after acetylation 24.11–35.10; average yield of oil 0.071%. A series of four oils distilled from the needles and twigs had the constants; $d_{15}°$ 0.8755–0.8844; $n_{D_{15}}°$ 1.4805–1.4838; $a_{D_{20}}°$–15.94 to –17.26; acid No. 0.67–0.87; ester No. 5.89–8.10; ester No. after acetylation 25.14–35.68; average yield of oil 0.114%.

The oils contained very little α-pinene. After repeated fractionation only 1.5% was obtained distilling between 157 and 160°, having the constants, $d_{15}°$ 0.8660 and $a_{D_{23}}$–27.0°. The pinene nitrosochloride obtained melted at 102.5°. Camphene was not found. β-pinene was present in large amounts; it was oxidized to nopinic acid, m. p. 126°, and to nopinone, m. p. of semicarbazone 188°. Dipentene was present as shown by the tetrabromide m. p. 124°.

Borneol was detected with difficulty. After saponification of the ester fraction, the liberated alcohols were heated with phthalic anhydride and the ester purified in the usual way. On subsequent saponification an oil was obtained; on oxidizing this oil a few crystals having the appearance and odor of camphor were formed. After sublimation the crystals melted at about 160°. Formic and acetic acids were present in the oil both in the free and combined state. The fraction b. p. 255–285° contained the "green oil" found in so many of the needle oils.

The composition of the oil is approximately as follows: l-α-pinene 2%; l-β-pinene 75%; dipentene 6%; bornyl acetate 2%; free alcohol as borneol 7%; "green oil" 3%.

THE CONE OIL OF WESTERN YELLOW PINE (*Pinus ponderosa* Laws.)[1]

The pale green oil had the following properties: $d_{15}°$ 0.8757; $n_{D_{15}}°$ 1.4789; $a_{D_{20}}°$–11.48; acid No. 1.27; ester No. 7.20; ester No. after acetylation 22.41; yield of oil 0.063%.

Furfural was detected colorimetrically. The fraction b. p.

[1] Jour. Ind. Eng. Chem. 6 (1914) 893.

159–164°, $a_{D_{25}}$°–25.33° contained α-pinene; m. p. of nitrosochloride 103°. β-pinene was shown to be present by obtaining nopinic acid melting at 126–7°. The fraction b. p. 170–173° was examined for phellandrene with negative results, while the fraction b. p. 173–176° gave a good yield of dipentene dihydrochloride, m. p. 50°.

The ester fraction was too small for further examination. "Green oil" was present in the higher fraction.

The composition of the oil is the following: furfural, trace; l-α-pinene 6.0%; l-β-pinene 60%; dipentene 12–13%; ester as bornyl acetate 2.5%; free alcohol as borneol 4%; "green oil" 3–4%.

THE LEAF AND TWIG OIL OF SUGAR PINE (*Pinus Lambertiana* Dougl.)[1]

The seven oils examined had the following range of properties: d_{15}° 0.8676–0.8738; $n_{D_{15}}$° 1.4777–1.4795; $a_{D_{20}}$°–11.07 to –16.50°; acid No. 0.68–2.38; ester No. 2.22–5.91; ester No. after acetylation 23.25–32.04; average yield of oil 0.090%.

The fraction b. p. 156–160° contained furfural and l-α-pinene, the pinene nitrolpiperidine melting at 119°. The greater portion of the oil distilled between 164–167° and contained l-β-pinene. The oil when oxidized in the customary manner gave nopinic acid, m. p. 126°, and nopinone, the semicarbazone melting at 188.5°. Dipentene was present in the fractions b. p. 170–178°, the tetrabromide melting at 124°. The dihydrochloride was also prepared. Since this compound melted at 50°, sylvestrene was evidently absent.

Borneol was present as shown by oxidation to l-camphor melting at 167–170°. The silver salts prepared from the acids obtained from the ester fraction contained 64.86%, 40.80%, and 35.27% Ag respectively. Acetic acid was accordingly present along with higher acids. "Green oil" was again present in the higher fractions.

The composition of the oil is the following: furfural, trace; l-α-pinene 21%; l-β-pinene 51%; dipentene 12%; bornyl acetate 1.5%; free l-borneol 8%; "green oil" 1%.

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[1] Jour. Ind. Eng. Chem. 6 (1914) 893.

THE CONE OIL OF SUGAR PINE *(Pinus lambertiana* Dougal.).[1]

The light green oil had the following constants: d_{15° 0.8692; n_{D15° 1.4771; a_{D20° –23.18°; acid No. 0.63; ester No. 3.75; ester No. after acetylation 17.04; yield of oil 0.318%.

Furfural was present in the first fraction along with l-α-pinene. The pinene nitrosochloride melted at 98–99°, and the nitrolpiperidine at 116°. The l-rotary fraction b. p. 160–163° contained camphene. This terpene was identified by conversion into isoborneol melting at 211–212° in a sealed tube. β-pinene was present as usual. For identification the nopinene semicarbazone melting at 188–188.5° was prepared. The small fraction b. p. 170–180° gave dipentene dihydrochloride, m. p. 49–50°, when treated with hydrochloric acid gas.

The ester fraction was too small for examination. A yellow oil was obtained boiling between 255–290° that may be a sesquiterpene. When dissolved in ether and saturated with HCl gas the solution became deep purple. A solid hydrochloride could not be obtained.

The composition of the cone oil is approximately the following: furfural, trace; l-α-pinene 22%; l-camphene 21%; l-β-pinene 39–40%; dipentene 4–5%; ester as bornyl acetate 1.5%; free alcohol as l-borneol 3.5%; sesquiterpene *(?)* 1%.

THE LEAF AND TWIG OIL OF DIGGER PINE *(Pinus sabiniana* Dougl.)[2]

Three samples of the oil had the following constants: d_{15° 0.8517–0.8566; n_{D15° 1.4670–1.4708; a_{D20} –20.93° to –38.36°; acid No. 1.47–2.05; ester No. 6.77–11.98; ester No. after acetylation 25.86–37.16; average yield of oil 0.09%.

The oil began to distill at 100° and 6% was collected up to 152°. This fraction was repeatedly treated with concentrated sulphuric to remove terpenes. The residual oil amounting to 3% consisted of heptane as shown by the following properties: b. p. 98.5–101°, d_{15° 0.7013. The twigs present in the distillation material without doubt were the source of this small amount of heptane, since the oil from the oleoresin obtained from the wood of this species consists almost entirely of n-heptane.

The fraction b. p. 156–157°, d_{15° 0.8618, and a_{D20} –26.24°

[1] Jour. Ind. Eng. Chem. 6 (1914) 893.
[2] Jour. Ind. Eng. Chem. 7 (1915) 24.

contained α-pinene; m. p. of nitrasochloride 104–105°; m. p. of nitrolpiperidine 117°. The small amount of oil distilling between 160–170° was examined for β-pinene with negative results. Limonene was present, m. p. of tetrabromide 124°, while phellandrene and sylvestrene were absent.

The oil obtained by saponification of the ester fractions was oxidized with a saturated solution of potassium permanganate. Steam distillation yielded a small amount of oil having a strong odor of camphor. From the oxidation liquors an acid was obtained that sublimed readily and crystallized from water in thin needles. By titration of a known weight with standard alkali and by determination of its melting point, 183–184°, this acid was found to be anisic acid, indicating the presence of methylchavicol in the oil. A small amount of green oil was present in the higher fractions.

The fact that the oil from the needles of this species consists of terpenes while the oil from the wood is mainly n-heptane has a particular significance. It proves that in the digger pine at least, if not in all conifers, that the phytochemical processes taking place in the leaves and in the wood are entirely different.

The composition of the leaf and twig oil is the following: n-heptane 3%; l-α-pinene 58–59%; l-limonene 18%; ester as bornyl acetate 3.5%; free alcohol as borneol 6%; methylchavicol (?); "green oil" 2–3%.

THE LEAF AND TWIG OIL OF LODGEPOLE PINE (*Pinus contorta* Loud.)[1]

The sample examined had the following constants: $d_{15}°$ 0.8690; $n_{D_{15}}°$ 1.4831; $a_{D_{20}}°$–17.84°; acid No. 0.90; ester No. 6.02; ester No. after acetylation 32.30; yield of oil 0.234%.

An aqueous extract of the first fraction contained furfural. This fraction b. p. 156–160°, $d_{15}°$ 0.8662, $a_{D_{25}}°$–24.85°, consisted chiefly of α-pinene; m. p. of nitrosochloride 103.0–103.5°; m. p. of nitrolpiperidine 118°. Camphene was shown to be present by conversion into isoborneol m. p. 205–207° after one crystallization. The principal terpene in the oil was β-pinene, the nopinic acid melting at 127°. The fraction b. p. 170–180° consisted largely of l-phellandrene, the nitrite of which melted at 102°. Phellandrene is the chief terpene present in the turpen-

[1] Jour. Ind. Eng. Chem. 7 (1915) 24.

tine oil of this species. Bromination of portions of the oil collected between 170–180° did not yield a solid dipentene tetrabromide, owing possibly to the large amount of phellandrene present; however, dipentene dihydrochloride melting at 49° was obtained.

Oxidation of the alcohols obtained by saponification of the ester fractions gave a small amount of an oil having a strong odor of camphor. The oxidation liquors contained anisic acid, m. p. 183–184°. As in the case of the leaf and twig oil of digger pine anisic acid indicates the probable presence of methyl chavicol. The fraction boiling between 265 and 284° and having the rotation $a_{D_{21}}$°+14.69 was rich in cadinene. The cadinene dihydrochloride obtained melted at 117–118° and had the specific rotation [a]D –45.66° It may be mentioned that all the cadinene fractions from the various oils examined were d-rotatory while the cadinene dihydrochlorides obtained were always l-rotatory.

The oil has the following composition: furfural, trace; l-α-pinene 3%; l-β-pinene 49–50%; l-phellandrene and dipentene 19%; ester as bornyl acetate 2.0%; free alcohol as l-borneol 7.5%; methyl chavicol (?); cadinene 7%.

THE LEAF AND TWIG OIL OF RED FIR (*Abies magnifica* Murr.)[1]

The properties of the oil were the following: d_{15}° 0.8665; $n_{D_{15}}$° 1.4861; $a_{D_{20}}$°–16.70° acid No. 0.75; ester No. 9.93; ester No. after acetylation 36.22; yield of oil 0.154%.

The oil did not begin to distill until a temperature of 167° was reached. By repeated fractionation 3.6 grams of oil were obtained distilling between 160–164°. On treatment with ethyl nitrite and hydrochloric acid, the intense green coloration characteristic of the formation of pinene nitrosochloride was obtained but none of the solid derivative separated out. Oxidation of the oil distilling between 164–168° gave nopinic acid melting at 126–127°; this proves the presence of β-pinene. Phellandrene was the only additional terpene that could be detected. Large amounts of phellandrene nitrite melting at 102–103° were obtained. Dipentene could not be detected as either the tetrabromide or dihydrochloride.

The oil obtained by saponifying the ester fractions gave on oxidation so small an amount of solid camphor that it could not

[1] Jour. Ind. Eng. Chem. 7 (1915) 24.

be further characterized. About 13% of "green oil" was present in the higher fractions. It had the following properties: b. p. 255–260°; $d_{15}°$ 0.8963; $n_{D_{15}}°$ 1.4952; specific rotation $[a]_D$ –6.05°. A drop of the oil when dissolved in glacial acetic acid and then treated with bromine vapors, gave a purple solution, becoming deep blue. . Attempts to prepare solid derivatives such as the bromide, hydrochloride, nitrite, nitrosochloride, etc., were unsuccessful. A careful study of this "green oil", characteristic of so many of the conifer leaf oils, should prove to be highly interesting. The material, however, has been difficult to obtain.

The "green oil" will probably prove to be related to the "blue oil" (azulene) found in numerous volatile oils.

The composition of the oil is approximately the following: furfural, trace; α-pinene (?); l-β-pinene 16–18%; l-phellandrene 52%; ester as bornylacetate 3.5%; free alcohol as borneol 75%; "green oil" 13%.

THE LEAF AND TWIG OIL OF INCENSE *(Libocedrus decurrens* Torrey).[1]

Nine samples of oil distilled from normal material in the regular manner had the following range of properties: $d_{15}°$ 0.8655–0.8733; $n_{D_{15}}°$ 1.4754–1.4775; $a_{D_{20}}°$ –3.20 to +38.68°; acid No. 0.48–0.74; ester No. 19.19–24.27; ester No. after acetylation 28.64–39.83; average yield of oil 0.225%. The variation in the optical rotation is very pronounced.

A quantity of leaves and twigs that had been thoroughly mixed was divided into three portions; the first portion was distilled while fresh, and the second and third portions were distilled after having been stored two and four weeks respectively in the open air. Analysis of the three oils obtained showed a remarkably close agreement in all of their constants, showing that the storage had been without perceptible influence. It is interesting to note that there was a slight increase in the yield of oil from the stored material even when the calculation was based on the original green weight.

In one case the distillate from a charge of leaves and twigs was caught in four fractions and these fractions were examined separately. The properties of the first fraction differed slightly

[1] Jour. Ind. Eng. Chem. 8 (1916) 22.

from the remainder, but even in this case the difference was less than anticipated.

The distillation experiments lasted from May to November. The yield of oil was greatest during May and November but the total borneol content of the oils was greatest in August and September.

The first fraction consisted of l-α-pinene and contained a small amount of furfural. Regardless of the rotation of the original oil the pinene fractions were decidedly l-rotatory. For example, an oil having the rotation $a_{D_{20}}°$ + 38.68° gave a fraction having the boiling point of pinene and the rotation $a_{D_{24}}°$ –19.88° Pinene nitrolpiperidine melting at 117–118° was prepared from it. β-pinene and camphene were not found.

The oil distilling between 170–180° contained dipentene, limonene and sylvestrene. The first two terpenes were shown to be present by obtaining two tetrabromides by fractional crystallization melting at about 113° and 123.5°. Sylvestrene was identified by preparation of the dihydrochloride melting at 72–72.5° and by the deep blue color which the sylvestrene, regenerated from the dihydrochloride, gave with acetic anhydride and concentrated sulphuric acid. Sylvestrene is one of the rarer terpenes and this appears to be the first record of its occurrence in any American oil.

Borneol was identified by oxidation to camphor melting at 173–174°. The combined acids consisted of acetic and caproic acids. After removal of the esters a sesquiterpene was obtained in the fraction b. p. 250–280° and a deep green oil between 280 and 310°. The sesquiterpene had the following properties: b. p. 260–280°; $d_{20}°$ 0.9292; $n_{D_{26}}°$ 1.4994; $a_{D_{26}}°$ +6.4°. The hydrochloride prepared from this fraction crystallized from ethyl acetate in thin plates and melted at 132–133°. This sesquiterpene, "libocedrene", could not be identified with any of the sesquiterpenes recorded in the literature. Lack of material prevented a more detailed study.

The composition of the oil is the following: furfural, trace; l-α-pinene 12–16%; d-sylvestrene, d-limonene and dipentene 54–58%; bornyl acetate 8%; free borneol 4%; libocedrene 6–7%; "green oil" 2%.

The Bark Oil of Incense Cedar (*Libocedrus decurrens* Torrey.)[1]

The oil has the following constants; $d_{15}°$ 0.8621; $n_{D16}°$ 1.4716; $a_{D20}o$ +1.10; acid No. 0.60; ester No. 3.22; ester No. after acetylation 9.53; yield of oil 0.14%.

Furfural was detected colorimetrically. The oil consisted largely of α-pinene, the nitrolpiperidine melting at 117–118°. The small amount of oil distilling between 160–168° was examined for β-pinene with negative results. The oil distilling between 168-173° gave dipentene dihydrochloride melting at 48–49°. The melting point of the dihydrochloride indicates the absence of sylvestrene.

The ester fraction was too small for identification of the constituents. "Green oil" was again present in the high boiling fractions.

The oil has the following composition: furfural, trace; α-pinene 75–85%; dipentene 5–6%; ester as bornyl acetate 1%; free alcohol as borneol 2%; "green oil" 3%.

The Oleoresin of Digger Pine (*Pinus sabiniana Dougl.*)[2]

The oleoresin contained 11.4% of oil having the constants: $d_{15}°$ 0.6971; $n_{D15}°$ 1.3903.

About 95% of the oil distilled between 96.1 and 98.8°. This oil consisted of n-heptane as shown by determination of the physical properties.

Rabak[3] states that both the oleoresin and rosin are optically inactive but this was not found to be true of either substance. A 5.58% alcoholic solution of the rosin had the rotation $a_{D20}°$ +0.38°.

All attempts to obtain a crystalline resin acid from the original rosin were unsuccessful. At 10 mm. pressure, the rosin distilled between 240 and 250° with only slight decomposition. The distillate cooled to a hard transparent mass that crystallized readily from acetone. The crystals melted at 151–152°. The silver salt contained 26.15% of silver showing that the resin acid had the formula of abietic acid, $C_{20}H_{30}O_2$, the silver salt of which requires 26.37% silver.

Resin crystals removed from the original oleoresin by suction

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[1] Jour. Ind. Eng. Chem. 8 (1916) 22.
[2] Forest Service—Bulletin 119, p. 18.
[3] Pharm. Rev. 25 (1907) 212.

and then repeatedly crystallized from acetone and methyl alcohol melted at 131° and had the specific rotation [a] $_D$–95.82°. When a portion of the same crystals were crystallized from methyl alcohol containing hydrochloric acid triangular crystals melting at 158–159° resulted. The molecular rearrangement produced by hydrochloric acid in the case of resin acids is very marked. The silver salt contained 26.44% Ag showing that the resin acid had the formula $C_{20}H_{30}O_2$.

It is shown that in accordance with the observations of previous investigators the volatile consists largely of n-heptane. Resin acids having the formula $C_{20}H_{30}O_2$ were isolated from the crude oleoresin and from the colophony distilled under reduced pressure. The colophony could not be made to crystallize in its original state.

The Oleoresin of Sugar Pine (*Pinus lambertiana* Dougl.)[1]

The oleoresin contained 16.4% volatile oil, 75.3% rosin, and 8.3% water and foreign matter.

The oil had the following constants: $d_{15}°$ 1.4727–1.4728; [a]$_D$+10.42°. The oil consisted largely of d-α-pinene, the nitrosochloride of which melted at 103°. The small fraction distilling between 160–168° contained some β-pinene since a small amount of nopinic acid melting at 125° was obtained on oxidation. By repeated fractionation about 10 cc. of oil having the specific gravity 0.8550 was collected between 169–174.5°. Bromine did not give a solid derivative but a copious precipitate was obtained with nitrous acid. The crystals when filtered off with a force pump suddenly decomposed into an amorphous mass that could not be obtained again in a srystalline state. It is probable that a small amount of phellandrene or terpinene is present.

A fraction boiling between 110 and 130° at 25 mm. contained an aliphatic hydrocarbon. After repeated treatment with concentrated sulphuric acid, it had the constants: b. p. 194 to 201° at 742.7 mm.; $d_{15}°$ 0.7549; $n_{D15}°$ 1.4249. It is possible that the container in which the oleoresin had been shipped contained a small amount of a petroleum hydrocarbon.

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[1] Forest Service Bulletin 119, p. 22.
[2] Jour. and Proc. Roy. Soc. N. S. W. 25 (1901) 124.

The hydrocarbon in the highest boiling fractions resembled the sesquiterpene, aromadendrene, described by Smith.[2] It had the following properties: b. p. 144–148° at 30 mm. (250–255° at 739.9mm.); $d_{15}°$ 0.9238; $n_{D15}°$ 1.5006; $[a]_D$ +37.88°. No solid derivatives were obtained.

Attempt to crystallize the original colophony as well as the product obtained by distilling it under reduced pressure were unsuccessful.

The volatile oil had approximately the following composition: 70–75% d-α-pinene; 5% β-pinene; 2 to 3% of a terpene possibly phellandrene; 2 to 3% of an aliphatic hydrocarbon probably an impurity; and 10 to 12% of a sesquiterpene.

The Oleoresin of Western Pine (*Pinus ponderosa* Laws.)[1]

The six samples of oleoresin examined from California contained an average of 17.8% of oil, having the following properties: $d_{15°}$ 0.8625 – 0.8688; $n_{D15°}$ 1.4772 – 1.4793: $a_{D21°}$ – 12.41 to – 26.52°.

α-pinene was identified by conversion into the nitrosochloride, m. p. 103°, and the nitrolpiperidine, m. p. 118°. The oil consisted largely of β-pinene. After repeated fractionation 40% of the oil distilled between 166.6° and 167.6°; it had the constants; $d_{15°}$ 0.8670; $n_{D15°}$ 1.4762; $[a]_D$ –15.33°. On oxidation about 22% of sodium nopinate was obtained. The nopinic acid melted at 126°. It is a curious fact that in spite of the wide distribution of β-pinene in the conifer oils the l-rotatory form has so far been met with. Limonene was found in the oil boiling at about 175°. The tetrabromide melted at 104° and the dihydrochloride at 50°.

The first sample examined contained about 7% of oil boiling above 180° that appeared to be mainly polymerization products; it had a rotation of –0.86°. Later an oil was found containing about 13% distilling above 200°. This residue distilled mainly between 250–280°, and had the constants: $d_{15°}$ 0.9276; $a_{D20°}$+17.68°. When the etheal solution was saturated with HCl gas a crystalline dihydrochloride could not be obtained from the residue left after evaporation of the solvent. The behavior when inoculated with crystalline hydrochlorides was interesting. When a crystal of dipentene dihydrochloride, m. p. 49–50°, was added, a small amount of crystals was ob-

[1] Forest Service Bulletin 119, p. 11; Proc. Soc. Am. For. 11 (1916) 36.

tainted that melted at 102–106° after two crystallizations from alcohol; after a third crystallization the m. p. was 101–103°. When inoculated with cadinene dihydrochloride, m. p. 118°, a crop of crystals were obtained that melted at 118°. It is probable that a small amount of cadinene is present, although the dihydrochloride of this sesquiterpene usually crystallizes with ease.

According to patents held by Schering and Company,[1] β-pinene is stated to give much larger yields of isoprene than α-pinene. Since the oil of western yellow pine could be rendered available in large quantities and since it contained so large a proportion of β-pinene it was desirable to investigate the above statement. An improved form of the Harries isoprene lamp was constructed. With this apparatus, however, it was found that both α-pinene and β-pinene gave practically the same yield of isoprene, namely 10%.[2]

The colophony had the specific rotation $[a]_D$ – 12.88°. The crystals obtained by digesting the powdered colophony with alcohol containing hydrochloride acid followed by recrystallization from acetone melted at 159–160° and had the specific rotation $[a]_D$–78.44°. The crystals obtained from dilute acetone had the characteristic shape of abietic acid. The identification was checked by analysis of the silver salt; found 26.25% Ag; calculated 26.37%Ag. The resin crystals obtained from the rosin distilled under reduced pressure melted at 150–151° and had the specific rotation $[a]_D$–54.28°.

The volatile oil[3] contains about 5% l-α-pinene; 60% l-β-pinene; 20% l-limonene; and about 10% of a sesquisterpene which appears to be cadinene. The rosin contains about 90% abietic acid.

The Oleoresin of Western Yellow Pine, Variety *Scopulorum* (*Pinus ponderosa scopulorum* Englem)[4]

The oils contained from oleoresins collected in Arizona had the following properties: $d_{15}°$ 0.8639–0.8672; $n_{D_{15}}°$ 1.4723–1.4729; $[a]_D$+12.86 to + 13.03°.

[1] German Patent 260,934; K. Stephan, U. S. Patent 1,057,680 (1913) (Assignor to Schoring and Company).

[2] Jour. Ind. Eng. Chem. 7 (1915) 924; with R. Sayre.

[3] Adams [Jour. Ind. Eng. Chem. 7 (1915) 957] working in Wallach's laboratory reached the conclusion that the oils distilled from the wood of western yellow pine, digger pine, singleleaf pine, have about the same composition as the author had found for the oils from the oleoresins of the same species.

[4] Forest Service Bulletin 119, p. 15.

The oil consisted largely of α-pinene, the nitrosochloride melting at 103°. β-pinene was present in very small amounts in contrast with the oil of *P. ponderosa.* The nopinic acid obtained melted at 125°. Limonene was identified by means of the tetrabromide, m. p. 104.5, and dihydrochloride, m. p. 50°.

It will be noted that the turpentine oils from *Pinus ponderosa* and its variety *P. p. scopulorum* are distinctly different; the oil from the former is l-rotatory and consists mainly of β-pinene, while the oil from the latter is d-rotatory and consists largely of α-pinene. The decided difference between these oils is good evidence that the distinction between the species and subspecies should be maintained.[1]

The resin had the specific rotation $[a]_D$ –30.95°. After preliminary digestion with alcohol containing hydrochloric acid, crystals of abietic acid were obtained melting at 159°. Three silver salts prepared from the abietic acid had an average silver content of 26.25%; silver abietate, Ag $(C_{20}H_{29}O_2)$, requires 26.37% silver.

The turpentine oil of *P. p. scopulorum* has the following composition: 60–70% d-α-pinene; 5% β-pinene; and 20–25% limonene. The rosin consists of abietic acid.

THE OLEORESIN OF LODGEPOLE (*Pinus contorta* Loud.)[2]

The oleoresin gave on steam distillation 14.7% of oil having the following constants: $d_{15}°$ 0.8518–0.8549; $n_{D15}°$ 1.4860–1.4862; $[a]_D$–20.12°.

About 82% of the oil distilled between 170–180° at atmospheric pressure. The residue remaining in the flask and amounting to 15% solidified on cooling to a hard amber-colored mass. The high degree of polymerization pointed to phellandrene. This was confirmed by preparation of the nitrite melting at 103°. A carefully purified sample of the phellandrene had the following properties: b. p. 60° at 11 mm.; $d_{\frac{21°}{15°}}$ 0.8460; $n_{D15}°$ 1.4861; $[a]_D$ –12.36°. No additional terpenes could be detected. This occurrence of phellandrene is very interesting since it is the first recorded occurrence of phellandrene in the turpentine oils of any of the *Pinus* family.

[1] Schorger, Proc. Soc. Am. Foresters 11 (1916) 33.
[2] Forest Service Bulletin 119, p. 25.

The colophony contained some of the polymerized phellandrene. About 80% of abietic acid crystals were obtained from the colophony by crystallization from alcohol containing hydrochloric acid. The triangular plates melted at 159–160°, The silver salt contained 26.15% Ag in agreement with the formula Ag ($C_{20}H_{29}O_2$).

The turpentine oil of lodgepole pine accordingly consists mainly of l-β-phellandrene and the colophony of abietic acid.

THE OLEORESIN OF PINON PINE (*Pinus edulis* Engelm)[1]

The oleoresins of piñon pine and singleleaf pine are very similar in odor, appearance and composition. The oleoresin of piñon pine contained 76.5% colophony and 20% of a volatile oil having the following properties: $d_{15}°$ 0.8680; $n_{D_{15}°}$ 1.4707; $[a]_D$+19.26°.

The oil consisted largely of d-α-pinene the nitrosochloride melting at 103°. β-pinene was detected with difficulty, the few crystals of nopinic acid obtained melting at 123°. The high boiling fractions contained the sesquiterpene cadinene: b. p. 135–140° at 20 mm.; $d_{15}°$ 0.9173; $n_{D_{15}°}$ 1.4926; $[a]_D$ + 15.41°. The dihydrochloride melted at 118° and was l-rotatory. So far as known this was the first case in which cadinene had been found in a turpentine oil.

All attempts to obtain crystalline a resin acid from the colophony were unsuccessful. The crystals obtained from the crude oleoresin melted at 129–130° after four crystallizations from acetone. When these were dissolved in methyl alcohol and hydrochloric acid was added, triangular plates melting at 137° were obtained. The latter crystals had the specific rotation $[a]_D$ –52.77° and the silver salt contained 26.46% Ag. The acid accordingly has the empirical formula of abietic acid.

The volatile oil of this species contains 70–75% d-α-pinene, about 5% β-pinene, and 15–20% d-cadinene. The resin acid in the oleoresin is isomeric with abietic acid.

[1] Forest Service Bulletin, 119, p. 28.

PART II

Analysis of Woods

The methods for the analysis of woods are largely empirical. The only determination that may be considered as accurately showing the amount of a definite group present is the methoxy determination according to Zeisel. The acetic acid obtained on digestion with dilute sulphuric acid may be considered as derived from acetyl groups (CH_3CO-) and acetic acid residues ($-CH_2CO-$). The decomposition of the wood is evidently considerable since birch sawdust loses about 30% by the above digestion.

The estimation of pentosans and methylpentosans by determining the amounts of furfural and methyl furfural formed on distillation with 12% hydrochloric acid gives closely agreeing results when the procedure worked out by Tollens and his pupils is followed. In the case of woods the difficulty lies in determining the proper source of the furfural. According to Cross and Bevan, in addition to the pentosans, wood also contains "furfuroids," while to the cellulose is assigned the structure of an oxycellulose which in turn gives furfural. All the furfural obtained is usually calculated as pentosan though it is evident that this procedure is not strictly correct. It is a striking fact that the pentosan content of the isolated cellulose as calculated from the yield of furfural is practically the same as that of the original wood. Whether the furfural originates from the cellulose proper or from pentosan residues in the cellulose has not been definitely decided.

The cellulose was determined by the chlorine method. As originally described by Cross and Bevan[1] it calls for a preliminary boiling of the ligneous material with 100cc. of 1% NaOH. Also following chlorination and the addition of sodium sulphite solution, the latter is brought to boiling, 0.2% of NaOH are added and the solution is boiled for five minutes. According to the investigations of Renker[2] the preliminary treatment

[1] "Cellulose", p. 95.
[2] "Bestimmungsmethoden der Cellulose", (1910) p. 44.

with NaOH as well as the subsequent addition of NaOH to the sulphite solution causes an attack of the cellulose resulting in a lower yield. Both these observations have been confirmed. Since solutions of sodium sulphite have a decidedly alkaline reaction as a result of hydrolysis, there was a possibility that even the sodium sulphite attacked the cellulose. This was found to be the case since when the solution was kept saturated with sulphur dioxide during heating the yield of cellulose was increased 1–2% in some cases.

The chlorination was limited to 30 minute periods since in many cases a first chlorination lasting one hour as usually recommended is too long. The length of time and manner of heating the sulphite solutions was also fixed to 30 minutes heating in a water bath. Renker recommends heating the sulphite solution on the steam bath for "1 to 2 hours." On account of the alkaline reaction of the sodium sulphite, the period of heating should be as short as possible.

The conifers are much more resistant to the action of chlorine than the broad-leaved trees, showing that there is a difference in the lignin. The lignin of the conifers contains fewer methoxy groups and gives less acetic acid on hydrolysis. There is also a wide difference in the pentosan content of the two classes of woods. Yellow birch, for example, contains four times the amount of pentosan found in Douglas fir. As previously mentioned the cellulose from the various species give about the same amount of furfural as the original woods.

When the percentage of cellulose is based on the wood free from materials soluble in hot water and ether the following is obtained.

Western larch	66.40	
Longleaf pine	67.20	
Douglas fir	66.30	Mean 65.92
White spruce	63.79	
Sugar maple	63.43	
Yellow birch	64.38	Mean 64.26
Basswood	64.97	

The conifers accordingly contain a slightly greater amount of cellulose.

The methods of analysis being largely empirical, they are given in considerable detail. In order to obtain closely agreeing results, it is necessary, especially in the case of woods, to follow the methods exactly as described.

Method of Analysis

Sampling—A cross-sectional disc about two inches thick is taken from the tree about 20 feet from the ground and from this disc two diagonally opposite sectors are split out, the size of the sectors depending upon the diameter of the trees. The material employed for analysis consists of two forms—*thin* shavings and sawdust. The shavings are obtained by planing off a radical face from each of the sectors previously described. The damp shavings are then passed through a grinder having a shredding effect, the resulting fragments being 3–5mm. long and 1–2mm. wide. The material after air drying is then screened and all that passes through a 40-mesh sieve is rejected. The residual material is then thoroughly mixed to insure a uniform sample. The remaining portions of the sectors are then cut into sawdust and the sawdust thoroughly mixed. A portion of the sawdust from coniferous woods will be kept in a sealed container (Mason jars are very convenient) for the determination of moisture by the xylol method and the determination of volatile oil, while the remainder after air drying is so ground in a mill as to pass through a 40-mesh sieve. All the moisture content being determined in a separate sample. the material used for analysis should be in the air-dry form, the moisture content being determined in a separate sample. All results are calculated on the oven-dry basis.

The 40-mest sawdust should be kept in a rubber-stoppered flask so that the moisture having once been determined the samples taken out for analysis can be easily reduced to the dry weight by calculation.

Moisture—Three grams of 40-mesh sawdust are weighed out in a glass-stoppered weighing bottle and dried to constant weight in an air oven at 105° C. Dry wood is very hygroscopic and should always be weighed in a closed vessel. In the case of coniferous woods the moisture figure must be corrected for volatile oil.

Volatile Oil—Twenty-five grams of sawdust from the sealed container are quickly weighed, placed in a 250 c.c. Erlenmeyer

flask, and 75 c.c. of water saturated xylol added. On distillation the xylol and water distill over together, the distillate being collected in a graduated funnel. The amount of water present can then be read off directly. (For details of this method see Forest Service Circular 134).

Ten grams of sawdust are weighed into a tared wide-mouthed, stoppered Erlenmeyer flask. The flask is then provided with a rubber stopper containing a tube extending nearly to the bottom of the flask for the introduction of steam and an outlet tube for connection with a condenser. The flask is heated in an oil bath maintained at 110° C. and steam is passed in gently until oil ceases to pass over. This point can be readily ascertained by catching a few cc of the distillate in a test tube in which case even traces of oil are distinguishable on the surface. When all the oil has been driven over the stopper is withdrawn and any adhering sawdust is washed down into the flask. Continue heating the flask in the oil bath until practically all the water is expelled. This operation is greatly facilitated by inserting a tube into the mouth of the flask and applying suction with a water pump. The exterior of the flask is then carefully cleaned and the drying completed in the air oven. The stoppered flask is then weighed after cooling.

In this way the weight of wood substance is obtained, the water and volatile oil having been removed. Since the moisture content of the original sample has been determined by the xylol method, subtracting the combined weight of residual wood substance and moisture from the original weight of the sample gives the amount of volatile oil.

The determination of volatile oil by heating a sample in the oven and subtracting from the total loss in weight the water found by the xylol method usually does not give the true oil content. The "pine oil" of longleaf pine can be quite readily expelled with steam but only partially by heating for a brief period in the oven.

The volatile oil determination may be neglected in the case of only slightly resinous conifers.

Waxes, Fats, Resins—3–4 grams of 40-mesh sawdust are extracted with ether in a Soxhlet extractor, the amount of material extracted being determined by weighing the residue remaining after evaporation of the solvent. Calculations should be based on dry wood.

Ash—Five grams of sawdust are incinerated in a shallow platinum dish in the electric muffle at a dull red heat. The contents of the dish should be stirred occasionally, if necessary, to insure complete combusion of the carbon. If the combustion is incomplete the carbon will appear as a black suspended material on treatment with dilute hydrochloric acid.

Alkali Soluble—Two grams of 40-mesh sawdust are placed in a 250 cc beaker, 100 cc of 1 per cent NaOH added, covered with a watch glass, and placed in a pan of boiling distilled water for exactly one hour, the height of the water in the pan being maintained level with the solution in the beaker by addition of boiling distilled water. The contents of the beaker are occasionally stirred. The material is then collected in a tared alundun crucible, washed consecutively with hot distilled water, one per cent acetic acid, and hot water; it is then dried. The difference is the portion soluble in alkali and consists of pentosans, lignin, resin acids, etc.

Hot Water Soluble—Two grams of 40-mesh sawdust are digested with 100 cc of H_2O in a 300 cc Erlenmeyer flask provided with a reflux condenser. After the water has been boiled gently for three hours, the contents are transferred to a tared alundum, crucible, washed with hot water, dried and weighed.

Cold Water Soluble—Two grams of 40-mesh sawdust are placed in a 400 cc beaker, 300 cc of water added, and allowed to digest with frequent stirring for 48 hours. The sawdust is then transferred to a tared alundrum crucible, washed with cold distilled water, dried and weighed in a weighing bottle.

Pentosan and Methyl Pentosan—Two grams of 40-mesh sawdust from coniferous woods (1 g. from hardwoods) are placed in a 250 cc flask provided with a separatory funnel and attached to a condenser. Add 100 cc of 12 per cent hydrochloric acid (sp. gr. 1.06)[1] and distill at the rate of 30 cc of distillate in ten minutes. The distillate should pass through a small filter before entering the receiver. As soon as 30 cc of distillate are collected, 30 cc of HCl are added to the distillation flask and the distillation is continued in this manner until 360 cc of dis-

[1] The solution of hydrochloric acid is conveniently prepared as follows: 300 cc of the ordinary concentrated HCl are diluted to 1000 cc and cooled to room temperature with tap water. A hydrometer reading 1.06 is suspended in the acid and by adding a small amount of either con. HCl or water, as necessary, the desired specific gravity 1.06 is easily obtained.

tillate are collected. To the total distillate, add 40cc of filtered phloroglucine solution that has been prepared at least a week previously by heating 11 grams of phloroglucine in a beaker with 300 cc of 12 per cent HCl, and after solution has taken place make up to 1500 cc with 12 per cent HCl. After addition of the phloroglucine, the solution soon turns greenish black. Let stand 16 hours, when the furfural phloroglucide will have settled to the bottom of the beaker. If a drop of the supernatant liquid gives a pink color with aniline acetate paper[2] the precipitation of the furfural is incomplete. A further amount of phloroglucine solution is then added and the beaker allowed to stand over night as formerly.

The furfural phloroglucide is filtered through a tared asbestos crucible and washed with exactly 150 cc of water. The crucible is then dried for four hours in a water oven and weighed in a weighing bottle.

The crucible is then placed in a small beaker and 20 cc of 95% alcohol are added to the crucible. The beaker is then placed in a water bath, maintained at 60°, for ten minutes. The alcohol is then removed with a suction pump and the process repeated (usually four or five times) until the alcohol that runs through is practically colorless.[1] The crucible is then dried for two hours in the water oven and again weighed. The weight of the residual phloroglucide subtracted from the weight of mixed phloroglucides gives the weight of methyl furfural-phloroglucide. From the weights of furfural-phloroglucide and methyl-furfural-phloroglucide obtained the amounts of pentosan and methyl-pentosan present in the wood are calculated from the tables of Kroeber and Tollens on pages 137 and 154 of Vol. II of Aberhalden's "Handbuch der Biochemischen Arbeitsmethoden."

Cellulose—Two grams of shavings in a tared alundum crucible are extracted in a Soxhlet extractor for 3 or 4 hours with a mixture of equal parts of alcohol and benzol. After removal of

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[2] The aniline acetate paper is conveniently prepared by dipping strips of filter paper into aniline acetate. The latter is prepared by adding acetic acid drop by drop to a mixture of equal parts of aniline and water until a clear solution is obtained.

[1] Extraction of the methyl-furfural-phloroglucide in a modified Soxhlet extractor as described by Ishida and Tollens (J. f. Landw. (1911) 59) in the author's experience does not give accurate results owing to the difficulty in determining when the extraction is completed.

the solvent the shavings are thoroughly washed with hot water using the suction pump. The moist shavings are then transferred to a 250 cc beaker with a pointed glass rod, evenly distributed over the bottom, and subjected to a slow stream of washed chlorine gas for half an hour. The end of the tube delivering the chlorine gas should be about one-half inch above the shavings. After the chlorine treatment the shavings are treated with a solution of SO_2 until the chlorine odor disappears, transferred to the alundum crucible, and washed with water. The shavings are again returned to the beaker with the glass rod, and 100 cc of a 2 per cent sodium sulphite solution are added. The covered beaker is then placed in a boiling water bath for 30 minutes, the water in the bath being maintained on a level with the solution in the beaker by the addition of hot distilled water. The fibers are then transferred to the crucible and washed with hot water. The above treatment is seldom sufficient to remove all the lignin, so that the treatment with chlorine and subsequent procedure as outlined above is repeated until the fibers are practically a uniform white. The second and following treatments with chlorine should not be longer than 15 to 30 minutes. After all the lignin has been removed the fibers are given a final bleaching with 10 cc of a 0.1 per cent solution of potassium permanganate, and rendered colorless with SO_2 solution. The fibers are then thoroughly washed with hot water, acetic acid, and alcohol, and finally with ether and dried at 105° in the air oven, the crucible being weighed in a weighing bottle.

Acid Hydrolysis—Approximately 2g. of 40-mesh sawdust are placed in a 250 c.c. Erlenmeyer flask and 100 c.c. of 2.5 per cent H_2SO_4 added. The flask is connected with a reflux condenser and the contents are boiled quietly for 3 hours and then allowed to cool. Wash down the interior of the condenser with a little distilled water and transfer the contents of the flask to a 250° c.c. graduated flask. Make up to the mark with distilled water free from carbon dioxide. Let the solution stand several hours with frequent shaking, and then filter.

A wide-mouthed, round-bottomed, 750 c.c. flask is provided with a rubber stopper containing (1) a dropping funnel; (2) a glass tube drawn out to a capillary, closed with a rubber tube and pinch cock, and extending to the bottom of the flask; and (3) a Soxhlet connecting bulb-tube. Use an ordinary condenser, to

the end of which is attached for a receiver a 500 c.c. distilling flask cooled with a stream of water and connected with a manometer and suction pump.

Place a few pieces of pumice in the boiling flask and then add 200 c.c. of the filtrate obtained above (in the case of hardwoods use 100 c.c.). The flask is heated in an oil bath maintained at 85° C. while the pressure is reduced to 40–50 mm. When the contents of the flask are reduced to about 20 c.c., add distilled water through the dropping funnel, drop by drop, at the same rate that distillation takes place. When 100 c.c. of wash water have been distilled over, titrate the distillate with N/10 NaOH using phenolpthalein as the indicator. If (a) 200 c.c. or (b) 100 c.c. of solution were taken for distillation, multiply the number of c.c. of NoOH used by (a) 5/4 or (b) 5/2 respectively, and calculate as acetic acid.

This method gives accurate results. Duplicate determinations should agree within 0.10 of 1 per cent. It is necessary to use low temperatures and pressures to prevent decomposition of the carbohydrates, etc., by the sulphuric acid before all the acetic acid is removed.

All the distilled water used in this determination should have been recently boiled to expel carbon dioxide.

Determination of Methoxy Group (CH_3O)—The principle of the methoxy determination depends upon heating the substance to be examined with hydriodic acid, whereby methyl iodide is formed. The methyl iodide is swept from the reaction flask into vessels containing a known volume of an alcoholc solution of N/10 silver nitrate, the methyl iodide being decomposed with the formation of silver iodine. The undecomposed silver nitrate is estimated volumetrically or the silver iodide formed is precipitated by diluting the solution, filtering, and weighing. Since 1 part of silver iodide is equivalent to 0.132 part of CH_3O or 1 part of Ag NO_3 is equivalent to 0.1823 part of CH_3O the percentage of methoxy groups in the sample can be easily calculated. The details of the Zeisel method may be found in most works on organic chemistry or organic analysis.

TABLE I.—*Analysis of Woods.*

Species	S'mple No.	Ash	Soluble in cold water	Soluble in hot water	Soluble in ether	Soluble in 1% NaOH	Acetic acid by hydrolysis	Methoxy groups CH_3O	Pentosan	Methyl Pentosan	Cellulose	Ash in Cellulose	Ash free Cellulose	Pentosan in Cellulose	Methyl pentosan in Cellulose	Volatile Oil
		%	%	%	%	%	%	%	%	%	%	%	%	%		%
Longleaf Pine (*Pinus palustris*)	11	0.40	7.75	8.20	6.70	24.62	0.70	5.00	7.33	3.48	55.33	0.31	55.02	8.38	1.26	1.60
	12	0.34	5.60	6.03	6.70	21.07	0.93	4.90	7.52	3.29	57.53	0.30	57.23	7.19	1.39	1.12
	13	0.35	5.40	6.78	2.65	18.89	0.62	5.26	7.57	3.87	61.41	0.32	61.09	7.39	1.03	0.87
	20	0.38	6.05	7.57	9.23	24.87	0.79	5.03	7.43	3.67	59.67	0.36	59.31	7.90	0.96	1.60
Douglas Fir (*Pseudotsuga taxifolia*)	Mean	0.37	6.20	7.15	6.32	22.36	0.76	5.05	7.46	3.60	58.48	0.32	58.16	7.71	1.16	1.30
	1	0.40	3.79	6.62	0.94	15.82	0.93	4.81	6.03	4.24	61.97	0.21	61.76	5.56	1.26	.....
	2	0.37	3.16	6.07	1.00	16.76	1.01	5.17	6.30	4.64	57.00	0.10	56.90	.....	.....	.....
	3	0.35	2.94	6.36	1.11	15.12	1.13	4.88	6.00	4.38	63.08	0.07	63.01	.....	.....	.....
	5	0.38	4.25	6.96	1.02	16.72	1.07	4.92	5.73	4.38	63.82	0.26	63.56	5.12	1.15	.....
Western Larch (*Larix occidentalis*)	Mean	0.38	3.54	6.50	1.02	16.11	1.04	4.95	6.02	4.41	61.47	0.16	61.31	5.34	1.20	.....
	1	0.21	10.45	12.57	0.72	22.07	0.61	5.08	11.15	2.47	58.25	0.24	58.01	9.12	1.40	.....
	2	0.32	11.00	12.40	0.74	21.93	0.91	4.91	11.04	2.83	58.71	0.18	58.53	8.41	1.22	.....
	3	0.22	8.16	10.08	0.93	19.44	0.76	5.08	10.22	3.14	60.91	0.29	60.62	8.67	1.24	.....
	4	0.16	12.83	15.30	0.83	25.11	0.55	5.05	10.78	2.80	58.31	0.45	52.86	9.55	0.90	.....
White Spruce (*Picea canadensis*)	Mean	0.23	10.61	12.59	0.81	22.14	0.71	5.03	10.80	2.81	57.80	0.29	57.51	8.94	1.19	.....
	1	0.33	1.28	1.88	1.95	11.33	1.58	5.31	10.78	3.08	62.61	0.27	62.34	10.26	0.83	.....
	2	0.29	0.92	2.28	0.90	11.58	1.57	5.26	10.31	3.52	63.29	0.39	62.90	9.29	0.68	.....
	3	0.30	1.45	2.52	0.97	12.75	1.49	5.29	10.04	3.95	60.43	0.25	60.18	.....	.....	.....
	4	0.32	0.82	1.88	1.63	10.63	1.73	5.32	10.42	3.64	61.09	0.30	60.79	9.33	0.66	.....
Basswood (*Tilia americana*	Mean	0.31	1.12	2.14	1.36	11.57	1.59	5.30	10.39	3.55	61.85	0.30	61.55	9.63	0.72	.....
	1	0.80	2.04	3.84	1.50	23.43	5.78	6.23	19.82	3.72	62.92	0.11	62.81	24.48	1.19	.....
	2	0.74	1.63	2.94	1.14	21.61	6.14	6.05	19.54	3.85	62.41	0.14	62.27	23.54	1.46	.....
	3	0.96	3.14	5.66	3.59	26.93	5.46	6.11	20.37	3.68	54.66	0.24	54.42	26.61	1.62	.. ..
	4	0.94	1.23	3.22	0.89	21.46	5.41	5.91	19.14	4.16	63.13	0.22	62.91	21.89	1.45	.....
	5	0.85	2.55	4.67	2.68	25.38	6.18	5.72	20.79	3.23	63.08	0.22	62.86	24.86	2.00	.....
Yellow Birch (*Betula lutea*)	Mean	0.86	2.12	4.07	1.96	23.76	5.79	6.00	19.93	3.73	61.24	0.19	61.05	24.28	1.54	.....
	1	0.58	2.88	4.21	0.55	20.02	3.99	6.12	24.26	3.18	60.49	0.11	60.38	28.40	1.11	.....
	2	0.57	2.58	3.87	0.67	20.20	4.39	6.03	25.40	3.12	61.08	0.12	60.96	29.96	1.32	....
	3	0.54	3.15	4.66	0.54	19.51	3.81	6.19	23.00	2.25	61.82	0.14	61.68	26.55	1.04	.....
	4	0.37	2.06	3.15	0.63	19.65	5.02	5.92	25.86	2.21	61.85	0.16	61.69	.....	.....	.....
Sugar Maple (*Acer saccharum*)	Mean	0.52	2.67	3.97	0.60	19.85	4.30	6.07	24.63	2.69	61.31	0.13	61.18	28.30	1.16	.....
	1	0.46	2.60	4.27	0.29	16.98	4.26	7.22	21.10	2.50	60.78	0.13	60.65	21.08	1.04	.....
	2	0.51	2.73	4.22	0.22	17.20	4.25	7.23	21.90	2.14	61.67	0.28	61.39	25.82	1.05	.....
	3	0.40	2.94	4.78	0.30	18.04	4.60	7.25	22.21	2.05	60.20	0.33	59.87	25.83	1.00	.....
	4	0.38	2.33	4.15	0.20	18.35	4.74	7.28	21.62	2.85	60.48	0.39	60.09	25.20	0.77	.....
	Mean	0.44	2.65	4.36	0.25	17.64	4.46	7.25	21.71	2.39	60.78	0.28	60.50	24.48	0.96	.....

PIGMENTS OF FLOWERING PLANTS.

By NELLIE A. WAKEMAN.

INTRODUCTORY CHAPTER.

THEORIES OF COLOR IN ORGANIC COMPOUNDS.

While the study of pigmentation in plants early attracted the attention of chemists as well as botanists, it was not until the introduction of synthetic dye stuffs in the latter part of the 19th century that any considerable amount of attention was directed to the determination of the cause of color in the pigment itself. Until this time indeed, the constitution of most of the natural dye stuffs was unknown, consequently any consideration of the relationship between color and molecular structure was impossible. With the introduction of dye stuffs of known constitution, however, the question not only presented itself but well nigh forced itself upon the attention of chemists. The earlier endeavors were, for the most part, directed toward determining a direct relationship between color and molecular constitution. This led to a study of so-called chromophorous groups and chromogens, a study which, while it has been fruitful of results in the manufacture of dye stuffs, has been of much less value in the study of plant pigments. Recently, however, these studies have assumed a more basal character and the subject has been approached through the general question of absorption spectra, the invisible as well as the visible portion of the spectrum being taken into consideration.

It is obvious that color does not inhere in the colored substance itself, but is the response of sensation to the stimulus of light which proceeds from the colored substance either by transmission or reflection. The different dyes and pigments possess their many varying hues because, by a process known as

selective absorption, each pigment absorbs certain definite colors from white light and transmits or reflects only those which it does not absorb. A transparent object takes on the color of the light which it transmits, while an opaque object takes on that of the light which it reflects.* If an object absorbs all of the radiations of white light and transmits none, it is black; if it absorbs all but the red radiations, for example, it is red; but if it absorbs none and transmits all of the radiations of white light, it appears to be colorless. Again, if light of only one color be absorbed, violet for example, its complementary color, yellow in this instance, will alone be visible. If on the other hand two complementary colors alone are absorbed, the object will appear relatively colorless. Many substances are quite transparent and colorless within the limits of the visible spectrum which show selective absorption in the infra-red or ultra-violet regions. Such substances only appear colorless. In a physical sense they are similar to colored substances, for, physically, there is little difference whether absorption is of radiant energy of short wave length in the ultra violet, of medium wave length in the visible portion of the spectrum, or of long wave length in the infra-red.

That a substance shows selective absorption is probably due to the fact that the oscillation frequencies of its molecules corspond to certain definite wave lengths of radiant energy, the energy corresponding to such wave lengths being absorbed by the molecules. In accordance with this theory of color in material objects, much attention has been paid in recent years to the question of what molecular structures are likely to correspond to more or less definite periods of molecular vibrations, thus producing selective absorption. Narrowed down to the study of pigments, the question has taken the form of what particular configurations, as well as the introduction of what group or groups of elements, will throw this selective absorption into the region of the visible spectrum where color will be produced. This brings the whole subject of chromophorous groups into a new light and explains how the introduction of a given group, supposed to aid in the production

* Comparatively few substances, e. g. the metals, some solid organic dyes, feathers, etc., which have a metallic lustre, owe their color to reflection. Most substances generally considered opaque transmit light for some distance below the surface. Color in such substances is a transmission rather than reflection phenomenon.

of color, might result in one case in the change of a colorless substance to a colored one, while in another case it might have the opposite result of taking away the color from a colored substance. It shows, in fact, that color in a substance is not a function of a particular group of elements but of the structure of the entire molecule.

The first attempt to show the relationship between constitution and color in organic compounds appears to have been that of Graebe and Liebermann[1] in 1868. These investigators laid down the rule as of general application that, if the colored metallic salts of colorless organic acids are excepted, all colored organic compounds are rendered colorless by reducing agents, and that in this reduction the compound adds on hydrogen without the elimination of any other elements from the molecule. As illustrations they quote quinone, $C_6H_4\left\{\begin{matrix}O\\O\end{matrix}\right\rangle$ reduced to hydroquinone, $C_6H_6\left\{\begin{matrix}OH\\OH\end{matrix}\right.$; azobenzene, $C_6H_5N{=}N{-}C_6H_5$; reduced to hydrazobenzene, $C_6H_5\underset{\underset{H}{|}}{\overset{\overset{1}{|}}{N}}{-}\underset{\underset{H}{|}}{\overset{\overset{1}{|}}{N}}{-}C_6H_5$, etc.

From these reactions they infer that colored compounds either contain elements with incompletely saturated affinities, or that some of the atoms are more intimately bound than is necessary for their retention in the molecule; furthermore, that the physical property of color depends upon the manner in which the oxygen or nitrogen atom is combined, in the colored compounds these elements being in more intimate combination than in the colorless compounds. In the case of colored nitro and nitroso compounds, which are rendered colorless by reduction to amido compounds, it is the intimate association of the oxygen and nitrogen to form a group which renders the substance colored.

Graebe and Liebermann's theory was formulated before the present diketone formula for quinones was accepted, their conception of a quinone being that of a benzene nucleus with two oxygen atoms linked together, hence the idea of "more intimate, or internal, combination."

In 1876 Witt[2] advanced an entirely different explanation.

[1] Ber., 1, p. 106.
[2] Ber., 9, p. 522.

Color in a substance, according to Witt, is due to the presence of a chromophore group in the molecule. Such a substance, though generally colored, is not a dyestuff and is called by Witt a chromogen. It becomes a dye by the introduction of a salt forming auxochrome group. According to Witt the principal chromophore groups are the nitro $-N\overset{O}{\underset{O}{=}}$, the nitroso, $-N=O$, the carbonyl $-C=O$, and the allied group the thio carbonyl, $=C=S$; and also the azo menthin group $-C=N-$, the azo group $-N=N-$. The principal auxochrome groups are the hydroxyl, the amino, and the mono- and di- alkyl amino groups. Witt's theory, while it proved of great value in the synthesis of artificial dyes, as stated before, has been of comparatively little use in the study of plant pigments, since only the corbonyl group among the so called chromophore groups and the hydroxy among the auxochrome groups are of at all frequent occurrence in the plant pigments of known constitution. Moreover the mere presence of these two groups, or of multiples of one or both, is not sufficient to explain the phenomenon of color in the plant pigment molecule.

From time to time other chromophore groups have been added to those named by Witt. Principal among these are the ethylene[3] group $=C=C=$, the azoxy[4] group $-N-N-$ (O) or $-N=N-$ (O), or $-N\equiv N-$ (O), and combinations of some of the above named groups.

In 1888 Armstrong[5] introduced his quinone theory of color. Since dyestuffs in general can, by the addition of hydrogen, be reduced to the corresponding leuco bases, Armstrong considered all colored compounds to be quinones and the corresponding colorless compounds to be hydroquinones. Using the Fittig diketone formula for quinones, Armstrong attempted to show that the structure of colored compounds in general may be represented by a quinoidal formula. Under the term quinoidal formula Armstrong included all structures containing

[3] Ber., 33, p. 666.
[4] Ber., 31, p. 1361; 33, p. 123; 29, p. 2413.
[5] Proc. Chem. Soc., 4, p. 27; 8, pp. 101, 143, 189, 194.

either the para quinone or the ortho quinone grouping, the double bonds being satisfied by oxygen or any other divalent element or group or by combinations of any of them.

Armstrong's theory has received a great deal of attention, much evidence both for and against it having been produced. Though our present knowledge of the structure of colored compounds would indicate that by no means all colored substances are of quinoidal character, yet a surprisingly large number, if not all, of the substances of a quinoidal configuration are colored. The quinoidal configuration is in fact one of the best known and most reliable chromogens.

In the study of plant pigments the quinone theory has proved of much more value than Witt's theory of chromophorous groups. The constitution and the properties of a very large number of vegetable dyestuffs, and of other colored substances derived from plants, have been accounted for by assuming a quinoidal structure. Moreover the closely related quinhydrone[6] hypothesis of pigmentation in some plants promises to explain biochemically the existence of many colors and shades of color, as well as many changes of color, which have hitherto been unaccounted for.

In considering quinone and quinhydrone hypotheses of pigmentation mention should be made of Richter's[7] oxonium theory of quinones. According to Richter the characteristics of oxonium salts, namely simple addition of the components in their formation, ready decomposition in solution, and upon sublimation, and marked increase in intensity of color, are also those of the quinhydrones. For these and other reasons hydroquinones, phenoquinones, etc., are regarded by Richter as oxonium compounds formed by the addition of pnenols, etc., to the tetravalent oxygen of quinones.

Assuming that the doubly bound oxygen of quinones acts as a tetravalent oxygen does not impair the validity of the quinhydrone hypothesis as suggested by Kremers and Brandel. It brings the quinone pigments into line with the anthocyanin pigments, as interpreted by Willstaetter, and explains how many quinones, as well as many flavone and xanthone derivatives, also haematin and brazilin, dissolve in acids with an in-

[6] Ph. Rev., 19, p. 200.
[7] Ber., 43, p. 3603.

tense color, but are precipitated unchanged from this solution by the addition of water. It is easy to understand how the tetravalent oxygen in quinones, being basic, might add on the elements of phenols as well as acids or how it might add on the elements of a molecule of water. While it would not be wise, however, without very careful investigation, to say it were impossible, it is not easy, without altering our present conception of the tetravalent oxygen, to see how the same oxygen could be able to form addition products with organic nitrogen bases, such as phenylendiamine, and even with potassium hydroxide, which Richter represents it as doing.

In 1879 Nietzki[8] formulated a rule, supposed to be of general application, that the pigments of most simple construction are yellow and by increase of molecular weight they gradually change from yellow to red, then to violet, then to blue.

Schuetze[9] in 1892 found that Nietzki's rule holds only in certain cases; but that changes in color in general are the results of changes in selective absorption in the regions of the visible spectrum. The results of Schuetze's investigations are summed up as follows:

1. A change of absorption from violet toward red usually causes the following changes in color; greenish yellow, yellow, orange, red, reddish violet, violet, bluish violet, blue, bluish green, etc. Passing through the colors in this direction Schuetze calls deepening or lowering the tint; in the opposite direction, raising the tint.

2. Definite atoms and atomic groups by their entrance into the molecule cause, for compounds of the same chromophore in the same solvent, a characteristic deepening or raising of the tint. Those which deepen the tint are called "bathochrome" groups or elements, those which raise the tint are called "hypsochrome" groups or elements.

3. Hydrocarbon radicles are always bathochromic. Consequently in homologous series the shade always deepens as the molecular weight increases.

4. The same deepening of color is caused in the groups of the periodic series as the atomic weights increase.

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[8] Verhandl. des Vereins fur Befoerderung der Gewerbfleisses., 58, p. 231 (Quoted by Schuetze, Zeit. fur Phys, Chem., 9, p. 109).

[9] Zeit. fur Phys. Chem., 9, p. 109.

5. The addition of hydrogen always results in raising the tint.

6. The rise or fall of the tint (the passage of absorption from violet to red) by substitution of hypsochrome or bathochrome groups, or by the addition or loss of hydrogen, is the greater the nearer the atoms affected by the change are to the chromophore group. From this it would appear that in the bi-derivitives of benzene the substituents in para position are nearer to each other than those in ortho position.

7. These rules hold only for monochromophoric compounds and for symmetrical dichromophoric compounds. The color of an unsymmetrical dichromophoric compound, Y, A, X, A, Z, is approximately the same as that of a mixture of the two symmetrical compounds, Y, A, X, A, Y, and Z, A, X, A, Z.

In general the term bathochrome group is interpreted to mean a group which swings the absorption toward the red, and a hypsochrome group, one which swings the absorption toward the violet. Among the former are the hydrocarbon radicles, the halogens, and the salt forming groups with the exception of the amino group. Among the latter are the acetyl and the benzoyl groups, the alkyl-oxy groups, hydrogen, and the amino group. The sulpho group is sometimes one and sometimes the other.

The effect of the introduction of bathochrome and hypsochrome groups upon the color of the original substance depends upon the original molecule, the position of the group, and the number of groups introduced. It is easy to understand how the introduction of one bathochrome group might deepen the color by throwing the absorption into the red, while the introduction of two or three such groups would remove it by throwing the absorption wholly outside the visible spectrum. Again the same two or three groups might be required to render another molecule colored. Here again we find additional evidence that the color of a substance is not conditioned by the presence of a definite group, or groups, but by the entire structure of the molecule.

In 1904 Baly and Desch[10] in the course of a study of the ultraviolet spectrum of certain enol-keto tautomerides brought forth evidence to support the view that the absorption bands exhibited by these substances are due to the equilibrium existing

[10] Proc. Chem. Soc., 85, p. 1029.

between the two possible tautomeric forms. Neither of the two substances in a pure state exhibits absorption but when the two are present in mutual equilibrium, that is, when a number of molecules are changing from one form to another, a very decided absorption band is formed. In 1905[11] they stated further:

1. No organic substance shows an absorption band unless a possibility for tautomerism exists in the molecule.

2. This tautomerism may not be due to a labile atom, but may be of the same order as that occurring in those aromatic compounds containing the true benzenoid structure.

3. In all cases of the simpler tautomeric molecule the vibration frequencies of the absorption bands are very nearly the same.

4. An increase in the mass of the molecule causes a decrease in the oscillation frequencies of the absorption band, i. e. a shifting toward the red.

Baly and Desch account for these facts and explain the formation of the absorption band by the same theory as that advanced by physicists to explain the phenomena of radio activity, emission spectra, etc., namely the electron theory.

In 1906 Baly and Stewart[12] applied the principle involved in the foregoing to many colored compouds. As a result of their investigations they conclude:

The color of diketones and quinones is due to an oscillation or isorropesis between the residual affinities of the oxygen atoms which results in the absorption of light in the visible spectrum. Also that in order to start the oscillation it is necessary that some influence should be present to disturb the residual affinities of the oxygen atoms. When this disturbing influence is present there is no doubt that the principle may be extended, and that visible color is due to the oscillations between the residual affinities on atoms or groups of atoms in juxtapposition. They also call attention to the fact that the assumption that two compounds must be fundamentally different in constitution if one is colored and the other not is quite untrustworthy. Many compounds can and do exist with all the conditions for isorropesis and yet there is lacking the influence to disturb the equilibrium between the residual affinities and so the com-

[11] Proc. Chem. Soc., 87, p. 766.
[12] Jr. Chem. Soc., 89, pp. 502, 514, 966, 982.

pounds are colorless. They consider this principle to be the key to Armstrong' theory of color and as an explanation of the colors of many compounds which are difficult of interpretation by Armstrong's quinoid linking alone, for though Armstrong was perfectly right in concluding that color is due to quinoid linking, this formula gives no reasons why color is thus produced.

In 1907[13] Hale drew practically the same conclusions as those of Baly and his associates, namely that isorropesis is the cause of color in both the aromatic and the aliphatic series. By isorropesis is meant the making and breaking of contact between atoms thus giving them marked activity. This change of linkage which must accompany the transformation of one modification of the compound to the other is the source of the oscillations producing the absorption bands. If these oscillations are synchronous with light waves of a high frequency they give rise to absorption bands in the ultra violet and the compound is colorless. If, however, they are less frequent, the absorption band appears in the visible portion of the spectrum and this absorption of colored rays results in the compound taking on the complementary color.

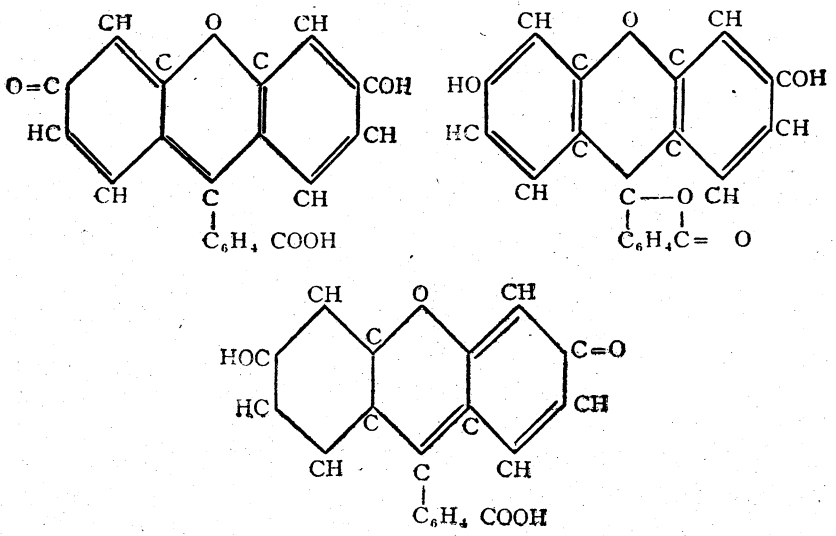
In 1907 Hewett and Mitchell[14] pointed out that in every case of colored compounds the molecules contain not merely double linkages but chains of alternate double and single linkages. Generally speaking the longer this chain of conjugate double linkages the slower the oscillation frequency of the molecule. This explains why a benzene nucleus gives absorption bands in the ultra violet and is colorless while a quinone nucleus gives absorption bands in the violet and is therefore colored yellow. In estimating the number of such alternate double and single linkages, when a benzene nucleus is encountered, one is justified in following the structure around one side of the ring only until para position is reached. The chain in the benzene nucleus, therefore, contains at best only two double linkages, while that of the quinone contains three.

Hewett and Mitchell also conclude that a radical change in the absorption spectrum of a compound when it undergoes salt formation generally means the radical alteration of its consti

[13] Pop. Sci. Mo., 72, p. 116.
[14] Jr. Chem. Soc., 91, p. 1251.

tution. Other groups or elements when introduced in place of hydrogen may diminish the oscillation frequency, but the effect in such cases must be slight and the general character of the absortion would remain unaltered. It is conceivable that such groups introduced into the molecule of a substance colorless in the ordinary sense, might, if its absorption occurs just outside the visible spectrum, render the substance colored by a slight shifting of the absorption band; but when a radical change in the color takes place on salt formation the salt is constituted differently from the parent substance.

In 1900 Hewett[15] advanced the idea that symmetrical compounds capable of equal tautomeric displacements in either of two directions should be fluorescent, for the molecule would swing between the two extremes like a pendulum, the energy absorbed in one wave length being degraded and given out with slower frequency.



Fluorescein.

In 1910 Porai-Koschitz[16] summed up the more recent views of the oscillation theory of the cause of color in organic compounds as follows: The change in color of a compound is due to the retarding of the oscillations or the setting up of a new

[15] Zeitschr. physikal. Chem., 34, p. 1. (See also Jr. Phys. Chem. 10, p. 375; Jr. Chem. Soc., 87, p. 768.)

[16] Jr. Russ. Phys. Chem. Soc., 42, p. 1237. (Jr. Chem. Soc., A. II, p. 3.)

type of oscillations within the molecule by the entrance of a new group, by the formation of a molecular compound, or by the associations of the molecule of a solute with those of its solvent. Three cases are possible:

1. The new oscillation may coincide with and increase the original oscillation; then the absorption band will move further toward the ultra-violet and the compound will remain, or become visibly colorless.

2. The new oscillation may be an entirely different type from the original, in which case new bands will appear, and since the original oscillation will be retarded to some extent, there will be a change but not a very considerable one in the visible color of the substance.

3. The new oscillation may combine with the original and retard it greatly, when there will be a considerable sharp change in color.

PREFACE.

Work upon plant pigments was begun by the writer during the summer of 1907 when, as an undergraduate student, her attention was directed to pigmentation in the Monarda species. Since that time, though the subject has been sometimes temporarily pushed aside by other interests, it has never been lost sight of and it has usually been the subject of most absorbing interest. At times the work has appeared to be of a purely chemical nature, without any biochemical significance, as in the study of thymoquinone, hydrothymoquinone, and the oxidation products of thymoquinone. Its object at these times has been to elucidate the behavior of certain plant pigments, i. e. those of the Monarda species. Sometimes it has been of a character usual in the study of plant pigments, the extraction of pigments from the plants themselves and an examination of the products obtained. At other times it has been of what is generally considered of a more purely biochemical character, a study of oxidases, water content, etc., and their relation to the formation of pigments in plants.

It has been found in the course of these investigations that no adequate and satisfactory knowledge of plant pigments can be

gained by a study of simply the pigments themselves; but that each pigment should be considered not only in relation to the other colored substances in the same and related plants, but also to the non-colored substances as well. A close and peculiar relationship has often been found to exist between the colored and the non-colored constituents not only of the same plant, but sometimes of the related species of a whole plant family.

As the work progressed a complete revision of the literature on plant pigments became necessary. Very little literature of a general nature upon plant pigments was found to exist, almost nothing in fact beyond Brandel's excellent monograph. Several treatises upon vegetable dye stuffs, it is true, are available. Among these may be mentioned two by Thomas, *Les Matiéres Colorantes Naturelles,* and *Les Planets Tinctoriales,* also Rupe's more recent *Chemie der natuerlichen Farbstoffe* (1909) and volume 6 of the *Biochemisches Hand-Lexicon, Farbstoffe der Pflanzen und der Tierwelt.* (1911), as well as chapters on natural dye stuffs in various treatises on dye stuffs in general. By far the larger literature on plant pigments, however, is scattered through the chemical and botanical journals of the past fifty years, some extending much further back. Before attempting to proceed further with work upon plants and plant products it seemed desirable to review this literature in order,

1. To avoid useless repetition of work already done.
2. To interpret new work in the light of the old, and the old in the light of recent experimentation.
3. To make comparisons, draw conclusions, and formulate theories as a guide to future work.

In the course of this review of the literature upon plant pigments it was found that by arranging the pigments according to the degree of saturation, as calculated from the underlying hydrocarbons, certain relationships were brought out which could not well be observed in any other way. Among the important relationships emphasized by this classification are:

1. The influence of unsaturation upon the production of color in a molecule.
2. The influence of so called chromophorous groups upon the production of color in a molecule.
3. The existence of homologous series of pigments.

4. The existence of series of pigments related to similar symmetrical, or almost symmetrical hydrocarbons of different degrees of saturation.

1. *The influence of unsaturation upon the production of color in a molecule.*

In this connection it should be pointed out that all organic pigment molecules are unsaturated. The highest degree of saturation known in a pigment molecule is C_nH_{2n-4}, and visible color exists in substances of this degree of saturation only when the quinone grouping is present, the quinone grouping being one of the best known and most reliable of the chromophorous groups.

Among substances referable to hydrocarbons of the degree of saturation C_nH_{2n-6} no substances colored in the ordinary sense are known to exist but several pigment producing substances are known. All substances, however, having a benzenoid grouping are colored in a physical sense, since they all exhibit selective absorption, not, it is true in the visible portion of the spectrum but just beyond it in the ultra violet.

The largest number by far of plant pigments are referable to hydrocarbons of the degrees of saturation C_nH_{2n-14} and C_nH_{2n-16}, through colored substances of known constitltion referable to hydrocarbons of higher unsaturation, up to C_nH_{2n-34}, have been isolated from plants. Moreover, all colored hydrocarbons, in which the production of color cannot be attributed to the usual chromophorous groups, are highly unsaturated, caroten and lycopen being of the degree of saturation C_nH_{2n-24}, while the blue hydrocarbon from oil of milfoil, having the formula $C_{15}H_{18}$, is apparently of the degree of saturation C_nH_{2n-12}.

2. *Influence of co-called chromophorous groups upon the production of color.*

As has been pointed out elsewhere in this paper, but little has been contributed to our knowledge of pigmentation in plants by a study of chromophorous groups, since only the corbonyl of the so-called chromophorous groups and the hydroxyl of the auxochrome groups are of at all frequent occurrence in plant pigments. Neither is the mere presence of either or both of these groups, or of multiples of one or both, sufficient to explain the phenomenon of color in any known plant pigment. In

many instances, however, the influence of both the presence and the position of these groups, especially of the hydroxy group, is evident. For example, the substitution of hydroxy groups for hydrogen in the xanthone or flavone pigments usually intensifies both the color and the dyeing properties of these substances, while the removal of such groups, either by replacement with hydrogen or by methylation, usually diminishes both. In no instance does the presence of the chromophorous group explain the color. In most cases it will be seen that it is not the mere presence of these so-called chromophorous groups but their relation to each other and to the rest of the molecule which postulates color in a substance. Color, in other words, appears to be a function, not of certain groups or elements but of the entire molecule.

3. *The existence of homologous series of plant pigments,* or more accurately, of pigments referable to homologous series of hydrocarbons is worthy of note. This homology is manifest in connection with every degree of saturation where a sufficiently large number of pigments, or of pigment forming substances, to admit of comparisons adequate to justify the drawing of conclusions, has been isolated.

Under the degree of saturation C_nH_{2n-4} we find evidence of the existence of quinone, methyl quinone and of methyl p-isopropyl quinone. Similarly, under the formula of saturation C_nH_{2n-6} the pigment forming substances hydroquinone, methyl hydroquinone, and methyl-p-isopropl hydroquinone are found. A similar homology is found to exist in connection with pigments referable to hydrocarbons of other degrees of saturation, especially to C_nH_{2n-16} where we find pigments referable to homologuus of diphenyl ethene, diphenyl propane, etc., as well as to homologous series of dihydroanthracenes.

A condition quite similar in many respects to homology, and sometimes confused with it, exists among the pigments falling under the degrees of saturation C_nH_{2n-14} and C_nH_{2n-16}. This is the existence of pigments referable to closely related series of hydrocarbons, not truly homologous yet differing from one another by CH_2, such as diphenyl, diphenyl methane, diphenyl ethane, diphenyl propane, among the former; and diphenyl ethene, diphenyl propene, and diphenyl butene among the latter, as well as alkyl substitution products of these

hydrocarbons. This relationship is very similar to that existing between pigments referable to hydrocarbons of different degrees of saturation discussed in the following paragraph.

4. *The existence of series of compounds referable to similar symmetrical, or nearly symmetrical hydrocarbons of different degrees of saturation.*

A relationship quite similar to that expressed by homology under the same degree of saturation is noted between the hydrocarbons of different degrees of saturation to which any of the plant pigments are referable. This relationship is best expressed by the accompanying chart of graphic formulae representing the hydrocarbons to which a large majority of the plant pigments falling under the degrees of saturation C_nH_{2n-10} to C_nH_{2n-18} are referable.

In addition to the hydrocarbons listed in this table, attention should here be called to pigments, or pigment forming substances, referable to benzene and dihydrobenzene, naphthalene and dihydronaphthalene anthracene and dihydroanthracene series of hydrocarbons. It is also interesting to note the symmetrical or almost symmetrical character of all of the above hydrocarbons. Whether this symmetry of arrangement of the underlying hydrocarbon is only coincident with the conditions which produce color in the molecule, or whether the symmetrical arrangement is itself one of the conditions does not become manifest.

In order to bring out the relationships just discussed the plant pigments of known constitution have here been classified according to the underlying hydrocarbon. A second classification, according to plant families, showing the relationship which exists between the pigments and the noncolored constituents of the same and related plants, was intended to be included. This classification was, however, found to be too long for the purposes of this paper, therefore will be published later as supplementary to it. The experimental work of the writer[1]. where heretofore published has been referred to in the same manner as that of other investigators. Some work not previously published has been briefly described, i. e. the study of the pigment of red geranium blossoms. In addition to the above a large amount of

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[1] Quantitative determinatino of oxidase in the leaves of Monarda fistulosa.
Ph. Rev., 26, p. 314.
Thymoquinone and Hydrothmoquinone.
Ph. Rev., 26, p.
Higher oxidation products of thymoquinone.
Proc. A. Ph. A., 58, p. 979.
The Monardas, a phytochemical study.
Bull. of Univ. of Wis., Sci. Ser., Vol. 4, No. 4, pp. 81–128.

SYMMETRICAL CONFIGURATIONS OF HYDROCARBONS UNDERLYING PIGMENT MOLECULES.

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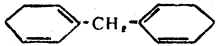
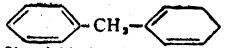
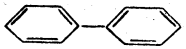
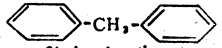
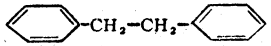
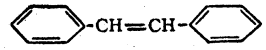
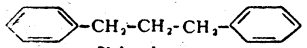
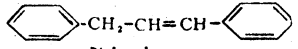
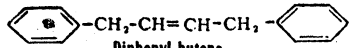
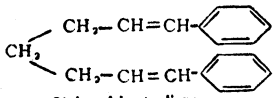
 di-dihydro phenyl-methane	 Phenyl-dihydro phenyl-methane	 Di phenyl		
		 Di phenyl methane		
		 Diphenyl ethane	 Diphenyl ethene	
		 Diphenyl propane	 Diphenyl propene	
			 Diphenyl butene	
				 Diphenyl hepta diene

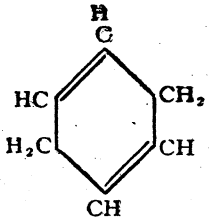
material has been collected and studied for the purpose of making comparisons and verifying conclusions.

PLANT PIGMENTS.

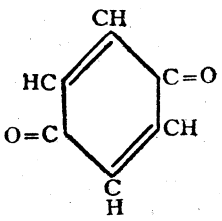
PIGMENTS REFERABLE TO HYDROCARBONS OF THE FORMULA OF SATURATION C_nH_{2n-4}.

All of the known plant pigments of this degree of saturation are quinones or more particularly their quinhydrone or phenoquinone addition products, and metallic derivatives of the latter, and are referable to dihydro benzene, dihydro toluene and dihydro cymene.

Pigments referable to dihydrobenzene.



Dihydro benzene

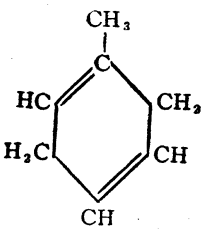


Benzo quinone

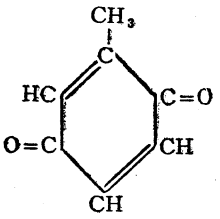
The only plant pigment referable to dihydro benzene, of whose existence in plants we have any evidence, is the ordinary quinone, or benzoquinone. However, its occurrence is purely hypothetical. Though benzoquinone has never yet been isolated from a plant, its dihydro derivative, hydroquinone, is known to occur in several species and under such conditions as would suggest the formation of quinone and quinhydrone as a possible explanation of the pigmentation which exists there. For example, the glucosides arbutin and methyl arbutin occur in the leaves of *Gaultheria procumbens, Uva ursi,* and several other species of the Ericaceae. Arbutin upon hydrolysis yields hydroquinone. Hydroquinone by the action of oxidases, known to occur in *Gaultheria* and, no doubt, present in the other arbutin containing plants, is readily converted into quinone with the formation of quinhydrone as an intermediate product. The presence of benzoquinhydrone, which is brownish-red in color, would afford an explanation of the reddish tint commonly acquired by the leaves and stems of these plants in the fall. It might also account for the remarkable colorations of the *Madrones* and *Manzanitas* so well known upon the Pacific coast, since both are species of *Arbutus* and closely related to the above named plants. Arbutin has been isolated from the leaves of at least one of the manzanitas, *Arctostaphlos glauca.*

Hydroquinone also exists as the mono methyl ether in the oil of star anise. *Illicium verum*, a member of the family *Magnoliaceae.*[1]

Pigments referable to dihydrotoluene



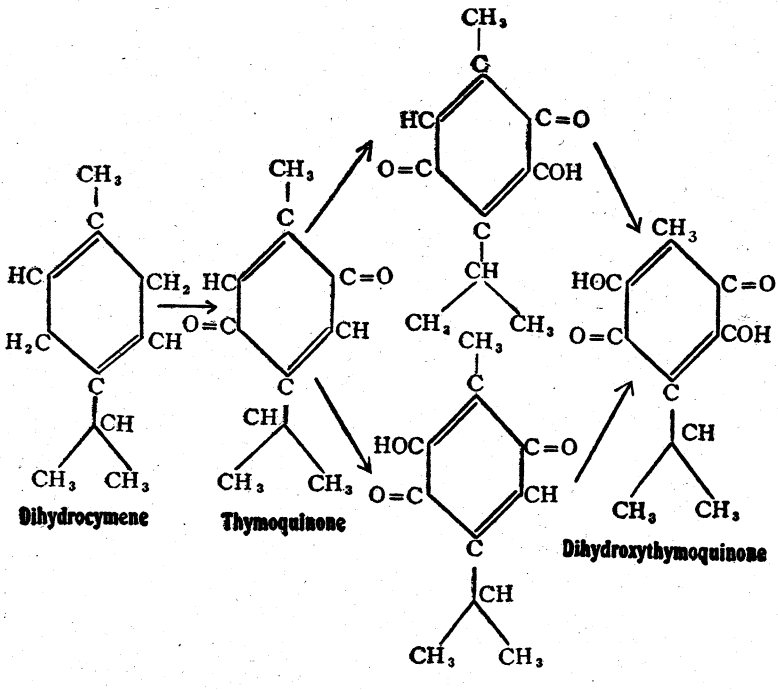
Dihydrotoluene



Methyl benzoquinone

As has been pointed out in connection with quinone, methyl hydroquinone exists potentially in *Gaultheria procumbens* and other species of the *Ericaceae* as the glucoside methyl arbutin. The same possibilities for forming pigments by hydrolysis, oxidation, and addition exist in both quinone and methyl quinone.

Pigments referable to dihydrocymene



[1] For references see under Hydroquinone, formula of saturation Cn H_{2n-6}.

Of the oxidation products of dihydrocymene, three, and possibly four, are believed to exist in plants. Of these thymoquinone and dihydroxy thymoquinone have actually been isolated, while there are strong reasons for believing that one or both of the monohydroxy thymoquinones occur in *Monarda* species either in the free state or as labile compounds.

Thymoquinone together with the corresponding hydroquinone has been isolated from several species of *Monarda,* also from the oil from the wood of *Thuja articulata.*[2] Hydrothymoquinone also exists in the oil from the fruit of *Foeniculum vulgare,*[3] also as dimethyl ether in the oil of *Eupatorium triplinerve*[4] (*E. Ayapana*) and in the oil from *Eupatorium capillifolium.*[5] Inasmuch as in the diethers the original phenol hydrogens are replaced by alkyl radicals they are not prone to oxidation in like manner as the phenols, hence, presumably do not take part in pigment formation.

Monohydroxy thymoquinone[6]) is believed to occur in *Monarda fistulosa, Monarda citriodora,* and perhaps in other species of *Monarda.*

Dihydroxy thymoquinone[7]) has been isolated from the volatile oils of *Monarda fistulosa* and *Monarda citriodora.* Its presence has been indicated in *Monarda didyma.*

The chemical relationship of the thymoquinones to some of the other constituents of the *Monardas* is very close and is worthy of notice here. From the volatile oils of the several species of *Monarda* so far examined, have been isolated both of the monohydroxy phenols, thymol and carvacrol, and probably cymene[8]) the hydrocarbon underlying not only these phenols but hydrothymoquinone as well.

The relation of the pigment substances to each other and to the volatile constituents of the Monardas, also the role which some of the non-colored volatile and non-volatile substances

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[2] C. r., 139, 927.

[3] Schimmel, Gesch. Ber. 1906, Apr. p. 28.

[4] Gildemeister—The Volatile Oils, p. 479.

[5] Personal communication from Prof. E. R. Miller, Laboratory of Plant Chemistry, University of Wisconsin.

[6] Bulletin of the University of Wisconsin No. 448, p. 31–34.

[7] Bulletin of the University of Wisconsin No. 448, p. 31–34.

[8] Ph. Rund., 13, p. 207; Ph. Rev., 14, p. 223. (The writer has not succeeded in identifying cymene in her study of the hydrocarbons in the oil of M. punctata.)

play in the formation of the pigments, can best be illustrated by a series of graphical formulas given on the accompanying chart.

From this chart it becomes apparent that here we have to deal with white (or colorless), yellow, orange, and red substances, all of which are very closely related to each other. Moreover, the thymoquinone, monohydroxythymoquinone and dihydroxythymoquinone, have the capacity of adding monatomic phenols, thus yielding highly colored phenoquinones; also diatomic phenols thus yielding the equally highly colored quinhydrones.

Thymoquinhydrone has actually been isolated from the corollas of Monarda fistulosa while the formation of phenoquinones and quinhydrones of mono- and dihydroxythymoquinone has been considered as a probable explanation of the complexity of the mixture of crystalline pigment originally referred to as "alizarin-like[9])." The following table illustrates the phenoquinone and quinhydrone pigments that can result from the addition of the phenols to the quinones thus far observed in the Monardas.

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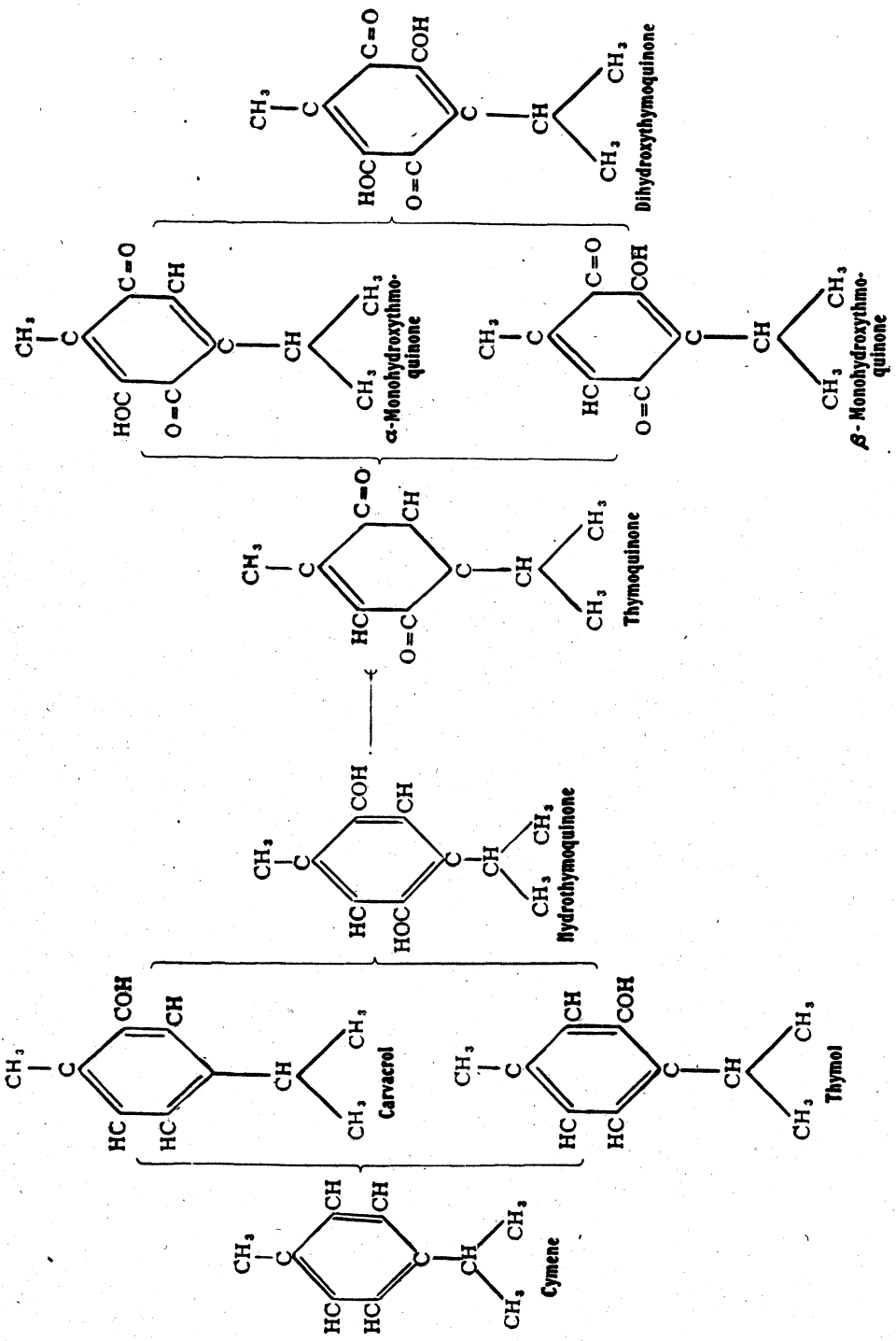
[9] Ph. Rev., 19, p. 244; Mid. Drug., Ph. Rev., 44, p. 342; Bulletin of the Univ. of Wis., No. 448, p. 22.


Dihydroxythymoquinone
α-Monohydroxythmo-quinone
β-Monohydroxythmo-quinone
Thymoquinone
Hydrothymoquinone
Carvacrol
Thymol
Cymene

Quinones	*Phenoquinones*	*Quinhydrones*
		1.) With hydrothymoquinone.
		2.) With dihydroxythymoquinone.
	1.) With two molecules of thymol.	
	2.) With two molecules of carvacrol.	
	3.) With one molecule each of thymol & carvacrol.	
Thymoquinone	4.) With two molecules of α-monohydroxythymoquinone.	
	5.) With two molecules of β-monohydroxythymoquinone	
	6.) With one molecule each of α- & β-monohydroxythymoquinone.	
	7.) With one molecule each of α-monohydroxythymoquinone and thymol.	
	8.) With one molecule each of α-monohydroxythymoquinone and carvacrol.	
	9.) With one molecule each of β-monohydroxythymoquinone and thymol.	
	10.) With one molecule each of β-monohydroxythymoquinone and carvacrol.	

Quinones	*Phenoquinones*	*Quinhydrones*
		1.) With hydrothymoquinone.
		2.) With dihydroxythymoquinone.
	1.) With two molecules of thymol.	
	2.) With two molecules of carvacrol.	
α-Hydroxythymoquinone.	3.) With one molecule each of thymol and carvacrol.	
	4.) With two molecules of α-monohydroxythymoquinone.	
	5.) With two molecules of β-monohydroxythymoquinone	
	6.) With one molecule each of α- and β-monohydroxythymoquinone.	
	7.) With one molecule each of α-monohydroxythymoquinone and thymol.	
	8.) With one molecule each of β-monohydroxythymoquinone and carvacrol.	
	9.) With one molecule each of β-monohydroxythymoquinone and thymol.	
	10.) With one molecule each of β-mono-hydroxythymoquinone and carvacrol.	

Quinones	*Phenoquinones*	*Quinhydrones*
		1.) With hydrothymoquinone.
		2.) With dihydroxythymoquinone.
	1.) With two molecules of thymol.	
	1.) With two molecules of carvacrol.	
	3.) With one molecule each of thymol and carvacrol.	
β-Hydroxythymoquinone.	4.) With two molecules of α-monohydroxythymoquinone.	
	5.) With two molecules of β-monohydroxythymoquinone.	
	6.) With one molecule each of α- and β-monohydroxythymoquinone.	
	7.) With one molecule each of α-monohydroxythymoquinone and thymol.	
	8.) With one molecule each of α-monohydroxythymoquinone and carvacrol.	
	9.) With one molecule each of β-monohydroxythymoquinone and thymol.	
	10.) With one molecule each of β-monohydroxythymoquinone and carvacrol.	

Quinones	*Phenoquinones*	*Quinhydrones*
		1.) With hydrothymoquinone.
		2.) With dihydroxythymoquinone.
	1.) With two molecules of thymol.	
	2.) With two molecules of carvacrol.	
	3.) With one molecule each of thymol and carvacrol.	
Dihydroxythymoquinone.	4.) With two molecules of α-monohydroxythymoquinone.	
	5.) With two molecules of β-monohydroxythymoquinone.	
	6.) With one molecule each of α- and β-monohydroxythymoquinone.	
	7.) One molecule each of α-monohydroxythymoquinone and thymol.	
	8.) With one molecule each of β-monohydroxythymoquinone and thymol.	
	9.) With one molecule each of α-monohydroxythymoquinone and carvacrol.	
	10.) With one molecule each of β-monohydroxythymoquinone and carvacrol.	

Taking into consideration only those compounds that have been isolated, (hydrothymoquinone, thymoquinone, and dihydroxythymoquinone) or whose presence has been indicated (monohydroxythymoquinone) in the Monardas thus far, the number of possible pigments becomes truly bewildering. A consideration of these possibilities of easily decomposable phenoquinones and quinhydrones readily explains why a crystalline pigment, seemingly a chemical unit, upon recrystallization from such a solvent as ether yields several kinds of crystals of different shades of red and purple. To attempt the isolation of a number of these pigments would seem a thankless task. Indeed, after they had been isolated the question might pertinently be asked whether the combination as isolated existed as such in the plant or whether it had been formed because of a change of solvents.

However, the subject of the pigmentation of the Monardas is not solved even after the numerous combinations of phenoquinones and quinhydrones have been worked out. Most of these pigments are phenolic in character and hence can combine with metallic constituents, ammonia and organic nitrogen bases of the plants giving rise to different shades of the original pigment.

This is shown by the varying shades of color produced by treating solutions of these phenols with solutions of basic metallic compounds, etc. However, nothing definite is known of the particular kind of metallic and other derivatives which may be found in the various parts of the several Monarda species.

Another possible influence of basic inorganic material remains to be referred to, viz: the stimulating influence some of them, such as potassium hydroxide and calcium hydroxide, exert on oxidizing reactions, e. g. the oxidation of thymoquinone. That even very dilute basic solutions exert such an influence has been shown by the action of lime water upon aqueous thymoquinhydrone solution. It has further been demonstrated by Schaer[10] and others that traces of basic substances, organic as well as inorganic, stimulate the action of oxidases.

Assuming that much of the pigmentation of plants containing quinones or hydroquinones is due to the formation of quinhydrones or phenoquinones, the intense coloration of the

[10] From a reprint from the Pharm. Inst. Strassburg, 1902.

lower surfaces of the leaves, and often of the entire shoots of *Monarda fistulosa,* and the general reddish appearance of the young plants of *Monarda punctata* in spring can readily be accounted for by the greater oxidase content of the vigorous young tissue and the consequent greater chemical activity.[1] A similar phenomenon in the young plants or shoots after the fall rains may be explained in the same way.

On the other hand, the fall coloration of arbutin containing foliage may be explained by assuming that as the synthetic life process of the plant grow sluggish, the reserve carbohydrates stored away in the glucoside are rendered available as food material by hydrolysis. This latter process would set free the hydroquinone as well as the sugar. The former (chromogen) in turn would be oxidized to pigment. If this line of reasoning may be applied to the madrones as well as to the other species of arbutus, the brilliant coloration of both the enormous leaf buds in spring and of the leaves and the freshly peeled trunk in autumn may be accounted for.

It has been pointed out above that the diethers of the hydrothymoquinone, being deprived of their phenolic hydrogen are no longer prone to oxidation, hence to quinhydrone pigment formation. It is, therefore, not surprising that plants characterized by the presence of the dimethyl ether of hydrothymoquinone are not conspicuously colored. The only pigmentation of the Eupatorium species which could be attributed to quinhydrone formation is the occasional purplish coloration of the stems.

This purplish stem coloration though not conspicuous deserves special notice since most of the plants considered in this chapter are remarkable at some period of their development, generally late in the season, for conspicuously colored stems, the members of the Ericaceae, trailing arbutus, wintergreen, manzinitas, and mandrones for red or red brown stems, the characteristic color of benzo quinhydrone, while the stems of the Monardas are often conspicuously purple, the color of thymoquinhydrone.

Many investigators have inferred that ferments—hydrolases, oxidases, and reductases—play an important role in pigment

[1] The oxidase content of the Monardas has been studied both quantitatively and qualitatively by F. Rabak, Ph. Rev., 22, p. 190; Swingle, Ph Rev., 22, p. 193; Wakeman, Ph. Rev., 26, p. 314.

formation. No where would this part seem more conspicuous than in the formation of quinhydrone and pheno-quinone pigments. Indeed one of the first questions which arises in the biochemical study of any such series of related compounds as the cymene, thymol, carvacrol, hydrothymoquinone, monohydroxy thymoquinone and dihydroxy thymoquinone series in the Monarda species is which of these complex cyclic substances was first formed from the simple chain products of photo synthesis? Being accustomed for purposes of classification to look upon the hydrocarbons as basal compounds and to consider all other compounds as being derived from the hydrocarbons, it is easy to regard such a series as being formed in this order. However, it is highly improbable that the plant works in this way. An oxidase which oxidises hydrothymoquinone to thymoquinone exists in several species of Monarda, but up to the present time no oxygen conveyer has been found which oxidizes thymol or carvacrol to hydrothymoquinone, all attempts in this direction having been attended with negative results, or in the case of thymol, sometimes, with the formation of dithymol. It is not at all improbable that the monatomic phenols and the hydrocarbons are reduction products, possibly by products of autoxidation. The large amounts, however, in which the monatomic phenols are found in comparison with the amounts of thymoquinone and its oxidation products present does not encourage this assumption.

Of almost equal interest with the thymoquinone series of compounds in the Monarda species are the less complete, possibly because less closely investigated, series of carvacrol, hydrothymoquinone, and thymoquinone in *Callistris quadrivalvis* (*Thuja Articulata*) and the cymene, hydrothymoquinone series of Foeniculum vulgare. Another similar example which should be mentioned here is *Thymus vulgaris*. The oil of thyme is known to contain cymene, thymol, and sometimes carvacrol. Other members of the series, if not present in the original oil, are possibly produced upon standing, since oil of thyme, quite colorless when freshly distilled, often, upon standing, takes on a red color quite similar to the color of the oil from *Monarda fistulosa* from which dihydroxythymoquinone has been separated.

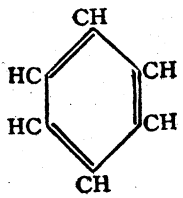
The fact that so frequently the pigment forming substance does not occur alone but is associated with other closely related,

colored or non colored, compounds is of much biochemical significance as will be pointed out in succeeding chapters.

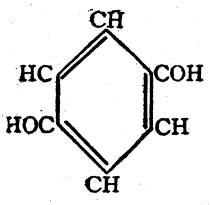
PIGMENTS REFERABLE TO HYDROCARBONS OF THE DEGREE OF SATURATION $C_n H_{2n-6}$.

There exist in plants several compounds referable to hydrocarbonss falling under this degree of saturation, being substitution products of benzene, toluene and cymene, which though colorless in themselves are readily oxidized to pigments. Moreover, being hydroquinones, they are capable of forming highly colored phenoquinones and quinhydrones by addition with their oxidation products the quinones. These compounds occur in a large number of plants either in the free state, as alkyl ethers, or in sugar ether combination as glucosides and they may be looked upon as pigment forming substances, commonly designated chromogens in pigment literature.

While no attempt is being made here at a discussion of the pigments of non-flowering plants it is interesting to note that there exist in several species of lichens pigments and pigment forming substances referrable to the hydrocarbons toluene, o-xylene, p-xylene and trimethyl 1, 2, 4 benzene.



Benzene



Hydroquinone

The only known pigment, or pigment forming substance, referable to benzene as the underlying hydrocarbon is the ordinary hydroquinone, or hydrobenzoquinone. The occurrence of hydroquinone as the glucoside arbutin in several species of *Ericaceae* and the possibility of its forming the corresponding quinone and quinhydrone through oxidation thus furnishing an explanation for the pigmentation of several species has been referred to under benzoquinone.

Arbutin occurs in *Ledum palustre*[1]); *Rhododendron maxi-*

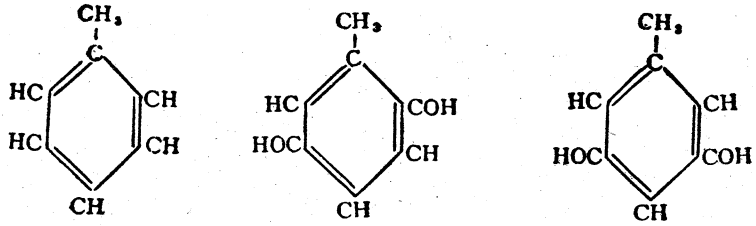
[1] Am. Jr. Ph., 46, p. 314.

mum,[2]) *Kalmia latifolia,*[3]) *Kalmia angustifolia,*[4]) *Gaultheria procumbens,*[5]) *Arctostaphylos Uva Uris,*[6]) *Arctostaphylos glauca,*[7]) *Vaccinium Myrtillus,*[8]) *Vaccinium vitis,*[9]) *Vaccinium macrocarpum,*[10]) *Vaccinium arctostaphylos,*[11]) *Calluna vulgaris,*[12]) *Erica herbacea,*[13]) *Pinus communis,*[14]) *Protea millifera,*[15]) *Chimaphila umbellata,*[16]) *Chimaphila maculata,*[17]) and *Pirola uniflora.*[18])

In addition to the above, the mono-ethyl ether of hydroquinone has been found in the oil of star anise, *Illicium verum.*[19]) Furthermore it has been pointed out that the pentatomic alcohol of hexahydrobenzene, quercite,[20]) may lose three of its hydroxy groups in the form of water and form hydroquinone as indicated by the following formula:

$$C_6H_7(OH)_5 \xrightarrow{-3H_2O} C_6H_4(OH)_2$$

Pigments referable to toluene



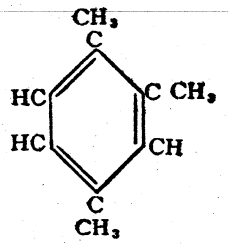
Toluene **Hydrotoluquinone or Methyl hydrobenzoquinone or Methyl hydroquinone** **Orcinol**

[2] Wehmer, Die Pflanzenstoffe, p. 570.
[3] Am. J. Ph., 47, p. 5.
[4] Am. J. Ph., 58, p. 417.
[5] Am. Jr. Ph., 46, p. 314.
[6] Arch. Pharm., 227, p. 164; Am. Jr. Ph., 46, p. 314.
[7] Am. Jr. Ph., 46, p. 314.
[8] Monatsh f. Chem., 30, p. 77.
[9] Arch. exper. Path., u. Pharm., 50, 46.
[10] Chem. News, 52, 78.
[11] Apoth. Ztg., 16, 694.
[12] Am. Jr. Ph., 46, 314.
[13] S. Ber. Wien. Acad., 9, 308.
[14] Jr. Pharm. Chim. (7) 2, 248.
[15] B. 29, R. P. 416.
[16] An. Chim., 129, 203.
[17] Am. Jr. Ph., 46, 314.
[18] Am. Jr. Ph., 11, 549.
[19] Schimmel & Co., Oct., 1895, p. 6.
[20] Plant Pigments, p. 11.

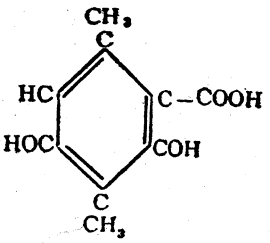
Methyl hydroquinone occurs as the glucoside methyl arbutin in several species of *Ericaceae.*[21]) It is also known to exist in several species of *Pirola*[22]) and in *Pirus communis.*[23])

Orcinol an isomer of methyl hydroquinone and referable to toluene is found in many lichens of the varieties *Rocella* and *Lecenora.* Orcinol[24]) when allowed to stand exposed to air and ammonia forms orcein $C_7H_7No_3$, the principal constituent of the coloring matter archil, called also persio, cudbear and nurpur. Azolithmin[25]) $C_7H_7No_4$, the coloring principle of litmus and an oxidation product of orcein, is also produced from these orcinol containing lichens by the action of ammonia and potassium carbonate.

Pigments referable to trimethyl 1, 2, 4 benzene.

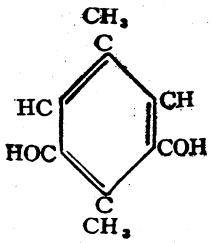


Trimethyl I, 2, 4, benzene



Methyl orsellinic acid

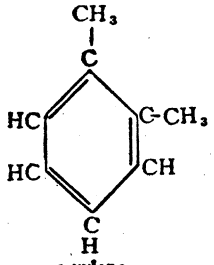
In one variety of *Rocella* there occurs a homologue of erythrin known as betaerythrin.[26]) This upon hybined to form the betaerythrin is therefore drolysis yields not orcinol but beta orcinol, or methyl orcinol, p-xylol orcinol. At least one molecule of the simple acids comprobably methyl orsellinic acid, referable to trimethyl 1, 2, 3 benzene.



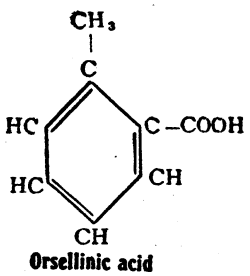
Methyl orcinol

[21] Ann., 206, 159; Ann., 177, 934.
[22] Am. J. Ph., 11, p. 549.
[23] Jr. Pharm. Chim., (7) 2, 248; C. r., 151, p. 444.
[24] Ann., 41, p. 157; 54, p. 261; 59, p. 72; Jr. Prakt. Chem., 44, p. 18;
[25] Ann., 39, p. 25; Czapek, Biochemie der Pflanzen, p. 508.
[26] Czapek Brochemie der Pflanzen, p. 507.

Pigments referable to o-xylene



o-xylene



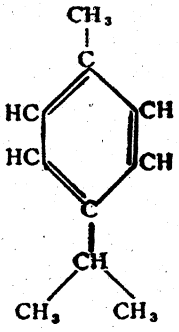
Orsellinic acid

Lecanoric acid,[27]) another pigment forming substances from some varieties of Roccella, Lecanora, and Variolaria, is probably a condensation product of two molecules of orsellinic acid, referable to o-xylene, dimethyl 1, 2, benzene.

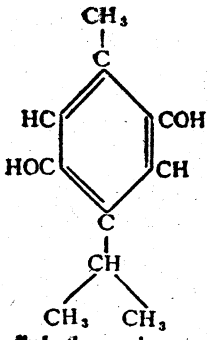
Lecanoric acid crystallizes in colorless crystals. With alkalies it gives a beautiful rose like color, with calcium chloride, a blood red color. It occurs combined with erythrite as the ester, lecanoryl erythrite, also known as erythrin.

The constituents of other similar pigments found in these lichens is not known.

Pigments referable to cymene



Cymene



Hydrothymoquinone

Hydrothymoquinone has already been discussed in connection with thymoquinone in the preceding chapter. It occurs

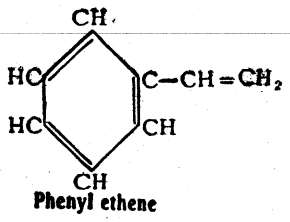
[27] Ann., 295, p. 278; 41, p. 157; 54, p. 261; 61, p. 72; Jr. Prakt. Chim. 44, p. 18; Czapek, Biochemie der Pflanzen, p. 507.

along with thymoquinone in several species of *Monarda,* also in *Thuja articulata.* Its occurrence as dimethyl ether[28]) in the oils of *Eupatorium triplinerve* and *Eupatorium capillifolium,* as well as in the oil from *Arnica Montana* has also been noted.

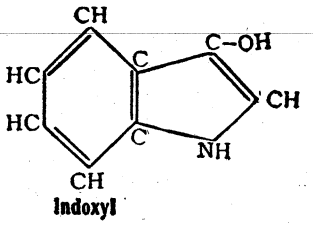
PIGMENTS REFERABLE TO HYDROCARBONS OF THE FORMULA OF SATURATION $C_n H_{2n-8}$.

The only plant pigments of known constitution referable to hydrocarbons falling under this degree of saturation are substitution products of phenyl ethene and phenyl propene, allyl benzene.

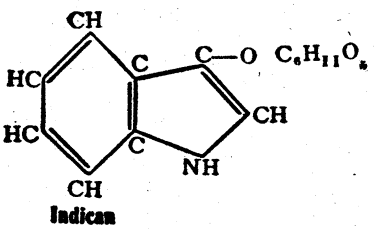
Pigments referable to phenyl ethene.



Phenyl ethene



Indoxyl



Indican

Indican occurs in indigo bearing plants almost exclusively in the form of the glucoside indican,* a sugar ether of indoxyl, referable to phenyl ethene. Upon treatment of the herb, or the indigo producing part thereof, with water the glucoside is extracted. This is hydrolized by an enzyme present in the plant. By contact with the air the indoxyl thus produced is oxidized to indigotin.

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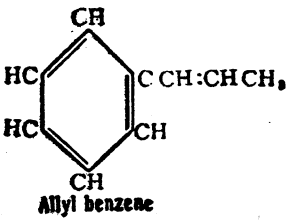
[28] See references to preceding chapter.

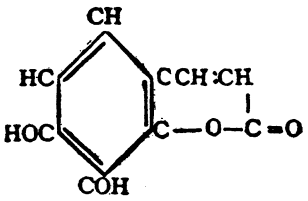
[29] Ann. Chem., 170, p. 345.

*For references to indican and indoxl see Indigotin, formula of saturation CnH2n-16.

Pigments referable to allyl benzene

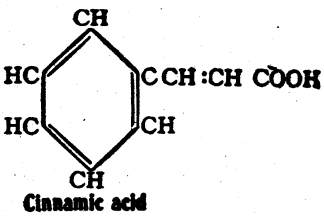


Allyl benzene

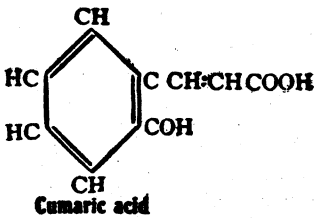


Daphnetin

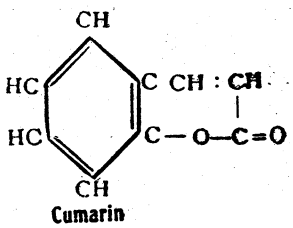
As will be seen from the structural formula, daphnetin may be looked upon as a dihydroxy cumarin, or as a product of the inner dehydration, a lactone, of tri-hydroxy cinnamic acid. Since the occurrence of daphentin in the plant is so frequently accompanied by that of cumarin, umbelliferone and other cinnamic acid derivatives, the importance of recognizing this relationship cannot be over estimated.



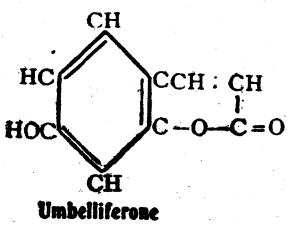
Cinnamic acid



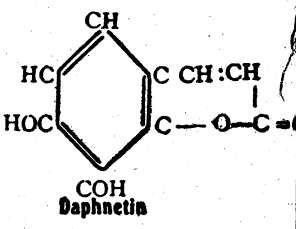
Cumaric acid



Cumarin



Umbelliferone



Daphnetin

Daphnetin which is yellow in color, occurs in the yellow flowers of sweet clover, *Melilotus officinalis.*[1].) together with cumarin, cumaric acid, and hydro cumaric[2]) acid. The frequency of the occurrence of these and related compounds in this and other members of the *Leguminosae* will be taken up in the consideration of pigmentation in that family.

Daphnetin, having two phenol hydrogens, is capable of forming metallic derivatives which may influence the color of the pigmented parts. With potassium it forms the socalled "semi-

[1] Berg. Jahresb., 14, 31[1].
[2] Richter, II, p. 280.

mono potassium salt'' $C_{18}H_{11}O_8K$ and the mono potassium salt $C_9H_5O_4K$. The former crystallizes in bright yellow and the latter in red crystals. To wools mordanted with chromium, aluminum, tin and iron it imparts various shades of olive and yellow.

Daphnetin occurs as the glucoside daphnin, in the bark and flowers of *Daphne mezerum*[3]) and in the leaves, bark and flowers of *Daphne Alpina.*[4]) In these plants, however, the odoriferous principle is not cumarin but umbelliferone, a 4-hydroxy cumarin.

Daphnin, or a glucoside similar to daphnin has, furthermore, been reported in *Panicum italicum*[5]) (Italian millet.)

Closely related to daphnetin are aesculetin, scopoletin, and fraxetin, substances which though not colored themselves form beautifully fluorescent solutions. Moreover, at least some of their metallic derivatives, in which form they would be likely to occur in plants, are colored.

Aesculetin is isomeric with daphnetin, being a 4, 5-dihydroxy cumarin. It forms a bright yellow potassium compound very similar to the corresponding daphnetin derivative. Its solutions show a beautiful blue fluorescence. Aesculetin occurs as the glucoside aesculin in *Aesculus hippocastanum*,[6]) the horse chestnut, and in *Gelsemium sempervirens.*[7]) In the free state, is found in *Euphorbia lathyris.*[8])

Scopoletin, a methyl ether of aesculetin occurs as the glucoside scopolin in *Gelsemium sempervirens*,[10]) and in several species of *Solonaceae, Atropa belladonna*,[11]) *Scopola japonica*,[12]) *Mandragora autumnalis*,[13]) and *Fabiana imbricata.*[14]) Scopoletin gives a blue fluorescence in solutions. Many of its metallic derivatives are colored.

Fraxetin, another beautiful fluorescent substance is a derivative of tetra hydroxy cinnamic acid. It may be considered as a

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[3] Ann., 84, 173.
[4] Zwenger's Ann., 115, 1.
[5] Ann. Chem. Jr., 20, 86.
[6] Arch. Pharm., 38, 330.
[7] Ber., 9, 1182.
[8] Ber., 23, 3347.
[9] Czapek—Biochemie der Pflanzen, p. 563.
[10] Am. Jr. Ph., 42, p. 1; 54, p. 337; Arch. Pharm., 236, p. 329.
[11] Arch. Pharm. 228, p. 438, 440.
[12] Same as 11.
[13] Jr. Prakt. Chem., 172, p. 274.
[14] Arch. Pharm., 237, p. 1.

methyl ether of hydroxy daphnetin or aesculetin, or as a methyl ether of trihydroxy cumarin.

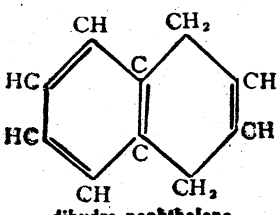
Fraxetin occurs along with aesculetin as the glucoside fraxin in the horse chestnut.[15]) It occurs as the glucoside fraxin in *Fraxinus excelsior*,[16]) *Fraxinus ornus*,[17]) also both in the free state and as glucoside in *Fraxinus americana*.[18])

Fraxetin gives a blue fluorescence in solutions. Many of its metallic derivatives are colored. The position of the methoxy group in fraxetin is not known.

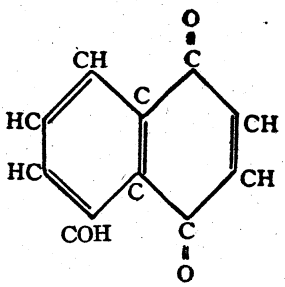
PIGMENTS REFERABLE TO HYDROCARBONS OF THE FORMULA OF SATURATION Cn H_{2n-10}.

Under this formula of saturation one pigment of known constitution, juglone, referable to dihydro naphthalene, and two others probably derivatives of methyl dihydro naphthalene have been isolated. All three of these compounds are hydroxy naphthaquinones, possessing both phenol and quinone properties and capable of forming phenoquinones and quinhydrones with themselves and with the corresponding hydroquinones. In addition to these naphthaquinone pigments one, referable to di-dihydro phenyl ethane has been isolated.

Pigments referable to dihydro naphthalene.



dihydro naphthalene



Juglone

Juglone, a hydroxy derivative of naphtha quinone, is found in all the green parts of the walnut tree, *Juglans regia*,[1]) and especially in the green shells of the nuts. Associated with it

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[15] Pogg. Ann., 107, p. 331.
[16] Pogg. Ann., 98, p. 637.
[17] Pogg. Ann., 98, p. 637; C. r. 51, p. 31.
[18] Am. Jr. Ph., 54, 282; 54, 99.
[1] C. N., 141, p. 838; Ber. Repert., 5, p. 106; 7, p. 1; Ber., 10, p. 1542.

in the twigs, bark, leaves and shell of the unripe fruit, but not in the shells of ripe nuts, α and β-hydrojuglones, trihydroxy naphthalenes, are also found. These colorless hydrojuglones, during the ripening of the nuts, are undoubtedly oxidized to the yellowish red juglone. Indeed the oxidation may well be carried farther, for juglone is readily oxidized by exposure to the air into hydroxy juglones which are still darker in color. There is no record, however, of the hydroxy juglones having been isolated from the walnut material. Juglone is also found in the green shells of the nuts and the bark of the twigs of *Juglans nigra*, the black walnut, *Juglans cinerea*,[2]) the butternut; in the leaves of *Carya olivaeformis*,[2]) the pecan; and in the bark of the twigs of *Pterocarya caucasia*.[2])

The possibilities for combination between juglone and the hydrojuglones must not be overlooked in considering the dark colored pigments in the walnut shells. Not only is there the possibility of juglone combining with each α and β-hydrojuglone to form the corresponding quinhydrones; but the additional possibilities of its forming phenoquinones with itself, through the addition of its phenol group to a carbonyl group, and also of forming phenoquinones with the hydro juglones. If juglone be oxidized in the plant to hydroxy juglones the possibilities for pheno quinone and quinhydrone formation become fully as great as with the thymoquinones in the *Monarda* species.

Pigments referable to a Methyl dihydro naphthalene

$C_{10} H_9 CH_3$	$C_{10} H_5 O_2 CH_3$
Methyldihydro-Naphthalene	Methyldihydro-Naphthaquinone
$C_{10} H_3 O_2 (OH)_2 CH_3$	$C_{10} H_2 (OH)_3 O_2 CH_3$
Dihydroxy-methyl	Trihydroxy-methyl
Naphthaquinone	Naphthaquinone

Two pigments, one orange red, crystallizing in needles, and the other red, crystallizing in plates, have been isolated from the root tubers of *Drosera Whittakeri*. The former is apparently dihydroxy methyl naphthaquinone and the latter trihydroxy methyl naphthaquinone.

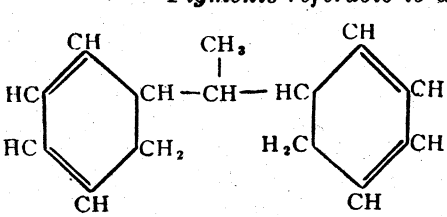
Pigmentation in Drosera Whittakeri seems to be confined to

[2] C. r., 141, p. 838.

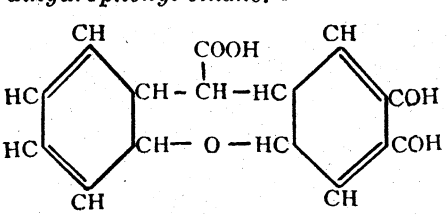
the tubers. According to Rennie[1]) who has made a study of these pigments, each plant is provided with one tuber attached to a stem at a depth of 3 to 4 inches. The tubers vary from ¼ to ¾ of an inch in diameter. Each consists of an inner solid but soft nucleus, full of a reddish sap, and an outer series of thin, more or less dry, layers of an almost black material. Between the layers is to be found, in small quantities, a brilliant red coloring matter, apparently most plentiful in the older tubers. The flowers of the species are white, resembling those of the white oxalis. The red pigment gives a violet, the orange red a deep red solution with ammonia and alkalies.

This remarkable form of pigmentation is doubly interesting when considered from the view point of the quinhydrone hypothesis. Both of the substances are quinones, and both have in addition phenol groups. The presence of the corresponding hydroquinones has not been indicated, though both substances may be reduced to hydroquinones. Whether the black outer layers owe their color to phenoquinones, quinhydrones or higher oxidation products of the known pigments does not become apparent from Rennie's investigations, though the red crystals between the dark layers are apparently the trihydroxy compound.

Pigments referable to di-dihydrophenyl-ethane.



Di-dihydro phenyl ethane



Phoeniceine

Phoeniceine[1].) occurs as the colorless leuco-compound Phoenin in the heart of wood of *Copaiefera bracteata,* the "purpurholz" "amaranth wood" or "blue ebony" of South America, comprising about two per cent of the wood. Phoenin, C_{14} H_{16} O_7, upon treatment with mineral acids gives up one

[1] Chem. News, 55, p. 115; Jr. Chem. Soc., 51, p. 371; 63, p. 1083.

[1] Kleerekoper, E. Neederl. Tijdschr. Pharm., 13, p. 245; 284, 303. (Chem. Centralbl. 72, II, p. 858, 1085).

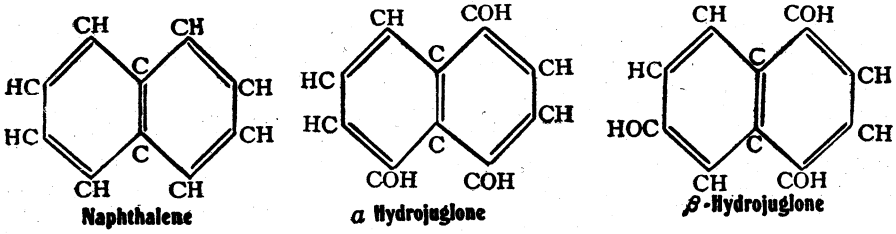
molecule of water forming quantitatively the red phoeniceine $C_{14}H_{14}O_6$. This reaction also takes place quantitatively upon long heating at 100° or heating for one hour at 150°–160°. Upon exposure to the air at ordinary temperature phoenin passes slowly to phoeniceine.

Upon treatment with alkalies phoeniceine turns blue, then violet, and finally brown in color. Its behavior toward alkalies and acids is similar to that of the flavone compounds containing two hydroxy groups in ortho position. Kleerekoper[1]) suggests the formula given above.

Pigments referable to hydrocarbons of the formula of saturation Cn H_{2n-12}

There exist in plants several pigments and pigment forming substances referable to hydrocarbons of this degree of saturation, all of which are substitution products of four different hydrocarbons, namely, naphthalene, two dihydro naphthalene derivatives with unsaturated side chains, and phenyl-diphenyl methane.

Pigments referable to Naphthalene.



Both of the hydro juglones exist, along with juglone, in all the green parts of the walnut tree, *Juglans regia.*[1]) Upon oxidation α hydrojuglone yields juglone. A discussion of the various quinhydrones and phenoquinones which may be formed by combination of the two hydrojuglones with juglone, also with possible higher oxidation products of juglone, has been given under juglone.

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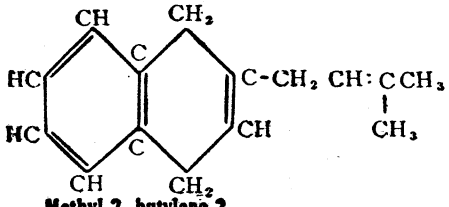
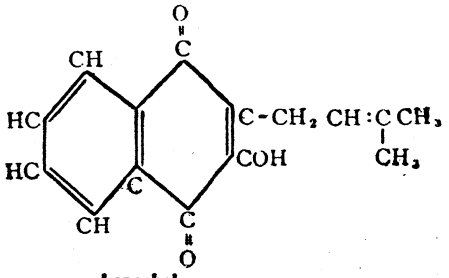
[1] Ber., 10, p. 1544; 17, p. 2411; 18, p. 204; 18, p. 474; 18, p. 2567.
[2] Jr. Chem. Soc., 69, p. 1355.

Pigments referable to methyl 2, butylene 2, dihydro naphthalene.



Methyl 2, butylene 2 dihydro naphthalene

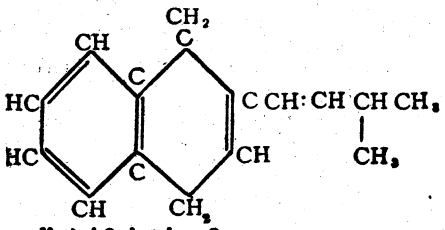


Lapachol

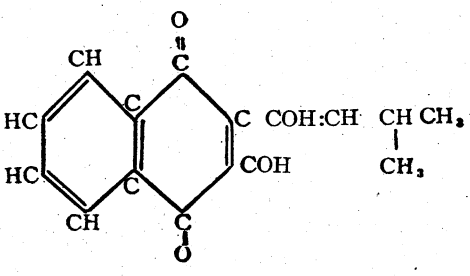
A hydroxy amylene naphthaquinone, lapachol, yellow in color, has been found in the lapacho[3]) wood, obtained from several species of South American *Bignoniaceae,* in the green heart of Surinam[4]) and in Bethabarra wood.[5])

Upon reduction with sodium, lapachol yields an unstable hydrolapachol. The metallic derivatives of lapachol are of various shades of red and orange red.

Pigments referable to methyl 2, butylene 3, dihydro naphthalene.



Methyl 2, butylene 3 dihydro naphthalene



Lomatiol

A yellow compound, lomatiol,[6]) hydroxy lapachol or oxyisolapachol, has been isolated from the seeds of *Lomatia ilicifolia* and *Lomatia longifolia.*

The metallic derivatives of lomatiol are red, orange or brown in color.

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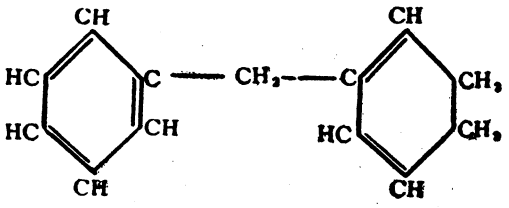
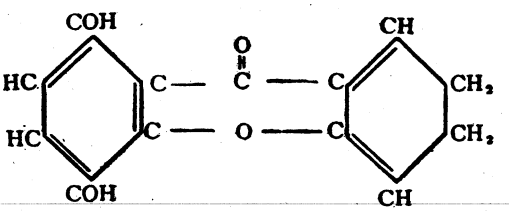
[3] Jahresb. u. d. Fortsch. d. Chem., (1858) p. 264.
[4] Ztsch. f. Chem., (1867) p. 92.
[5] Am. Chem., Jr., 11, p. 267.
[6] Jr. Chem. Soc., 67, 784.

Pigments referable to phenyl-dihydro phenyl methane.



Phenyl-dihydro phenyl-methane



Rhamnocitrin

A group of yellow pigments, some of them supposedly derivatives of the above named hydrocarbon, has been isolated from the berries of *Rhamnus cathartica.*[7]) These pigments are rhamnocitrin, β-rhamnocitrin, rhamnochrisin, rhamnolutin and rhamnonigrin. As will be seen from the formula assigned to rhamnocitrin above, these pigments resemble the xanthone derivatives, falling under the degree of saturation Cn H_{2n-14}, more closely than they do the remaining known pigments of this degree of saturation. They are indeed derivatives of a reduced xanthone nucleus.

Rhamnocitrin occurs, probably, in the free state. It crystallizes in golden yellow needles, and it forms metallic derivatives deeper in color than the compound itself.

β-Rhamnocitrin has the same empirical formula as rhamnocitrin which it closely resembles. To mordanted fabrics it imparts a more enduring color than does rhamnocitrin.

Rhamnochrysin, $C_{13}H_{12}O_7$ crystallizes in orange yellow crystals. It is looked upon as an oxidation product of rhamnocitrin. Whether the molecule is of phenyl-dihydro phenyl

[7] Bull. de Pharm., 4, p. 64; Journ. de Chim., Med., 6, p. 193; Journ. de Pharm., et de Chim., 11, p. 666; Arch. de Pharm., 113, p. 63; Arch. d Pharm., 238, p. 459.

methanone configuration, or contains the "chromone" group does not appear to have been determined. Since it contains two additional hydrogen atoms as well as the additional oxygen the former appears more probable, that is, the elimination of the elements of a molecule of water to form a heterocycle probably has not taken place.

The remaining pigments from *Rhamnus cathartica* plainly do not fall under this formula of saturation, therefore, will not be considered here.

PIGMENTS REFERABLE TO HYDROCARBONS OF THE DEGREE OF SATURATION C_nH_{2n-14}.

All of the pigments of known constitution falling under this degree of saturation fall into two closely related classes.

I. Derivatives of diphenyl and its homologues.

II. Derivatives of diphenyl methane series and their homologues.

Not only are the pigments referable to these closely related hydrocarbons but they are all hydroxy or methoxy—derivatives of these hydrocarbons. They occur in the plant either in the free state or as glucosides, and they resemble each other as closely in properties as they do in general structure.

I. Pigments referable to the diphenyl series and homologues.
   1. Pigments referable to ditolyl.
      a. Ellagic acid.

II. Pigments referable to diphenyl methane series and their homologues.
   A. Diphenylmethane series.
      1. Pigments referable to diphenyl methane.
         Cotoin.
         Euxanthone.
         Maclurin.
         Kinoin.
         Gentisein.
            Gentisin.
         Datiscetin.
      2. Pigments referable to phenyl-o-ethophenyl-methane.
         Catechin
         Cyanomaclurin.

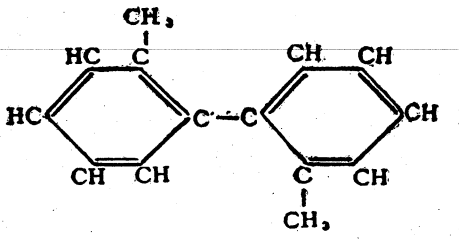
B. Diphenylethane series.
   1. Pigments referable to diphenyl ethane.
      Genistein.

C. Diphenylpropane series.
   1. Pigments referable to diphenyl propane.
      Phloretin.
      Butin.
      Saponarin and Vitexin.

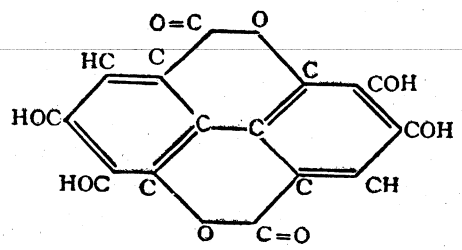
I. Pigments referable to the diphenyl series of hydrocarbons.

The only member of this series to which a plant pigment is known to be referable is a dimethyl homologue, the ditolpl.

Pigments referable to ditholyl.



Ditolyl



Ellagic acid

Ellagic acid occurs quite widely distributed in plants, usually accompanied by tannins. It is found in the leaves of *Juglans regia*[1] along with gallic acid and juglone; in the leaves of *Quercus pedunculata*,[2] *Quercus infectoria*,[3] *Caspinus betulus*,[4] *Haematoxylon campechianum*,[5] *Caesalpina brevifolia*,[5] *Caesalpinia coriaria*,[3] *coriaria myrtifolia*,[5] *Quebrachia lorentzii*,[6] *Tamarax gallica*,[7] *Tamarax africana*,[7] *Donabanga moluccana*,[8] *Punica granatum*,[3] *Terminalia chebula*,[9] *Vaccinium vitis ideaea*.[10]

Ellagic acid forms small yellowish crystals. It dissolves in concentrated sulphuric acid with a citron yellow color. From

[1] C. r., 141, p. 838.
[2] Z. physiol. Chem., 20, p. 511.
[3] Jr. Chem. Soc., 71, p. 1131.
[4] Arch. Pharm., 244, p. 575.
[5] Proc. Chem. Soc., 16, p. 45; Jr. Chem. Soc., 77, p. 426.
[6] Jr. Chem. Soc., 71, p. 1194.
[7] Jr. Chem. Soc., 73, p. 374.
[8] Nederl. Tijdschr. Pharm., 1887, p. 68.
[9] Ber., 42, p. 353.
[10] Chem. News., 52, p. 78; Pharm. Jr., 16, p. 92.

this solution it is precipitated unchanged by water. With potassium hydroxide solution it forms a deep yellow solution which upon exposure to the air goes to a deep reddish yellow. It dyes wools mordanted with chromium a deep olive yellow color.

The formula for ellagic acid given above was suggested by Graebe.[11]. Further work upon the constitution of ellagic acid by Goldschmidt,[12] also by Niernstein,[13] and by Herzig[14] has confirmed Graebe's formula.

II. DERIVATIVES OF THE DIPHENYL METHANE SERIES OF HYDROCARBONS AND THEIR HOMOLOGUES.

By far the greater number of pigments of known constitution falling under the degree of saturation $C_n H_{2n-14}$ are derivatives of the diphenyl methane series and their homologues. Of these diphenyl methane derivatives there are representatives of the diphenyl methane, diphenyl ethane, and diphenyl propane series; but by far the greater number are referable to diphenyl methane.

II. A. 1. *Pigments referable to diphenyl methane.*

All the plant pigments of known constitution referable to diphenyl methane are tri, tetra, penta, or hexa hydroxy substitution products of diphenyl methanone. In some instances it is true methoxy groups are substituted for hydroxy groups, while in others the elements of a molecule of water has been eliminated from the hydroxy groups, thus forming an oxide group. Indeed this latter condensation appears always to have taken place wherever the hydroxy groups are so located that by the elimination of the elements of a molecule of water there can be formed a cycle of six members. Thus some of the pigments of this group are dicyclic while others are tricyclic compounds, the third cycle being heterocyclic in as much as it contains an oxide oxygen. These pigments are all alike in that they form needle like crystals very similar in character, of a pale yellow color, (hence the name xanthone,) and of high melting point.

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[11] Ber., 36, p. 212.
[12] Monatsh. f. Chem., 26, p. 1143.
[13] Ber., 41, p. 1649.
[14] Monatsh. f. Chem., 29, p. 363.

Authorities differ somewhat as to what part or parts of the molecule the coloring properties are due. All seem to agree, however, that it depends largely upon the number of hydroxy groups. A study of the formulae reveals the fact that the property of color appears to depend upon the number of free hydroxy groups, or their oxide equivalent, rather than upon the number originally introduced into the molecule, the changing of the hydroxy groups into methoxy groups appears to diminish both the color of the compound and its dyeing properties. On the other hand the elimination of the elements of a molecule of water from two hydroxy groups to form the xanthone grouping appears to intensify both pigmentation and dyeing quality.

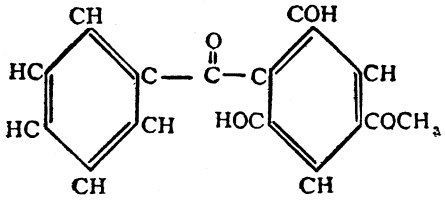
Most writers, in treating of these pigments, distinguish between diphenyl ketone and xanthone derivatives. However, since whether or not a compound falls into the xanthone group depends merely upon the position of hydroxy groups and the consequent elimination of the elements of a molecule of water and the formation of a heterocycle and not upon any more basal constitutional difference, there appears to be no sufficient point to this distinction. Therefore for the sake of simplicity as well as for observing genetic relationships, all of the members of this group, referable to diphenyl methane, will here be regarded as hydroxy derivatives of diphenyl methanone.

II. A. 1. *Pigments referable to diphenyl methane.*

a. Trihydroxides.
   Cotoin.
b. Tetrahydroxides.
   Euxanthone.
      Euxanthonic acid.
c. Penthydroxides.
   Maclurin.
   Kinoin.
d. Hexhydroxides.
   Gentiseine.
      Gentisin.
   Datiscetin.

II. A. 1. a.) *Trihydroxy derivatives of diphenyl methanone.*

Cotoin,—Dihydroxy-1, 5-methoxy-3-diphenyl methanone.



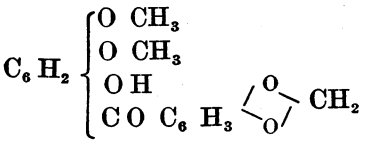
Cotoin is a trihydroxy derivative of diphenyl ketone, referable to a penthydroxide of the underlying hydrocarbon. It occurs in the bark of *coto*[1] and *para coto,* obtained from Brazil. Cotoin[2] forms colorless or only slightly yellow crystals which melt at 130°. With caustic alkalies it forms a yellow solution. In 1894 Ciamician and Silber[3] partially determined the constitution of cotoin. Pollock,[4] in 1901, completed this by determining the position of the hydroxy groups.

In the bark of *para coto* cotoin is accompanied by a series of closely related compounds, hydrocotoin, methyl hydrocotoin, protocotoin and methyl protocotoin. These compounds, all of which closely resemble cotoin, were first studied by Jobst and Hesse[5] in 1879. In 1891–1892 Ciamician and Silber[6] made a further investigation of this series of pigments and determined their relation to cotoin and to each other. This relationship is best illustrated by the following series of partly analyzed formulae:

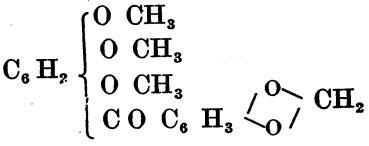
$$C_6H_2\begin{cases}O\ CH_3\\ O\ H\\ O\ H\\ C\ O\ C_6H_5\end{cases} \qquad C_6H_2\begin{cases}O\ CH_3\\ O\ CH_3\\ O\ H\\ C\ O\ C_6H_5\end{cases} \qquad C_6H_2\begin{cases}O\ CH_3\\ O\ CH_3\\ O\ CH_3\\ C\ O\ C_6H_5\end{cases}$$

Cotoin. Hydrocotoin Methyl hydrocotoin

[1] Neues. Rept. f. Pharm., 25, p. 23.
[2] Ann., 199, p. 17.
[3] Ber., 27, p. 1497.
[4] Monats., 18, p. 738; 22, p. 996.
[5] Ann., 199, p. 17.
[6] Ber., 24, p. 299, 2977; 25, p. 1119.



Protocotoin.



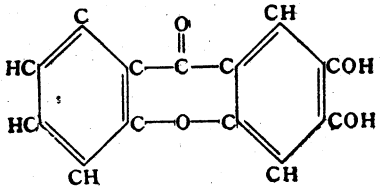
Methyl protocotoin.

It will be seen from the above formulae that hydrocotoin is not, as the name implies, a reduced cotoin; but, rather a methyl cotoin, or monohydroxy-dimethoxy-diphenylmethanone. Methyl hydrocotoin is dimethyl cotoin, or trimethoxy-diphenylmethanone. Protocotoin is the methylene ether of a dihydroxy-methylcotoin and methyl protocotoin is a methylene ether of a dihydroxy-dimethylcotoin.

The three compounds possessing free hydroxy groups form colored metallic derivatives. Further studies of cotoin, hydrocotoin and their derivatives have been made by Henrich,[7] Perkin,[8] and others.[9]

II. A. 1. b.) *Tetrahydroxy derivatives of diphenyl methanone.*

Euxanthone,—Dihydroxy-4, 5-diphenylene methanone oxide, or Dihydroxy—4, 5-xanthone.



Euxanthone exists partly in the free state and partly in combination with glucoronic acid in "*purree,*" or Indian yellow. Indian yellow is prepared from the urine of cattle fed upon

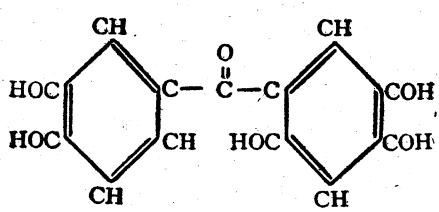
[7] B., 32, p. 3423.
[8] Jr. Chem. Soc., 71, p. 1194.
[9] Moants., 18, p. 142; Ann., 282, p. 191; B., 28, p. 1549; Pharm. Post., 18, p. 179.

mango leaves. Euxanthone was first studied by Stenhouse[1] in 1844 and a little later by Erdman[2] who named both the free euxanthone and the acid compound. In 1889 Graebe[3] synthesized euxanthone and made a study of its structure. The complete structure of the molecule was determined by Kostanecki[4] and Nessler, 1891–1894. As yet euxanthone does not appear to have been isolated directly from the plant in which it may or may not exist.

Euxanthone forms pale yellow needle like crystals which melt at 240°. It forms disodium and dipotassium[5] compounds which are red in color. Its monomethyl ether[6] is pale yellow in color and its dimethyl ether is colorless. Other derivatives of euxanthone have been studied by Perkin.[7]

II. A. 1. c.) *Penthydroxy derivatives of diphenyl methanone.*

Maclurin,—Penthydroxy—2, 4, 6, 3′, 4′—diphenyl Methanone.



Maclurin, also called penthydroxy benzophenone and moringa tannic acid, occurs in *Morus tinctoria*,[1] along with morin. Maclurin has been known for a long time and has called forth a large number of investigations. It was first isolated by Wagner[2] in 1850. Wagner considered the substance to be a tribasic acid isomeric with morin. Hlasiwetz and Pfaundler[3] in 1863 recognized the fact that maclurin is not an acid. Bene-

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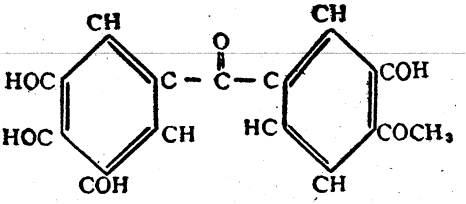
[1] Ann., 51, p. 423.
[2] Jr. Prakt Chem., 33, p. 190.
[3] Ann., 54, p. 265.
[4] Ber., 24, p. 3980; 27, p. 1989.
[5] Ann., 290, p. 156.
[6] Ann., 318, p. 365.
[7] Jr. Chem. Soc., 73, p. 671.
[1] Czapek, II., p. 521.
[2] Jr. Prakt. Chem., 51, p. 82; 52, p. 449.
[3] Ann., 127, p. 354; 134, p. 122.

dict[4] in 1877 confirmed the work of Hlasiwetz and Pfaundler, Ciamician and Silber[5] in 1894 attacked the problem of its constitution and partially elucidated its structure. Koenig and Kostanecki[6] in the same year completed this task. Other studies of maclurin have been made by Delffs[7] in 1862; Liebig[8] in 1860; Bedford and Perkin[9] in 1895; and by Perkin in 1897.

Maclurin forms fine pale yellow crystals which melt at 200°. It dissolves in caustic alkalies forming a yellow solution which turns brown upon exposure to the air. Lead acetate gives a yellow precipitate.

Kinoin,—Tetrahydroxy — 2, 3, 4 2′ — methoxy-3′ — diphenyl methanone.



Kinoin[1] occurs in the dried juice of *Pterocarpus erinaceus, Pterocarpus marsupium,* and *Coccoloba uvifera,* also in several species of *Eucalyptus,* and in *Butea frondosa.*

In 1879, Etti[2] isolated from green kino a substance which he called kinoin and to which he assigned the formula $C_{14}H_{12}O_6$. This substance contains a hydroxy group and upon hydrolysis yields pyrocatechin and gallic acid. Thomas[3] in his book on the natural dyestuffs has suggested for kinoin the structural formula given above.

A considerable number of investigations of the various species of kino have been made. The results of most of these investigations do not agree with those of Etti, phloroglucin, pyrocatechin,

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[4] Ann., 185, p. 117.
[5] Ber., 27, p. 1627; 28, p. 1393.
[6] Ber., 27, p. 1996.
[7] Chem. Centrlbl., 1862, p. 284.
[8] Jahresber. d. Chem., 1860, p. 278.
[9] Jr. Chem. Soc., 67, p. 933.
[10] Jr. Chem. Soc., 71, p. 186.
[1] Pharm. Jr., 16, p. 676; Pharm. Ztg., 58, p. 593.
[2] Ber., 11, p. 1876; 17, II, p. 2241.
[3] Les Matieres Colorantes Naturelles, p. 22.

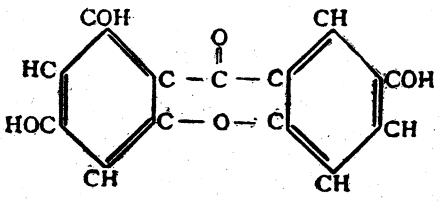
and protocatechuic acid being obtained as the products of hydrolysis.

The principal literature upon kinoin is given in the appended list.

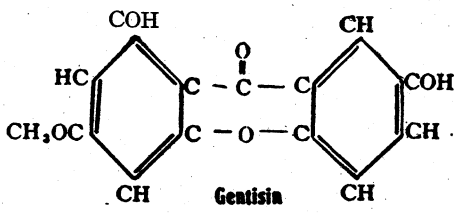
Literature upon kinoin.

Bergholz, Innaug. Dissert. Dorpat.; B., 5, p. 1.
Eissfeldt,—Ann., 134, p. 122.
Etti,—B., 11, p. 1879; 17, p. 2241.
Flueckiger,—B., 17, p. 2241.
Hlasiwetz,—Ann., 134, p. 122.
Kremler,—Pharm. Post., 16, p. 117.
White,—Pharm. Jr., 16, p. 676; 17, p. 702.

Gentisein,—Trihydroxy—1, 3, 2'—diphenyl methanone oxide, or trihylroxy—, 1, 3, 2' —xanthone.



Gentisein



Gentisin

Gentisein occurs as its methyl ether gentisin in the rhizome of *Gentiana*[1] *lutea*, also in the rhizome of *Frasera Walteri*. Gentisin was first isolated from the rhizome of *Gentiana lutea* in 1827 by Henry and Caventon. It was studied by Tromsdorff[2] in 1837 and by La Conte[3] in 1838. La Conte in his study points out the fact that the gentian plant from which the pigment is obtained, derives its name, according to Pliny, from the Illyrian king Gentis, or Gentius, who appears to have valued the root very highly as a remedy for certain illnesses epidemic in his time. In 1847 Baumert[4] made an extended study of gentisin

[1] Jr. de Pharm., (2) 7, p. 125.
[2] Am. Jr. Pharm., 52, 7.
[3] Jr. Pharm., (2) 7, p. 178.
[4] Ann., 21, p. 134.

and determined its empirical formula. Hlasiwetz[5] and Habermann, in 1874, took up the study of the constitution of gentisin. In 1876[6] they found that it contains a methoxy group and obtained gentisein by hydrolysis. In 1891 Kostanecki[7] turned his attention to the constitution of gentisin and in 1894[8] succeeded in synthesizing gentisein from phloroglucin and hydroquinone carboxylic acid. From this product he readily obtained gentisin by treatment with methyl iodide. The remaining question of the position of the methoxy group was answered by Perkin[9] in 1898.

Gentisein crystallizes in pale yellow crystals which melt at 315°. It is soluble in alkalies with a bright yellow color.

Gentisin forms fine needle like crystals of a pale yellow color. It forms well defined crystalline salts of sodium and potassium, $C_{14}H_9NaO_5$ and $C_{14}H_9KO_5$.

II. A. 1. d.) *Hexhydroxy derivatives of diphenyl methanone.*

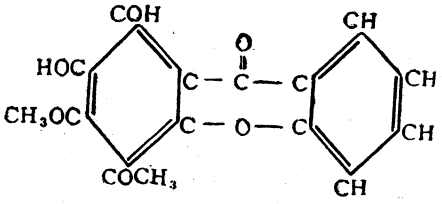
Datiscetin.

Datiscetin has been known for a long time in southern France where it has been used as a coloring agent for silk. It was first studied by Braconnet[1] in 1816. Later Stenhouse[2] showed that the substance to which the coloring properties are due is a glucoside which may be hydrolized into datiscetin and rhamnose. In 1893 and 1894 Marchlewski[3] and Schunk undertook the determination of the constitution of datiscetin and found thot it belongs to the xanthone group of pigments, assigning to it the generally accepted formula $C_{15}H_{12}O_6$ with two hydroxy and two methoxy groups, as shown below. Upon treatment with hydriodic acid datiscetin yields a tetra hydroxy xanthone of a yellow color.

More recently, 1907, Marchlewski[4] claims that datiscetin is of the formula $C_{15}H_{10}O_6$, that it melts at 268°–269° instead of

[5] Ann., 25, p. 200.
[6] Ann., 62, p. 106.
[7] Monats., 12, p. 205; 12, p. 318.
[8] Monats., 15, p. 1; 16, p. 919.
[9] Jr. Chem. Soc., 73, p. 673.
[1] Ann. Chim. et Phys., (2) 3, p. 277.
[2] Ann., 98, p. 167.
[3] Ann., 277, p. 261; 278, p. 346.
[4] Biochem. Zeit., 3, p. 287; Chem. Centralbl., 1906, II, p. 1265.

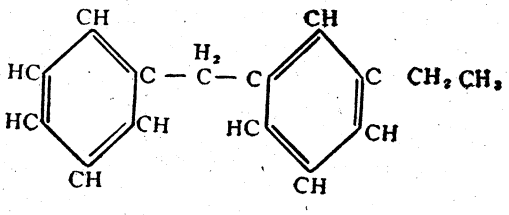
at 237° as generally given, and that it contains no methoxy groups. Also that it does not reduce Fehling's solution but readily reduces an ammoniacal silver solution, and that it forms tetra acetyl, tetra benzoyl, and tetra benzene sulphonal derivatives. Furthermore that when the glucoside datiscin is hydrolized, dextrose, not rhamnose is formed. Also that it is an isomer of luteolin and probably a flavone derivative.



Datiscetin

Datiscetin occurs as the glucoside datiscin in *Datisca cannabina.* It crystallizes in clear yellow needle like crystals which melt at 237°.

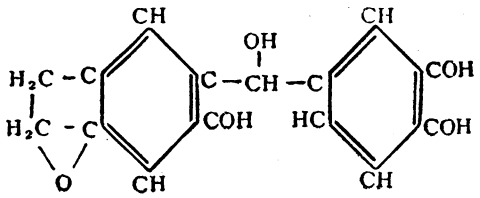
II. A. 2.) *Pigments referable to phenylethophenyl methane.*



Phenyl etho phenyl methane

One pigment of known constitution, catechin, is referable to the above hydrocarbon. Another, cyano maclurin, whose constitution is not yet fully determined is presumably derived from the same hydrocarbon. Cyano maclurin is accordingly placed with catechin in this classification.

Catechin.



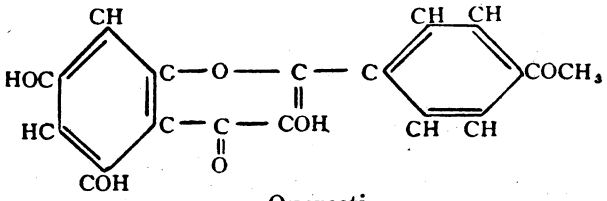
Catechu, also called catechinic acid and catechu tannic acid, was known by Runge[1] to exist in the heart wood of *Acacia catechu* as early as 1821. It has long been known as a dyestuff imparting yellow and brown tints to textile fabrics. The coloring principle, catechin, was probably first described by Nees van Esenbeck[2] in 1832. It has since been the subject of many chemical investigations, the results of which were for a long time so various that the chemistry of catechin remained in a very unsatisfactory condition. The majority of investigators of this pigment have considered but one catechin to exist, some, however, claim that three different catechins with different melting points, but with other properties similar, have been isolated. Perkin,[3] in 1902, described two catechins, with melting points of 175°–176°, and 235°–237° respectively, isolated from *Gambir catechu,* and another with a melting point of 204°–205°, from *Acacia catechu.*

Many different chemical formulae have been assigned to catechin by different chemists. The latest work by Perkin[4] upon this pigment, as well as the even more recent work of Kostanecki[5] and his collaborators, indicates $C_{15}H_{14}O_6$ with five hydroxy groups as the correct formula for the anhydrous compound. Perkin[6] calls attention to the great similarity of the catechins to quercetin, which accompanies them in the plant, probably as a glucoside. He points out the possibility of their being reduction products of quercetin. The later work of Kostanecki and Lampe, however, indicates the presence of a cumaran group, in which the six carbon ring contains only one

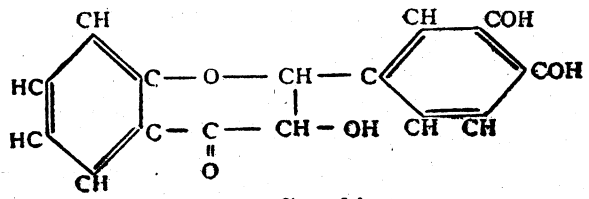
[1] Berz. Jahresber., 12, p. 250.
[2] Ann., 1, p. 243.
[3] Jr. Chem. Soc., 81, p. 1160.
[4] Proc. Chem. Soc., 20, p. 177.
[5] Ber., 39, p. 4007, 4014.
[6] Jr. Chem. Soc., 81, p. 1160.

unsubstituted hydrogen instead of the chroman group implied by Perkin's idea. This, they state would make catechin the cumaran derivative of leuco maclurin.

Perkin's suggestion.

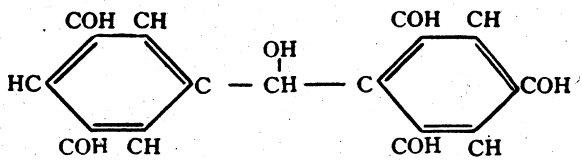


Quercetin

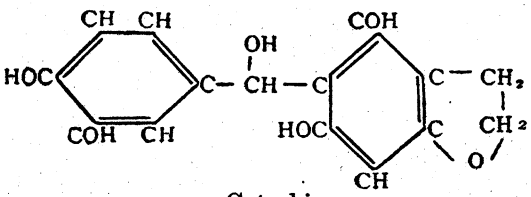


Catechin

Kostanecki's formula.



Leuco maclurin



Catechin

The more important contributions to the chemistry of catechin are included in the following list:

Catechin occurs in the heart wood of *Acacia catechu,* in the "Gambir" from *Ouruparia gambir,*[7] and in *Uncaria gambir,*[8]

[7] Czapek, II. p. 573.
[8] Jr. Chem. Soc., 81, p. 1160.

also in mahogany wood, and according to Gautier,[9] in various species of cahous, where he thinks there are several varieties of catechin of different melting points and characterized by a varying carbon content.

In a pure state catechin is composed of fine needle like colorless crystals, which by oxidation form dyes imparting yellow shades to textile fabrics. It is sparingly soluble in cold alcohol, readily soluble in hot alcohol. Air dried it dissolves in ethyl acetate, also to some extent in pure ether. Dried at 100° it is insoluble in both these solvents. In aqueous solutions lead acetate gives with catechin a colorless precipitate, ferric salts give a green color. In the presence of sodium acetate ferric chloride gives with catechin a deep violet coloration.

The more important of the contributions to the chemistry of catechin are included in the following list:

Literature on Catechin.

Clauser, — B., 36, p. 101.
Dellfs, — Pharm. Centrl., (1846), 604; Berz. Jahresb., 27, p. 284.
Doebereiner, — N. Jahresb. d. Chem. u. Pharm., (1831), p. 378; Berz. Jahresb., 12, p. 250; Schweigg. Jr., 61, p. 378.
Etti, — Monatsh., 2, p. 547; Wien, akad., 84, p. 553; A., 186, p. 327.
Gautier, — C. r., 85, p. 342; 86, p. 668.
Hagen, — Ann., 37, p. 320.
Hlasiwetz, — Ann., 134, p. 118.
Kostanecki and Lampe, — B., 39, p. 4007, 4014, 4022; 40, p. 720.
Kraut and Delden, — Ann., 128, p. 285.
Liebermann and Tauchert, — B., 13, p. 964.
Loewe, — Zeit. anal. Chem., 13, p. 113.
Ness van Esenbeck, — Ann., 1, p. 243.
Neubauer, — Ann., 96, p. 337.
Perkin, — Jr. Chem. Soc., 81, p. 1160; Proc. Chem. Soc., 20, p. 177.
Schuetzenberger and Bach, — Bull. Soc. Chem., 4, p. 51.
Swanberg, — Ann., 24, p. 215.
Wackenroder, — Ann., 37, p. 306.
Zwenger, — Ann., 37, p. 320.

[9] C. r., 85, p. 342; 86, p. 668.

Cyanomaclurin

Cyanomaclurin was isolated by Perkin and Cope,[1] in 1895, from *Artocarpus integrifolia,* where it exists along with the yellow pigment morin. Cyanomaclurin crystallizes in colorless crystals which dissolve in sulphuric acid with a beautiful crimson color. Ferric chloride colors an aqueous solution of cyanomaclurin a deep violet color. Dilute alkaline solutions, dissolve it with a deep indigo blue color which on standing changes to green, then brown. It does not combine with mordants to form a dye.

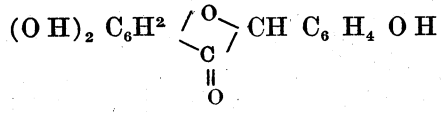
Cyanomaclurin[2] is isomeric with catechin. It is probably a catechin in which the catechol nucleus is replaced by resorcinol.

II. B. *Pigments referable to diphenyl ethane.*

Of the pigments of known constitution only one, Genistein, is believed to be referable to diphenyl ethane.

Genistein.

While the constitution of genistein has not yet been fully determined it is believed by Perkin and Newbury[1] to be represented by the formula



Genistein has been isolated, along with luteolin, from the leaves of *Genista tinctoria.*[2] It crystallizes in colorless needles. To fabrics mordanted with aluminum salts genistein imparts a yellow color.

II. C.) *Pigments referable to diphenyl propane.*

Three pigments, or pigment forming substances, of known constitution are referable to diphenyl propane. These are phloretin, butin, and saponarin or vitexin.

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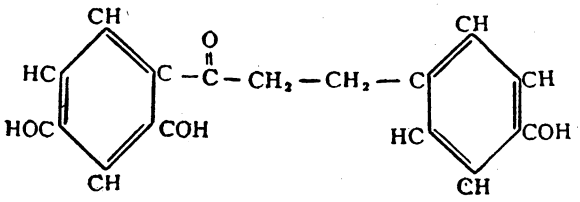
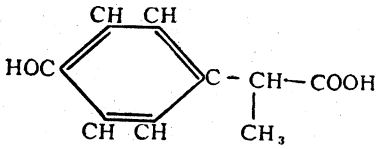
[1] Jr. Chem. Soc., 67, p. 939.
[1] Proc. Chem. Soc., 15, p. 179.
[2] Proc. Chem. Soc., 18, p. 138; 20, p. 170.
[2] Jr. Chem. Soc., 75, p. 832; 77, p. 1310.

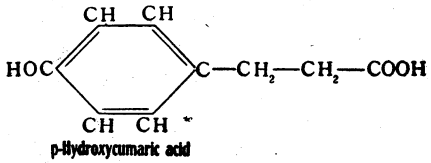
Phloretin.



Phloretin occurs as the glucoside phloridzin in several species of *Rosaceae,* especially in the leaf buds and the bark of *Pirus malus,*[1] the apple tree. Phloridzin was discovered in 1835 by De Koninck[2] and Stas in the bark of the apple tree. Later Stas,[3] 1839, made an extended study of phloridzin and recognized its glucosidal character, isolating glucose and phloretin. Further studies of phloretin and of phloridzin were made by Rennie,[4] Schiff,[5] Hesse,[6] and Fischer,[7] also by Schunck and Marchlewski.[8] In 1894 Michael[9] found that phloretin upon hydrolysis yields phloroglucin and phloritinic acid. The latter was at the time believed to be p-hydroxy-hydrotropic acid.



Later Bougult[10] found phloretinic acid to be identical with p-hydrocumaric acid.

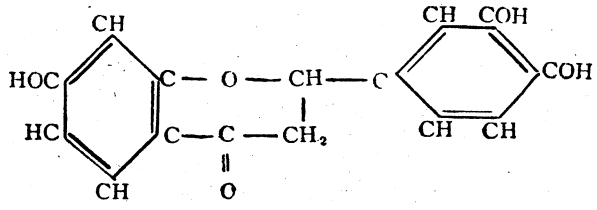


[1] Jr. Prakt. Chem., 98, p. 205; C. r, 139, p. 294.
[2] Ann., 15, p. 75, 258.
[3] Ann., 30, p. 200.
[4] Jr. Chem. Soc., 49, p. 860; 51, p. 636.
[5] Ann., 172, p. 357; 229; p. 374; B., 2, p. 743; 14, p. 303.
[6] Ann., 176, p. 288.
[7] Ber., 21, p. 288.
[8] Ber., 26, p. 942.
[9] Ber., 27, p. 2686.

Phloretin crystallizes in small colorless plates. It melts at 253° — 255°. Phloretin forms a tetra-methyl[11] and a tetra-acetyl[12] derivative,[12] therefore must contain four hydroxy groups.

Phloridzin crystallizes in fine silky needles, white or faintly yellow in color. It melts at 108° — 109°. With metals phloridzin forms colored compounds. Conspicuous among these are the dark red iron salt and the bright yellow calcium compound.

Butin,—Trihydroxy—3, 3', 4'—dihydro—α, β—flavone.



Butin, a penthydroxy derivative of diphenyl propanone, was first isolated from the flowers of *Butea frondosa* by Hummel and Cavallo[1] in 1894, and later, by Hill[2], in 1903. In 1904 a somewhat extended study of the pigment was made by Perkin[3] who showed that the substance isolated by Hummel and Cavallo and called by them butin was not a single compound but a mixture of two substances, one of which was colorless while the other was orange red in color. The colored substance Perkin named Butein, while to the colorless substance he assigned the original name of butin. Perkin showed further that while butin is a trihydroxy dihydro flavanone, butein is a tetrahydroxy derivative of diphenyl propene, benzyliden acetophenone (chalkon). This he proved by the synthesis of butein from monomethyl resacetophenone and dimethyl protocatechinic aldehyde according to the processes of Kostanecki[4] and his colleagues in their synthesis of the chalkon derivatives. After the synthesis of butein, butin was prepared from it by treatment with dilute sulphuric

[10] C. r., 131, p. 43.
[11] Ber., 28, p. 1396.
[12] Ber. 27, p. 2686.
[1] Proc. Chem. Soc., 10, p. 11.
[2] Proc. Chem. Soc., 19, p. 133.
[3] Jr. Chem. Soc., 85, p. 1459.
[4] Ber. 37, p. 773, 779, 784.

acid when the compound was, presumably, first hydrated and then dehydrated resulting in a rearrangement of the molecule.

Butin occurs with butein as a glucoside in the blossoms of *Butea frondosa,* a leguminous plant of India and Burma. It crystallizes in small colorless needles melting at 224°–226°. It is soluble in alcohol, sparingly soluble in acetic acid and ether, almost insoluble in benzene. With alcoholic lead acetate solution butin forms a pale yellow precipitate, with alcoholic ferric chloride a deep green coloration. With cold sulphuric acid it first turns red, then goes into solution with a pale yellow color. On fusion with caustic potash and a little water at 200°–220°, butin yileds protocatechuic acid and resorcinol.

Though butin is not itself a pigment it dyes mordanted fabrics exactly as buetin does. From this behavior it is believed that it is changed by the mordants into butein. When boiled with a solution of potassium hydroxide and then acidified a bright orange crystalline precipitate of butein immediately separates out.

Saponarin.

Certain plants contain in the cell sap of the epidermal cells of the leaves a substance which turns blue[1] with iodine. As in the case of starch this color disappears upon heating and returns again upon cooling. For this reason this substance is often mistaken for starch, as it was by its discoverer Sanio[2] in 1857. Schenk,[3] in the same year, doubted the identity of this substance with starch, and Naegeli[4] in 1860 showed that the two are not identical. Dufour,[5] in 1885, found this substance in about twenty species of plants.

In 1906 Barger[6] separated the above described substance from *Saponaria officinalis* and called it saponarin. He showed that the substance is a glucoside which upon hydrolysis yields glucose and another substance $C_{15}H_{14}O_7$, identical with vitexin from *Vitex littoralis.*

The substance which turns iodine blue, formerly known as soluble starch, has been found in *Gagea lutea,*[2] in *Ornithogalum*

[1] Rupe,—Der Natuerlichen Farbstoffe, 2, p. 42.
[2] Bot. Zeit., 15, p. 420.
[3] Bot. Zeit., 15, p. 497, 555.
[4] Zeit. zur. Wissensch. Bot., 2, p. 187.
[5] Bull. Soc. Sci. Nat., 21, p. 227.
[6] Jr. Chem. Soc., 89, p. 1210.

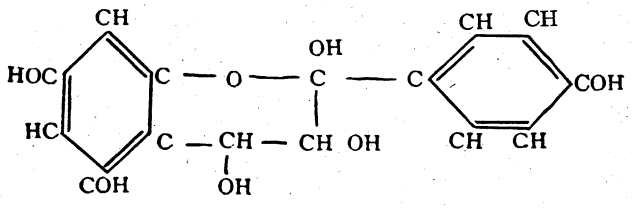
leaves,[7] in *Saponara officinalis,*[8] and in about twenty other species of phanerogams.[5] The identity of the peculiar substance from all these plants with saponarin has not been fully established.

Saponarin forms crystals which, dried in the air, are white, dried in a vacuum they are pale yellow. It is insoluble in cold water, soluble in solutions of caustic alkalies or alkaline carbonates with an intense yellow color. In mineral acids it gives a yellow solution. The solution in sulphuric acid has a blue fluorescence. Upon dilution the saponarin is not precipitated at once. This acid solution, upon the addition of iodine in potassium iodide solution, is colored blue or violet. The glucoside combines with nine acetyl radicals to form a nonacetyl derivative of saponarin.

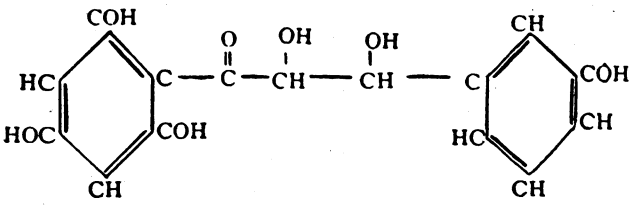
Vitexin.

Vitexin, $C_{15}H_{14}O_7$, occurs as a glucoside in the New Zealand dye wood *Puriri, Vitex litoralis,*[1] and as the glucoside saponarin in *saponara officinalis.*[2]

Vitexin crystallizes in microscopic, glistening plates of a pale yellow color. It melts at 260° with characteristic frothing. It is insoluble in water, slightly soluble in alcohol, soluble in pyridine and in solutions of alkalies with a golden yellow color. Vitexin forms a pentacetyl derivative. Upon treatment with nitric acid it forms a tetranitro apiginin. By decomposition with caustic alkalies it forms phloroglucin and p-hydroxy acetophenone. It is therefore closely related to apiginin from which it differs by the elements of two molecules of water. Since it forms phloroglucin and p-hydroxy benzoic acid the additional hydroxy groups are probably in the pyron cycle, or in a chain which would give rise to this cycle.

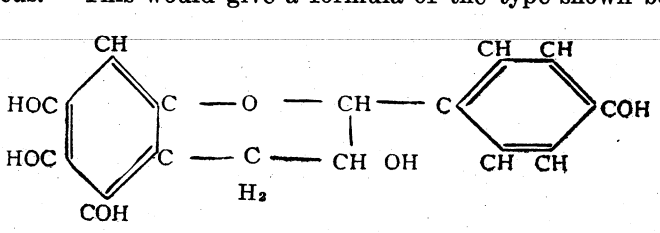


[7] Bull. Soc. Bot. de France., 5, p. 711.
[1] Jr. Chem. Soc., 73, p. 1019; 77, p. 422.
[2] Jr. Chem. Soc., 89, p. 1210..



Both the above formulas have, however, six hydroxy groups, whereas vitexin forms only a pentacetyl and saponarin only a nonacetyl derivative. Dehydration might, however, take place in the molecule during the process of acetylation.

To account for the formation of only the pentacetyl vitexin Perkin has suggested the presence of a reduced phloroglucinol nucleus. This would give a formula of the type shown below.



PIGMENTS REFERABLE TO HYDROCARBONS OF THE FORMULA OF SATURATION CH_{2n-16}.

Of all the pigments of known constitution occurring in plants by far the greater number are referable to hydrocarbons of the formula of saturation C_nH_{2n-16}. These pigments when referred to their underlying hydrocarbons fall into three classes.

1.) The diphenyl olefin derivatives.
2.) The dihydroanthracine derivatives.
3.) The methyl-phenyl hydrindine derivatives and their oxidation products.

In the first class are found the flavone derivatives, referable to diphenyl propene, and a large number of compounds referable to similar hydrocarbons. This group also includes the so-called anthocyanine pigments, so far as their structure has been determined.

The second class is made up of the anthraquinone and methyl anthraquinone derivatives, a group which includes, outside of the flavone derivatives, of the above class, the greater number of coloring matters, so far studied, falling under this degree of saturation.

The third class is a small one made up of the pigment forming substances brazilin and haematoxylin and the pigments brazilein and haematein.

I. PIGMENTS REFERABLE TO DIPHENYL OLEFINS AND HOMOLOGUES.

It was pointed out in connection with the pigments referable to hydrocarbons of the formula of saturation C_nH_{2n-14} that a conspicuously large number of these colored compounds were derivatives of the diphenyl and diphenyl methane series of hydrocarbons. The same relationship is found to exist among the pigments referable to hydrocarbons of the degree of saturation C_nH_{2n-16}, for here we find a considerable number of compounds referable to the diphenyl olefin series of hydrocarbons, having as their initial members diphenyl ethene, diphenyl propene, and diphenyl butene.

I. Pigments referable to the diphenyl olefin series of hydrocarbons.

A. Diphenyl ethene.

1.) Tolyl-ethophenyl-ethene.

Berberin.

B. Diphenyl 1, 3, propene.

1.) Flavone derivatives.

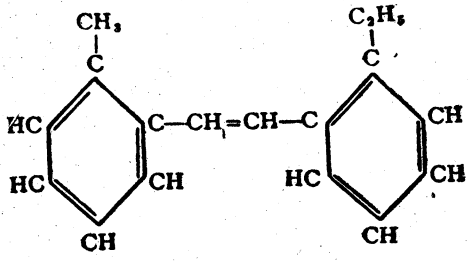
Chrysin
Tectochrysin
Apiginin
Acacetin
Galangin
Galangin meyhyl ether
Luteolin
Luteolin methyl ether
Lotoflavin
Fisetin
Kaempherol
Kaempherid
Quercetin
Rhamnetin
Isorhamnetin
Rhamnazin
Morin
Myricetin
Gossypetin

2.) Butein
3.) Anthocyanins.
Pelargonidin
Cyanidin
Paeonidin
Delphinidin
Myrtillidin
Malvidin
Oenidin

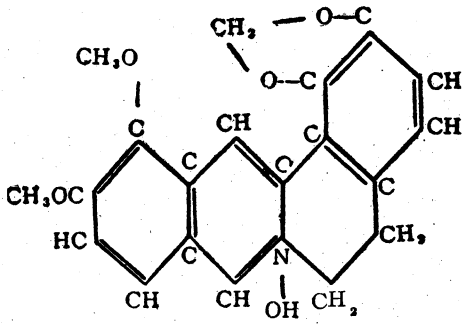
C. Diphenyl 1, 4, butene 2.
Indigotin.

I. A. *Pigments referable to the diphenyl ethene series of hydrocarbons.*

1.) The only members of this series to which a plant pigment is referable is a methyl ethyl homologue, the tolyl-etho phenyl-ethene.



Tolyl-etho phenyl-ethene



Berberin

Berberin, which is a basic plant pigment, an alkaloid, may be regarded as the product obtained by the deammoniation of a dimethyl, methylene either of a tetra hydroxy, triamido substitution product of the above hydrocarbon. The accepted for-formula[1] for berberin is based upon the investigations of the products which result from the *abbau* of the molecule by oxidation with potassium permangenate.

Berberin was isolated by Buchner[2] before 1837 from the root of *Berberis vulgaris* and described by him. Since then it has been the subject of a large number of investigations and has

[1] Jr. Chem. Soc., 55, p. 63; 57, p. 992; 97, p. 318.
[2] Ann., 24 p. 228.

been found to be widely distributed in nature. In addition to several species of barberry it is known to exist in *Hydrastis canadensis,*[3] *Coptis trifola,*[4] *Zanthorrhiza apiifolia,*[5] *Delphinium saniculaefolium,*[6] *Thalictrum flavum,*[7] *Adonis vernalis,*[7] *Mahonia aequifolium,*[8] *Jatrohiza palmata,*[9] *Tintospora rumphii,*[10] *Zylopia polycarpa,*[11] *Chelidonium majus,*[12] *Argemone mexicana,*[13] *Corydalis tuberosa,*[14] *Corydalis vernyi,*[15] *Andria inermis,*[16] *Xanthoxylum caribaeum,*[17] *Xanthoxylum perrottetii,*[18] *Xanthoxylum piperitum,*[19] *Evodia meliifolia,*[20] *Orixa japonica.*[21] Yellow pigments resembling berberin and believed to be identical with it have also been isolated from several other plants.

Berberin crystallizes from water in yellow crystals with six molecules of water of crystallization, from chloroform with one molecule of chloroform of crystallization. It is easily soluble in hot water, difficultly soluble in cold water or chloroform, and almost insoluble in ether, benzene, ligroin, and acetic acid.

A large number of derivatives of berberin have been prepared. It forms salts with acids similar to ammonium salts, also gold and platinum double salts.

Berberin is used for dyeing leather, especially for gloves, also silk and wool.

The following list includes the more important of the many chemical investigations of berberin:

Ahrens,—B., 29, p. 2996.

Bernheimer,—Gazz. Chim. Ital., 13, p. 345.

Buchner and Herbeiger,—Ann., 24, p. 288.

Chevalier and Pelletan,—Jr. Chem. Med., 2, p. 314.

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[3] Pharm. Z. F. Russl., 33, p. 770; Pharm. Jr. Trans., 3, p. 546.
[4] Arch. Pharm., 222, p. 747.
[5] Pharm. Jr. Trans., 3, p. 546 and 567.
[6] New Commerc. Drug. 1887; Draendorff, Heilpflanzen, p. 227.
[7] Monat. Scient., 5, p. 483.
[8] Pharm. Centralh., 1882, nr. 28.
[9] Arch. Pharm., 240, p. 146, 450.
[10] Bull. Inst. Botan. Buitenzorg., 1902, 14, p. 11.
[11] Ann., 105, p. 360.
[12] Am. Jr. Ph., 1902; Botan. Centralbl., 45, p. 187.
[13] Jr. Am. Chem. Soc., 24, p. 238.
[14] Beitr. z. Kennt. d. Corydalis cava., Disser. Dorpat., 1890.
[15] Arch. Pharm., 246, p. 461.
[16] B. Neues. Repert. Pharm., 14, p. 211.
[17] Jr. Chem. Soc., 15, p. 339.
[18] C. r., 98, p. 999.
[19] Dragendorff, Heilpflanzen, p. 350.
[20] Chem. News., 71, p. 207; Arch. Pharm., 213, p. 337.
[21] Nederl. Tijdschr. Pharm., 1884, p. 228.

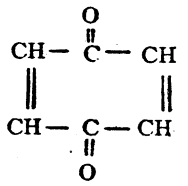
Dobbie and Lauder,—Proc. Chem. Soc., 17, p. 255.
Fleitmann,—Ann., 59, p. 160.
Freund,—Ann., 397, p. 1.
Freund and Beck,—B., 37, p. 4673.
Freund and Meyer,—B., 40, p. 2604.
Gadamer,—Chem. Ztg., 26, p. 291, 385; Arch. Pharm., 239, p. 648; 243, p. 12, 31, 43, 89, 246.
Gaze,—Beilstein, Handb. organ. Chem., 3, p. 800.
Gordin,—Arch. Pharm., 39, p. 638; 240, p. 146.
Hlasiwetz and Gilm,—Ann., 115, p. 45; 122, p. 256; Suppl. II. p. 133.
Henry,—Ann., 115, p. 133.
Link,—Arch. Pharm., 230, p. 734.
Mosse and Tauz,—Chem. Centralbl., 1901, II. p. 786.
Perkin, A. G.,—Jr. Chem. Soc., 67, p. 413; 71, p. 1189.
Perkin, H. W.,—Jr. Chem. Soc., 55, p. 78; 57, p. 1037.
Perkin, W. H. and Robinson,—Jr. Chem. Soc., 97, p. 305.
Perrins,—Ann., Suppl. II. p. 176.
Schlotterbeck,—Jr. Am. Chem. Soc., 24, p. 238.
Schmidt,—Handb. Organ. Chem., 3, Aufl. 3, p. 798.
Troege and Linde,—Arch. Pharm., 238, p. 6.
Weidel,—B., 12, p. 410.

I. B. *Pigments referable to diphenyl—1, 3—propene series of hydrocarbons.*

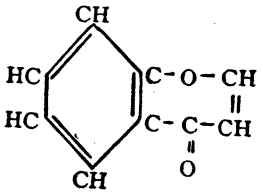
Just as the larger number of natural coloring matters of the degree of saturation $C_n H_{2n-14}$, may be referred to diphenyl methane, so by far the greater number of those of this degree of saturation, the flavone derivatives, may be referred to a similar hydrocarbon, diphenyl propene. Indeed the relationship of the flavone group to the xanthone group is much closer than would be inferred by simply referring the individual compounds to their underlying hydrocarbons. By comparing the structural formula of xanthone with that of flavone it will be seen that both compounds contain the γ-pyrone group: more than this, they contain the benzo γ-pyrone group, designated by Bloch and Kostanecki[1]. the chromone group. In the xanthone derivatives the chromone group is condensed with another benzene nucleus forming dibenzo γ-pyrone, while in the flavone derivatives it is united with a phenyl group forming phenyl-benzo-γ-pyrone. With this similarity in structure it is by

[1] B., 33, 471.

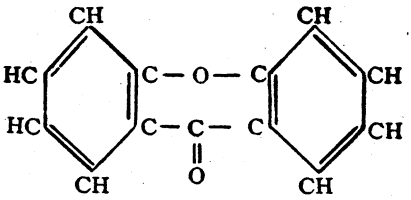
no means remarkable that flavone and xanthone derivatives so closely resemble each other in coloring properties.



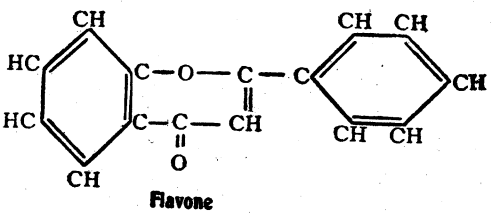
γ-pyrone



Chromone



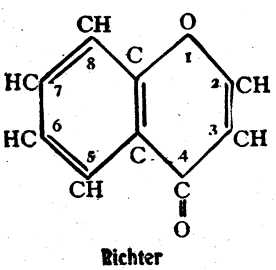
Xanthone



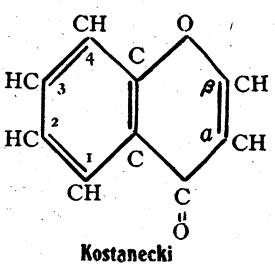
Flavone

There is some confusion as to the numbering of the carbon atoms in the benzo-γ-pyron group, called by Kostanecki the chromon group, and consequently in the numbering of those of the flavon and xanthon groups. According to Richter's *Lexikon der Kohlenstoff-Verbindungen* the oxide oxygen in the chromon group occupies position 1, and the carbon atoms are numbered from this. Kostanecki, however, assigns to the carbon atom adjacent to the carbonyl group the α position and the one adjacent to the oxide oxygen the β position, while the carbon atoms of the carbocyclic nucleus he numbers 1, 2, 3, and 4 respectively. This gives us two systems of numbering as indicated by the following structural formulae:

Chromon group numbered according to



Richter

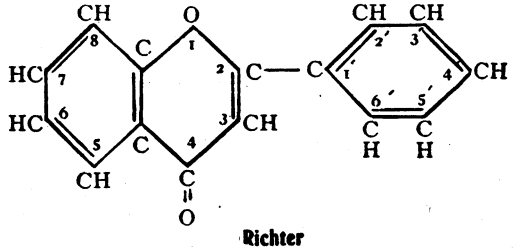


Kostanecki

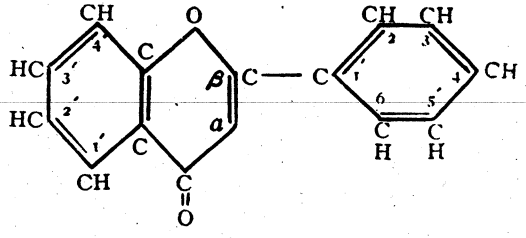
Whichever of these methods of numbering is followed, the positions of the carbon atoms of the phenyl group in the flavone

molecules are 1′, 2′, 3′, 4′, 5′, 6′, beginning with the carbon atom attached to the benzopyron group.

Flavon group numbered according to



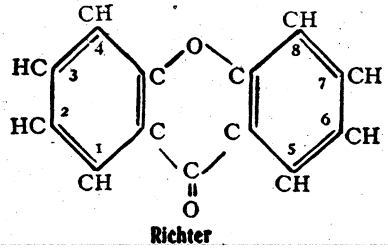
Richter



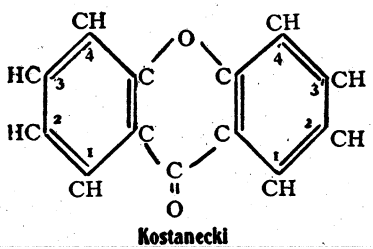
Kostanecki

Though the chromon grouping occurs in the xanthon molecule, as has been shown above, this fact does not seem to be recognized in the outline of cyclic systems as given in Richter's *Kohlenstoff-Verbindugen,* for here an entirely different seheme of numbering is employed for the xanthon grouping.

Xanthon group numbered according to



Richter



Kostanecki

While it might appear more rational than either of these systems to begin with some differentiated carbon atom and number each succeeding carbon atom in connection with which substitution can take place 1, 2, 3, ____8, 9, 10, etc., without resorting to other symbols yet Kostanecki's method undoubtedly

has its advantages since there are three different nuclei in which substitution may take place.

In naming the derivatives of these compound nuclei a distinction is sometimes made between hydroxy derivatives of the carbocyclic nuclei and the heterocyclic nucleus. The former are always regarded as flavones viz. hydroxy flavones, the latter, sometimes as flavonols.

The Pigments referable to diphenyl—1, 3—propene may be classified as follows:

1. The flavone derivatives (in the broader sense.)
   - a.) Dihydroxides.
     - Chrysin.
       - Tectochrysin.
   - b.) Trihydroxides.
     - Apiginin.
       - Acacetin.
     - Galangin.
       - Galangin methyl ether.
   - c.) Tetrahydroxides.
     - Luteolin.
       - Luteolin methyl ether.
     - Lotoflavin.
     - Fisetin.
     - Kaempherol.
       - Kaempherid.
   - d.) Penthydroxides.
     - Quercetin.
       - Rhamnetin.
       - Isorhamnetin.
       - Rhamnazin.
     - Morin.
   - e.) Hexhydroxides.
     - Myricetin.
     - Gossypetin.
2. Butein.
3. Anthocyanin pigments.
   - a.) Tetrahydroxides.
     - Pelargonidin.

b.) Penthydroxides.
Cyanidin.
Paeonidin.
c.) Hexhydroxides.
Delphinidin.
Myrtillidin.
Malvidin.
Oenidin.

Classified with reference to the position of one of the hydroxy groups, i. e. as to whether or not it is in *a* position thus producing a flavonol group, the flavone pigments, as already pointed out, fall into two classes.

1. The true flavones.
2. The flavonols.

This classification appears desirable inasmuch as Willstaetter looks upon the anthocyanin pigments as related to the flavonols but not to the true flavones.

True Flavones	*Flavonols*
Tetrahydroxides of Chalkon	
Dihydroxy flavones	
Chrysin	
Tectochrysin	
Penthydroxides of Chalkon	
Trihydroxy flavones	Dihydroxy-flavonols
Apiginin	Galangin
Acacetin	Galangin methyl ether
Hexhydroxides of Chalkon	
Tetrahydroxy-flavones	Trihydroxy-flavonols
Luteolin	Fisetin
Luteolin methyl ether	Kaempherol
Lotoflavin	Kaempherid

Hepthydroxides of Chalkon

Tetrahydroxy-flavonols

Quercetin
Rhamnetin
Isorhamnetin
Rhamnazin
Morin

Octhydroxides of Chalkon

Penthydroxy-flavanols

Myricetin

I. B. 1.) *The Flavone Pigments.*

The flavone group constitutes the largest known group of plant coloring matters. All of its members are di-, tri-, tetra-, pent-, and hexhydroxy substitution products of flavone or methyl ethers of these substitution products. Flavone itself is a dehydration product of a hydroxy derivative of diphenyl -1, 3-propene-1-one 3, (chalkone)[1] a compound which is yellow in color and the hydroxy derivatives of which (also yellow in color) have been used for the synthesis of practically of all of the members of this group.

The flavone derivatives are all, as the name indicates, yellow in color. The intensity of the coloration appears to depend somewhat upon both the number and the position of the OH groups and varies from the pale yellow, or almost colorless, apigenin and acacetin to the deep orange yellow myricetin—a hexhydroxy flavone.

The pigments belonging to this group are found in nearly all parts of the plant and both in the free condition and as glucosides. Chrysin, galangin, luteolin and kaempherid are reported as occuring only in the free state; quercetin, fisetin and kaempherol both as such and as glucosides, quercetin being found as the glucosides quercitrin, robinin, rutin, myticolorin, osyritrin. While luteolin is reported only in the free state, its (3) methyl ether occurs as glucoside in the leaves of parsley. All the other members of this group of coloring matters are reported as occurring potentially in the plant as glucosides,

[1] So called by Kostanecki to whose syntheses of the flavone coloring matters we are indebted for much of our knowledge of the structure of these compounds.

While the flavone pigments are found in all parts of the plant they occur most frequently in the roots, wood, bark and leaves. When they appear to be the pigment to which the flower owes its color, the blossoms are either pale yellow or almost white. In some instances, as the occurrence of kaempherol in the blue flowers of *Delphinium consolida* and *Delphinium zali* it is plainly evident that the color is not due to the presence of the flavone derivative but to some other pigment or pigments.

Willstaetter in his work upon anthocyanin has shown that in the *Delphiniums* the color is due to delphinidin, probably a potassium salt of delphinidin. Delphinidin, according to Willstaetter, is isomeric with quercetin and morin, both of which are hydroxy substitution products of kaempherol.

The flavone derivatives are not quinoid in character. All can be, however, theoretically, and some have been, actually, oxidized to quinoidal compounds (See Chrysin[2]. and quercetin[3].). These quinones are deep red in color and closely resemble, in their behavior toward reagents, the red and blue pigments of flowers (anthocyanins). These quinones can be reduced to the corresponding hydroquinones which form either colorless or pale yellow crystals.

From a consideration of the above reactions, also as a result of observations upon the distribution of anthocyanin, and from experimental evidence on the concentration of sugars and glucosides in various tissues, on the existence of enzymes, and on sugar feeding, there has recently been formulated a hypotheses (Miss Muriel Wheldale[4]) that: The soluble pigments in flowering plants, termed anthocyanin, are oxidation products of colorless chromogens, existing in the tissues as glucosides. The production of the glucoside from the chomogen and sugar is in the nature of a reversible enzyme reaction: chromogen + Sugar = glucoside + water, and the oxidation of the chromogen, which is effected by one or more enzymes, can take place only after its liberation from the glucoside.

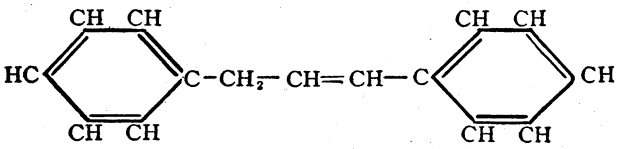
According to Miss Wheldale this hypothesis brings the formation of anthocyanine into line with that of pigments formed after the death of the plant (indigotin etc.) It is not opposed to the quinhydrone hypothesis of pigmentation and it is in ac-

[2] Ber., 45, 499.
[3] Ber., 44, 3487.
[4] Jr. Genetics, 1, 133, (Jr. Chem. Soc., A. II, 80).

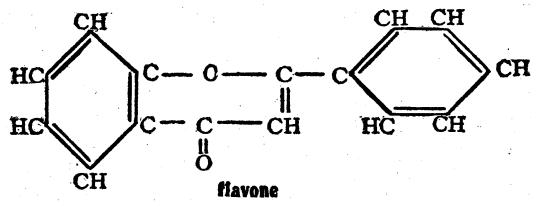
cord with the observations of Kastle[5]. and Hayden on the blue coloring matter of chicory blossoms. It is not in harmony, however, with the recent work of Willstaetter upon anthocyanins. According to Willstaetter it ought to be possible to produce anthocyanins by the reduction (not oxidation) of quercetin or other flavonols. Such an anthocyanin would be, not the quercetone or similar quinone of Niernstein and Wheldale, but an oxonium compound of a reduced quercetin or other flavonol.

Dihydroxy flavones

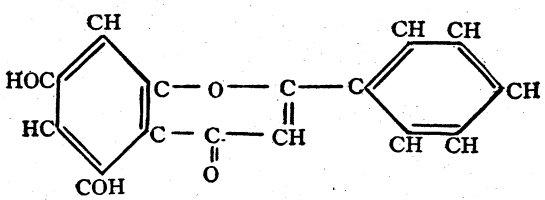
Chrysin,—Dihydroxy-1, 3-Flavone.



Diphenyl-I, 3-propene



flavone



Chrysin was probably first isolated by Hallwachs[1] in 1857 from the buds of *Populus nigra* or *Populus dilatata.* In 1864 Piccard[2] extracted chrysin from several varieties of poplar where he found it in the growing leaf buds. He named it *"chrisinsaeure"* to indicate both its yellow color and its salt forming properties. Several years later Piccard[3] undertook

[5] Am. Chem. Jr., 46, 315.
[1] Ann., 101, p. 872.
[2] Jr. f. Prakt. Chem., 93, p. 369.
[3] Ber., 6, p. 884; 7, p. 888; 10, p. 176.

a study of chrysin. In a series of articles he described its principal properties and attacked the problem of its constitution. This was determined later by Kostanecki[4] and his associates. (1893–1904) The work of Kostanecki was confirmed by that of Darier[5] in 1895.

Our present conception of the constitution of chrysin is based upon its decomposition by caustic alkalies into phloroglucin, acetic acid and benzoic acid, with small quantities of acetophenone. Also upon its synthesis from phloracetophenone trimethyl ether and ethyl benzoate.

Chrysin occurs in the buds of many species of poplar, including *Populus pyramidalis,*[6] *Populus nigra,*[7] *Populus monolifera,*[8] and *Populus balsamifera.*[9]

Chrysin forms clear yellow crystals. It melts at 275 and sublimes in fine needles at a temperature a little above the melting point. It is insoluble in water but soluble in both hot and cold alcohol, in aniline and acetic acid. It is difficultly soluble in ether and almost insoluble in carbon disulphide. In alkaline solutions it dissolves with a yellow color. Chrysin is precipitated from alcoholic solutions by lead acetate but dissolves in an excess of the reagent.

Treated with chromic acid and acetic acid chrysin is oxidized to chrysone,[10] a red amorphous powder which crystallizes in deep red needles melting above 360. Chrysone is insoluble in the ordinary organic solvents. It dissolves in concentrated sulphuric acid with a red color, in alkalies with a blue color. It forms a monoacetyl derivative which crystallizes in red needles. The acetyl derivative when reduced with zinc and acetic acid anhydride forms a white acetylated hydroxychrysin which, when hydrolized, crystallizes in small crystals melting at 304–305.

Tectochrysin, a methyl ether of chrysin.

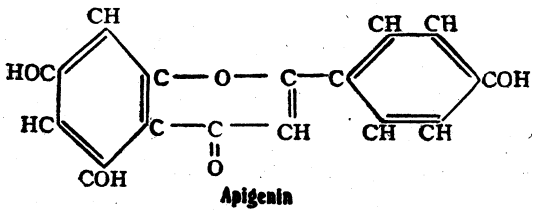
Tectochrysin[11] was first obtained by Piccard in the purification of chrysin. He called it tectochrysin from a Greek word

[4] Ber., 26, p. 2901; 32, p. 2260, 2449; 37, p. 3167.
[5] Ber., 27, p. 21.
[6] Ber., 6, p. 884.
[7] Ann., 101, p. 372.
[8] Ber., 6, 890; 7, p. 1485.
[9] Ber., 16, p. 176.
[10] Ber., 45, p. 499.
[11] Ber., 6, p. 888.

which means fusible, because its melting point is much lower than that of chrysin. Tectochrysin may be prepared synthetically by treating chrysin in alcoholic solution with methyl iodide. It is more readily soluble than chrysin, being easily soluble in benzine (distinction from chrysin.) Tectochrysin is sulphur yellow in color.

Trihydroxy flavones

Apigenin,—Trihydroxy—1, 3, 4'—flavone.



Apigenin

The glucoside apiin, of which apiginin is a component, seems to have been first isolated by Rump[1] in the course of his work on the chemical analysis of *Apium petroselinum*, but it was not until 1843 that Braconnot[2] first hydrolised this glucoside. Braconnot also applied the name apiin to the glucoside but made no analysis of the products of hydrolysis. In 1850 Planta and Williams[3] analysed apigenin and described it under the name of pure apiin. In 1867, Lindenhorn[4] showed the glucosidal character of apiin, and that by hydrolysis it gave glucose and a new substance to which he gave the name apigenin. Gerichten,[5] in 1876, took up the study of the constitution of apigenin, basing his conclusions upon its decomposition in the presence of alkalies. He ascribed to apigenin the formula $C_{15}H_{10}O_5$, a formula which has since been verified by the work of Perkin,[6] and more recently by that of Kostanecki and Tambor.[7]

Apigenin occurs as the glucoside apiin in *Petroselinum*

[1] Rep. f. Pharm., 6, p. 6.
[2] Ann. d. Phys. et Chim., 9, p. 250.
[3] Ber., 9, p. 112.
[4] Innaug. Dissert. Wuerzburg, 1867.
[5] Ber., 9, p. 259, 1121, 1477.
[6] Jr. Chem. Soc., 71, p. 807; 77, p. 420.
[7] Ber., 33, p. 1990.

sativum, Apium graveolens, and perhaps in other species of umbellifera.

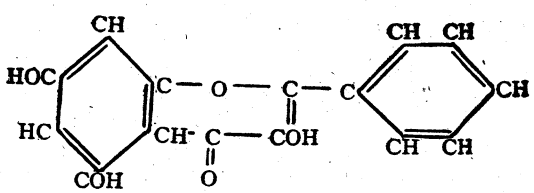
Apigenin forms crystals of a pale yellow color which melt at 212–215. It is difficultly soluble in water and in ether, more readily in alcohol. Alkalies dissolve it with a yellow color. In alcoholic solution it yields with lead acetate a yellow precipitate, with ferric chloride a red brown coloration.

Acacetin, a monomethyl ether of apigenin was named by Perkin[1] who first obtained it from the leaves of *Robina pseudacacia.*

Acacetin occurs in the leaves of the false acacia, *Robina pseudacacia.* It forms almost colorless needle like crystals which dissolve in alkaline solutions with a pale yellow color. From alcoholic solutions it is precipitated by lead acetate. With ferric chloride it gives a reddish brown color. It forms a diacetyl derivative which crystallizes in colorless needles which melt at 195–198. Fused with alkalies, acacetin yields phloroglucin and parahydroxybenzoic acid.

Galangin,—Trihydroxy—1, 3, —flavone or

Dihydroxy—1, 3—flavonol.



Galangin was first obtained by Brandes,[1] in 1839, together with kaempferid, from *Galanga* root, *Alpinia officinarum.* It was not, however, recognized by him as a distinct compound, and it was not until Jahns,[2] in 1881 showed that the kaempferid of Brandes was composed of a mixture of three substances which he called kaempferid, galangin, and alpinin, that galangin was actually isolated. In a later study (1900) of the colored compounds of *Galanga* root, Testoni[3] met with nothing correspond-

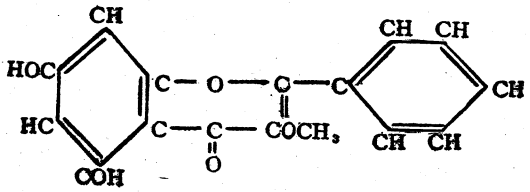
[1] Proc. Chem. Soc., 16, p. 45; Jr. Chem. Soc., 77, p. 430.
[1] Arch. der Pharm., (2) 19, p. 52.
[2] Ber., 14, p. 2305; 2807; Arch. der Pharm., 220, p. 161.
[3] Gazz. chim. ital., 30, (2) p. 327.

ing to the alpinin of Jahns, but found a methyl ether of galangin.

Jahns in his study of the constitution of galangin found its formula to be $C_{15}H_{10}O_5$, also that it has three hydroxy groups, and that upon fusion with potassium hydroxide it yields benzoic acid, oxalic acid and a phenol like substance. Kostanecki[4] and his associates by the hydrolysis, and subsequent synthesis of galangin, established its formula as given above.

Galangin crystallizes in yellowish white needles which melt at 214–215, and sublime with partial decomposition. It is almost insoluble in water, easily in ether, slightly in chloroform and benzene. It dissolves in alkalies with a yellow color. It yields a triacetyl and a tri methyl derivative, the latter, treated with acetic acid, yields a monoacetyl compound.[5]

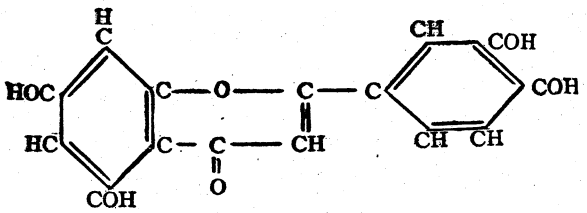
Galangin methyl ether.



A galangin methyl ether, probably with the methyl group in the position indicated above,[6] occurs along with galangin in the root of *Alpinia officinarum.*[7]

Tetrahydroxy flavones.

Luteoline,—Tetra hydroxy—1, 3, 3', 4'—flavone.



Luteoline was first isolated by Chevreul,[1] in 1830, and named by him from its source, *Reseda luteola.* Since that time it has been

[4] Ber., 37, p. 2803.
[5] Ber., 14, p. 2807.
[6] Czapek, Biochemie der Pflanzen, vol. 2, p. 523.
[7] Gazz. chim. ital., 30 (2) p. 327.
[1] Jr. Chim. Med. 6, p. 157.

studied by a number of chemists, Moldenhauer,[2] Schuetzenberger[3] and Paraf, Halsiwetz[4] and Pfaundler, Roechleder,[5] Adrian[6] and Trillot, Herzig,[7] Perkin,[8] and Kostanecki.[9]

Our present conception of the constitution of luteoline, like that of the other members of the flavone group, is based upon its decomposition by alkaline fusion when it yields phoroglucine and protocatechuic acid. Perkin, therefore ascribed to luteoline the foregoing formula which has since been verified by the synthesis, effected by Kostanecki[10] and his associates, of luteolin from phloracetophenone trimethyl ether and the diethyl ether of dihydroxy—3, 4—benzoic acid.

Luteoline occurs, as such, in *Reseda*[11] *luteola* in the leaves of *Digitalis*[12] *purpurea,* and in *Genista*[13] *tinctoria.* The 3-methyl ether of luteoline occurs as a glucoside in the leaves of parsley, *Petroselium sativum.*

Luteoline forms small quadrangular needles of a yellow color and bitter, astringent taste. They melt at 350° and sublime with partial decomposition. They are slightly soluble in cold water, better in warm water, alcohol, ether, and warm acetic acid.

Dry luteoline treated with phosphoric acid anhydride is changed to a red substance which dissolves in ammonia with a violet coloration. The aqueous solution of luteoline is colored first green, then reddish brown by ferric chloride, olive green by copper acetate. Luteoline dissolves in concentrated sulphuric acid with an orange red color and is precipitated unchanged by dilution. If to a saturated solution of luteolin in boiling acetic acid sulphuric acid is added, small orange red crystals, insoluble in acetic acid and decomposed by water into luteoline and sulphuric acid, are formed. Hydrobromides are formed in a similar manner with hydrobromide acid.

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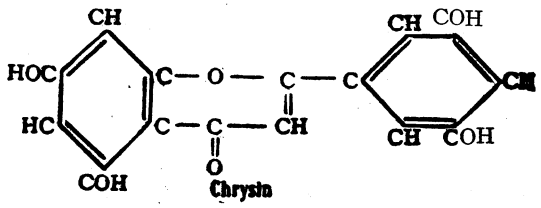
[2] Jr. Prakt. Chem., 70, p. 428.
[3] Bull. Soc. Chim., (1) p. 1861–18.
[4] Ann., 112, p. 107.
[5] Zeit. Anal. Chem. (1886) p. 602.
[6] C. r., 129, p. 889.
[7] Ber., 29, 1013; Monats., 17, p. 926.
[8] Jr. Chem. Soc., 69, p. 206, p. 1439.
[9] Ber., 32, p. 1184; 34, 1453; 37, 2625.
[10] Ber., 33, p. 3415.
[11] Ann., 100, p. 150; Jares., (1861) p. 707.
[12] Arch. Pharm., 383, p. 313; B., 32, p. 1184.
[13] Euler (1), p. 105.

Luteolin—methyl—ether is found as a glucoside in the green herb of parsley.

Lotoflavin,—Tetrahydroxy—1, 3, 2', 4'—flavone.

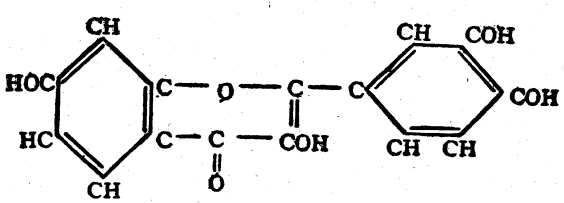


Lotoflavin was first met with in 1900 by Dunstan[1] in *Lotus arabicus*, a small leguminous plant growing abundantly in Egypt. This plant, which very closely resembles the common vetch, is commonly known as kuther. From the fact that fused with alkalies it yields β-resorcylic acid and phloroglucin, Dunstan and Henry[2] conclude that the structure of lotoflavin is as above.

Lotoflavin occurs in the *Lotus arabicus* in the form of a glucoside, lotosin, which by the action of dilute acids, or of a special enzyme, lotase, is hydrolised yielding lotoflavin, sucrose, and hydrocyanic acid.

Lotoflavin is a yellow crystalline substance readily soluble in alcohol or hot glacial acetic acid. It dissolves also in alkalies with a bright yellow color. It does not combine with mineral acids, but it forms a triacetyl derivative and two isomeric trimethyl ethers. By the action of fused alkalies it is converted into phloroglucin and resorcylic acid.

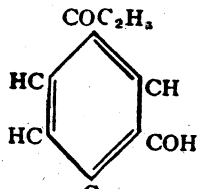
Fisetin,—Tetrahydroxy a—3', 4', a—flavone, or Trihydroxy—3, 3', 4' flavonol.



[1] Proc. Roy. Soc., 67, p. 224; 68, p. 374.
[2] Chem. News., 8, p. 301; 84, p. 26.

Chevruel[1] probably first extracted fisetin in the form of a tannin; though perhaps impure, from the fustel wood. Some years later it was again obtained from the same source by Bolley.[2] It was later isolated and studied by Schmidt,[3] Herzig,[4] Perkin,[5] and Kostanecki.[6]

Our ideas of the constitution of fisetin are based upon the work of Hirzig who showed that by boiling with alcoholic potassium hydroxide fisetin did not yield phloroglucin, but fisetol and protocatechuic acid, with traces of resorcin. By the synthesis of fisetol (ethylresorcylic acid), Kostanecki and Tambor confirmed the work of Herzig.



Fisetol.

Fisetin occurs as a glucoside in *Rhus cotinus*,[7] *Rhus rhodanthema*,[8] and *Querbracho colorado*.[9] It also occurs free in *Rhus rhodanthema*,[10] and in the blossoms of *Butea frondosa*.[11]

Crystallized from dilute alcohol fisetin forms small lemon yellow needle like crystals. From acetic acid it crystallizes in yellow prisms with six molecules of water of crystallization.

Fisetin is almost insoluble in water, easily soluble in alcohol, acetone and acetic acid. It is difficultly soluble in ether, benzene, petroleum ether and chloroform. Ferric chloride when added to fisetin solutions produces a dark green coloration and

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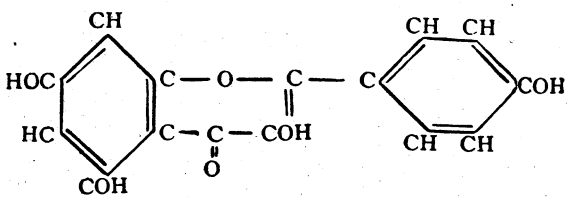
[1] Zeit. anal. Chem., 12, p. 127.
[2] Bull. Soc. Chim., 2, p. 479.
[3] Ber., 19, p. 1734.
[4] Monatsh., 12, p. 177.
[5] Jr. Chem. Soc., 67, p. 648; 69, p. 1304.
[6] Ber., 28, p. 2302; 37, p. 784; 38, p. 3587.
[7] Ber., 19, p. 1703.
[8] Jr. Chem. Soc., 71, p. 1194.
[9] Chem. News, 74, p. 120.
[10] Jr. Chem. Soc., 71, p. 1194.
[11] Proc. Chem. Soc., 19, p. 183; Jr. Chem. Soc., 85, p. 1459.

upon the addition of ammonia, a black precipitate. Lead acetate added to fisetin solutions forms an orange yellow precipitate which is easily soluble in acetic acid.

Fisetin forms tetramethyl, tetraethyl, tetrabenzoyl, and tetraacetyl derivatives. By fusion with alkalies it yields phloroglucin, resorcinol, and protocatechuic acid. Treated with chromic acid[12] it does not yield an oxidation product corresponding to those produced from chrysin and quercetin under the same condition. To fabrics mordanted with aluminum fisetin imparts an orange color, with tin a bright red or yellow red color, with chromium a brown color.

Kaempherol,—Tetrahydroxy a—1, 3, 4,—flavone, or—Trihydroxy—1, 3, 4′—flavonol.



Kaempherol was probably first extracted by Zwenger[1] and Dronk, in 1861, as the glucoside robinin from *Robinia pseudacacia.* It was considered by them, however, to be a glucoside of quercetin. It was first prepared from kaempherid, its 3-methyl ether, in 1897 by Gordin[2] who treated the crystalline kaempherid with strong hydriodic acid solution thus securing the free kaempherol. It was later isolated by Perkin (1900) from the flowers of *Delphinium consolida*[3] and by him identified. Perkin also isolated the glucoside robinin from the flowers of *Robinia pseudacacia.*[4] The constitutional formula of kaempherol follows from its preparation from kaempherid, and from its synthesis, along with that of kaempherid, by Kostanecki and others.

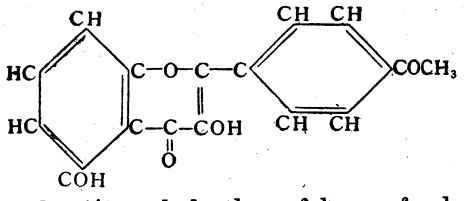
Kaempherol occurs both as such and combined as the gluco-

[12] Ber., 45, p. 499.
[1] Ann., Sup. 1, (1861) p. 257.
[2] Ber., 34, p. 3723.
[3] Jr. Chem. Soc., 81, p. 585.
[4] Proc. Chem. Soc., 17, p. 87.

side in the blue flowers of *Delphinium consolida*[5] and *Delphinium zali*,[6] as the glucoside in the white flowers of *Robinia pseudacacia*,[7] and, along with quercetin in the blossoms of *prunus spinosa*,[8] *Alpina officinarum*,[9] and *Rumex eckonianus*.[10] It has also been isolated from the indigo producing plants,[11] *Polygonum tinctorium* and *Indigofera amicta*, as the glucoside kaempheritrin. Scutellarein,[12] probably identical with kaempherol, is formed by the hydrolysis of the glucoside scutellarin which occurs in the epidermis of *Scutellaria caleopsis*, and *Teucrium* species.

Kaempherol crystallizes in pale yellow crystals which melt at 276–277. It is readily soluble in boiling alcohol, and soluble in alkalies with a pale yellow color. Alcoholic lead acetate solutions yield an orange red precipitate with kaempherol; alcoholic ferric chloride a greenish black coloration. Kaempherol dissolves in concentrated sulphuric acid forming a yellow solution which in a short time gives a blue fluorescence. To wools mordanted with aluminum kaempherol imparts a yellow color; with tin, a yellow color; with chromium, a brownish red color; and with iron, a deep olive brown.

Kampherid,—Trihydroxy—1, 3, α-methoxy-4′-flavone, or Dihydroxy-1, 3-methoxy-4′-flavonol.



Kampherid, the 4′ -methyl ether of kaempferol, was first extracted by Brandes[1] in 1839 from the rhizom of *Alpina officinarum*. Later, as has been shown in the chapter on galangin,

[5] Jr. Chem. Soc., 81, p. 585.
[6] Jr. Chem. Soc., 73, p. 267.
[7] Proc. Chem. Soc., 20, p. 172.
[8] Ann. (Sup.) 1, p. 257.
[9] Arch. d. Pharm., 247, p. 447.
[10] Jr. Chem. Soc., 97, p. 1.
[11] Jahresb. d. Chem., (1886) p. 573; Proc. Chem. Soc. 20, p. 172; 22, p. 198.
[12] Euler, p. 105.
[1] Arch. der Pharm., 67, p. 52.

this kaempferid of Brandes was found by Jahns[2] to be a mixture of three substances which he called kaempferid, galangin, and alpinin. Our ideas of the constitution of kaempferid, and also of kaempferol, are based upon its behavior with oxidizing substances and alkalies. By the action of oxidizing agents it yields para hydroxy benzoic acid and oxalic acid, fused with alkalies, oxalic acid, formic acid, and phloroglucine.

This conception of the formula of kaempferid and kaempferol is supported by the work of Kostanecki[3] and his associates, also by that of Gordin,[4] and of Cimician and Silber,[5] and it is confirmed by its synthesis by Kostanecki, Lampe and Tambor.[6] In this synthesis hydroxy-2′-trimethoxy-4′, 6′, 4-chalkon,[8] synthesized from phloracetophenone-dimethyl ether and anise aldehyde, treated in alcoholic solution with dilute sulphuric acid, yielded trimethoxy-1, 3, 4′-flavonon, which in turn gave the trimethoxy-1, 3, 4′-flavonol, and that gave the trihydroxy—1, 3, 4′—flavonol.

Kaempherid occurs, as has been already pointed out, in the rhizom of *Alpina officinarum.*

Kaempferid crystallizes in yellow plates which melt at 224–225. It is insoluble in water, slightly soluble in cold alcohol, chloroform and benzene, readily soluble in hot alcohol, ether, and sulphuric acid. It dissolves with an intense yellow color in solutions of the alkalies and the alkaline carbonates. In concentrated sulphuric acid it dissolves with a yellow color and a blue fluorescence. The alcoholic solution gives an olive green precipitate with ferric chloride, and a yellow precipitate with lead acetate. It reduces Fehling's solution when warmed.

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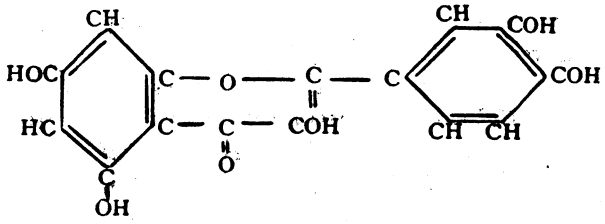
[2] Ber., 14, p. 2305, 2807; Gazz. chim ital., 30 (11) p. 327.
[3] Ber., 32, p. 318; 34, 3723; 28, p. 2302.
[4] Ber., 34, p. 3723; Dissertation, Berne, 1897.
[5] Ber., 32, p. 861.
[6] Ber., 37, p. 2096.
[7] Ber., 37, p. 192.

Penthydroxides of flavone.

Quercetin,—Penthydroxy—1, 2, 3', 4', α-flavone, or Tetrahydroxy—1, 2, 3', 4' -flavonol.



Quercetin which occurs very widely distributed throughout the plant kingdom, both in the free state and as a glucoside, has probably been more widely studied than any other vegetable coloring matter except chlorophyll and, perhaps indigo and alizarin. Quercetin was first extracted as a glucoside quercitrin by Chevreul[1] from the inner bark of *Quercus tinctoria* and later from the same source, also from the horse chestnut, by Rochleder.[2] The free quercetin was first obtained from the glucoside by Rigaud,[3] in 1854. The names of the various chemists who have since contributed to the literature of quercetin, with references to their published works are given in the appended list, which, although it contains the more important articles upon quercetin, is probably not at all complete.

Our conception of the structure of quercetin comes from the work of Hirzig,[4] also that of Kostanecki[5] and his associates. Fused with alkalies it yields phloroglucin, Protocatehuic acid, and glycolic acid.

$$C_{15}H_{10}O_7 + 3H_2O = C_6H_3(OH)_3 + C_6H_3(OH)_2COOH + CH_2 OH COOH.$$

Quercetin has been synthesized by Kostanecki and his collaborators in a manner quite similar to their synthesis of fisetin.[6]

Quercetin occurs very widely distributed in the free state, as alkyl ethers, and as glucosides. As a glucoside it is most

[1] Leçons de Chemie appliquée à la Teinture.
[2] Wien. Acad. Ber., 33, p. 565.
[3] Ann., 90, p. 283.
[4] Monatsh., 12, p. 177; 14, p. 38.
[5] Ber., 37, p. 784, 793.
[6] Ber., 37, p. 784, 793.

frequently met with combined with rhamnose though it often combines with other sugars, sometimes forming mixed glucosides with one or more molecules of rhamnose and one or more of another sugar, glucose or galactose.

As a glucoside quercetin is found in the bark of *Quercus tinctoria, Q. digitata,* or *Q. trifida,*[7] in the bark of *Carya tomentoria,*[8] in grape leaves,[9] *Viola tricolor,*[10] leaves of *Eucalyptus macrorrhyncha,*[11] leaves of *Ruta graveolens,*[12] buds of *Sophora japonica,*[13] leaves of *Colpoon compressum,*[14] *Arctostaphylos uva ursa,*[15] in North American *Chimaphila* species,[16] in *Calluna vulgaris,*[17] in the blossoms of *Tagetes patula,*[18] in horse chestnut,[19] in the leaves and blossoms of *Cherianthus cheri*[20] and of *Crataegus oxycanthus,*[21] in the blossoms of *Viola tricolor var. evensis,*[22] and in the blossoms of the cotton plant.[23]

In its free state quercetin has been found by Perkin[23] in *Rhamnus* (fruit), *Hippophae* (berries), *Rhus cotinus* (bark), Apple (bark), *Prunus spinosa* (blossoms), *Aesculus* (leaves and flowers), *Cornus* (flowers), Grape (leaves), *Allium cepa, Podophyllum,* and the fruit of *Rumex obtusifolia.*[24] It has been found by Horst[25] in *Polygonum persecaria,* by Weiss[26] in *Trifolium repens, Acacia, Gambircatechu,* flowers of *Crataegus,* and leaves of *Myrtus checken;* by Loewe[27] in *Catechu;* by Hummel[28] in the leaves of *Cherianthus cheri;* by Pilgrim[29] in the coloring matter of *Delphinium zali;* and by Perkin and Wood[30] in the leaves

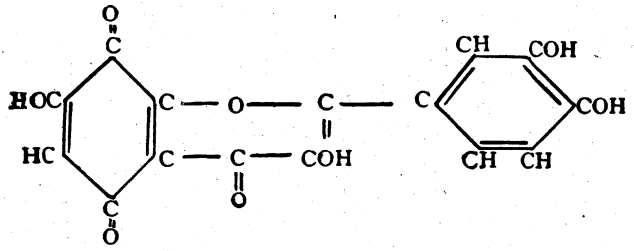
[7] Ann., 37, p. 101; Monatsh, 5, p. 72.
[8] Am. Jr. Pharm., 51, p. 118.
[9] C. Neubaur, Versuchrt, 16, p. 427.
[10] Jr. Chem. Soc., 71, p. 1131.
[11] Jr. Chem. Soc., 73, p. 697.
[12] Ann., 82, p. 197; Apoth. Zeit., (1901 p. 351.
[13] Jr. Chem. Soc., 67, p. 30.
[14] Jr. Chem. Soc., 71, p. 1131.
[15] Proc. Chem. Soc., 16, p. 295.
[16] Am. Jr. Pharm., 64, p. 295.
[17] Proc. Chem. Soc., 15, p. 179.
[18] Bull. Soc. Chim., 28, p. 337.
[19] Wien. Acad. Ber., 33, p. 565.
[20] Jr. Chem. Soc., 69, p 1295.
[21] Jr. Chem. Soc., 81, p. 477.
[22] Jr. Chem. Soc., 95, p. 2181.
[23] Proc. Chem. Soc., 19, p. 284; Jr. Chem. Soc., 49, p. 1295, 1556.
[24] Jr. Chem. Soc., 71, p. 1194.
[25] Chem. Ztg., 25, p. 2055.
[26] Arch. Pharm., (3) 26, p. 665.
[27] Zeit. f. Anal. Chem., 12, p. 127.
[28] Jr. Chem. Soc., 69, p. 1568.
[29] Jr. Chem. Soc., 73, p. 273.
[30] Jr. Chem. Soc., 73, p. 381.

of *Ailanthus glandulosa,* also in the leaves of *Rhus rhodanthema.*[31]

Pure quercetin presents the appearance of a lemon yellow crystalline powder made up of tiny needle like crystals. It is almost insoluble in cold water, soluble in alcohol, very difficultly soluble in ether, and easily soluble in dilute alkalies. It crystallizes with three molecules of water of crystallization which it loses at 130°. In alcoholic solution it gives a dark green coloration with ferric chloride which turns black upon heating. With lead acetate it gives a red precipitate. It reduces silver solutions when cold and Fehling's solution when heated. Quercetin melts at 250°. When treated with chromic acid and acetic acid it is oxidized to quercetone.

To fabrics mordanted with aluminum quercetin imparts a brownish yellow color; with chromium, a deep orange color; with iron, a dark olive; and with tin, a bright orange yellow.

Quercetone.



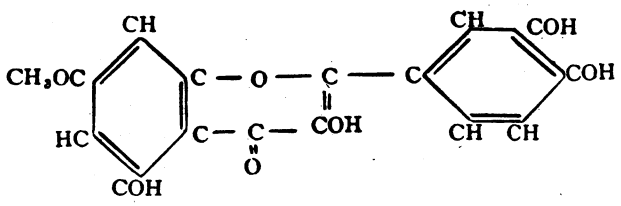
Quercetone,[32] the oxidation product of quercetin, crystallizes in small deep red needle like crystals which melt above 360°. It dissolves in alkalies with a blue, and in concentrated sulphuric acid with a red coloration. When heated with acetic acid and zinc dust acetylated hydroxy quercetin is obtained as a colorless, amorphous powder which yields upon hydrolysis penthydroxy-1, 3, 4, 3′, 4′-flavonol. This crystallizes in small yellow needles which lose a molecule of water at 160°, and melt at 352°–355°. Both alkaline hydroxides and sulphuric acid dissolve it with a yellow color. Pentmethoxy flavonol forms small colorless crystals which melt at 147°–149°.

[31] Jr. Chem. Soc., 73, p. 1017.
[32] Ber., 44, p. 3487.

Literature on Quercetin.

Bartolloti, — Gazz. chim. ital., 24, II. p. 480.
Bolley, — Ann., 37, p. 101; 125, p. 54.
Bolley and Mylinus, — Schweitzerische Poly. Ziet., 9, p. 22.
Bolley, — Jahresber d. Chem., 1861, p. 709.
Chevreul, — Lecons de Chem, app. a la Teinture.
Dunstan and Henry, — Jr. Chem. Soc., 73, p. 219.
Foerster, — Ber., 15, p. 214.
Gintl, — Jahresber. d. Chem., 1868, p. 801.
Herzig, — C. r., 5, p. 72; 6, p. 863; 9, p. 537; 548; 10, p. 561; 12, p. 172; 14, p. 39, 53; 15, p. 683; 16, p. 312; 17, p. 421; 18, p. 700.
Herzig, — Monatsh., 6, p. 863; 9, p. 541; 15, p. 696.
Hlasiwetz, — C. r., 29, p. 10.
Hlasiwetz, — Ann., p. 102.
Hlasiwetz, — Jahresber. d. Chem., 1864, p. 564; 1867, p. 732.
Kostanecki, — Ber., 37, p. 793, 1402.
Liebermann, — C. r., 16, p. 180.
Liebermann and Hamburger, — B., 12, p. 1179.
Liebermann and Hoerman, — Ann., 196, p. 299, 338.
Loewe, — Zeit. f. analyt. Chem., 12, p. 127, 233.
Lushing, — Dingler's Poly. Jr., 139, p. 1319.
Mandeline, — Pharm. Ztg. f. Russ., 22, p. 329.
Niernstein and Wheldale, — B., 44, p. 3487.
Perkin, — Jr Chem. Soc., 67, p. 644; 69, p. 1295; 71, p. 1131, 1135, 1191; 73, p. 2–1, 237, 381, 1017, 1135; 74, p. 278; 75, p. 837; 77, p. 426; 81, p. 477; 85, p. 56; 95, p. 1855, 2181.
Rigaud,— Ann., 90, p. 283.
Roechleder, — C. r., 33, p. 365.
Roechleder, — Jahresber. d. Chem., 1859, p. 523; 1866, p. 654; 1867, p. 731.
Rudolph, — Pharm. Post., 26, p. 529.
Schuetzenberger and Paraf, — Zeit. f. Chem., 1826, p. 41.
Schunck, — Chem. Gazette., 399, p. 20.
Stein, — Zeit. f. prakt. Chem., 1863, p. 467.
Stein, — Jr. f. prakt. Chem., 58, 399; 85, p. 351; 88, p. 280; 89, p. 491.
Wagner, — Chem. Centrlbl., 1873, p. 586.
Zwenger and Dronke, — Ann., (Suppl. I.) p. 257.

Rhamnetin,—Quercetin-3-monomethyl ether, or Trihydroxy-1, 3', 4'-methoxy -3-flavonol.



Rhametin was known as early as 1841 in the form of glucoside then called rhamnin[1] but now known as xanthorhamnin. It was hydrolized in 1858 by Gellatly,[2] and the sugar was identified as rhamnose by Berend[3] in 1878. Later Tanret[4] found that xanthorhamnin was a mixed glucoside containing two molecules of rhamnose and one of galactose. The constitution[5] of rhamnetin and other methyl ethers of quercetin has been the subject of considerable chemical study, the question under consideration being the position of the methoxy groups. Perkin,[6] in 1902 showed that by careful decomposition with alkalies the monomethyl ether of phloroglucin is obtained and that the methoxy group must therefore be in that part of the molecule from which the phloroglucinol is obtained. The formula for rhamnetin according to Perkin is given above.

According to Czapek, rhamnetin occurs as the glucoside in the fruit and in the bark of several species of *Rhamnus*. Kane,[7] Gellatly,[8] Schuetzenberger,[9] and Liebermann[10] find it in the "Gelbeern" or "Avignonkoerner," the fruit of *Rhamnus infectoria* and *R. tinctoria*.

Rhamnetin crystallizes best from phenol, in which it is easily soluble when heated. It separates on cooling in small bright lemon yellow crystals. It is sparingly soluble in warm water and very slightly soluble in the ordinary organic solvents. It

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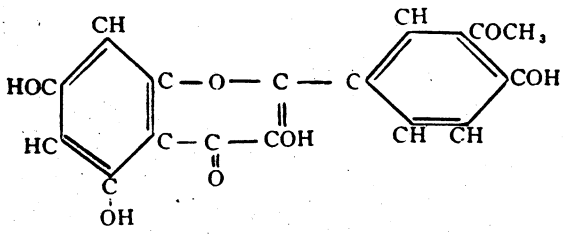
[1] Jr. Chem. Soc., 27, p. 666.
[2] Chem. Centrlbl., 29, p. 477.
[3] Ber., 9, p. 1353.
[4] C. r., 129, p. 725; Bull. Soc. Chim., (3) 21, p. 1073.
[5] Monats., 4, p. 889; 9, p. 548; 10, p. 561.
[6] Jr. Chem. Soc., 81, p. 569.
[7] Berz. Jahresb. 24, 505.
[8] Jahresb., 1838, p. 474.
[9] Jahresb., 1868. p. 774.
[10] Ann., 196, p. 313.

dissolves readily in alkalies with a yellow color. In alcoholic solutions it yields a brownish green color with ferric chloride, an orange yellow color with lead acetate and a reddish brown precipitate with lime or baryta water. It reduces an ammonical silver solution in the cold, Fehling's solution when warmed.

Isorhamnetin,—Quercetin-3'-monomethyl ether, or Trihydroxy- 1, 3, 4'-methoxy-3'-flavonol.



Isorhamnetin was first isolated by Perkin and Hummel[1] in 1896, from the petals of the yellow wallflower, *Cherianthus cheri,* and later by Perkin and Pilgrim[2] from the flowers of *Delphinium zali.* Because by oxidation in alkaline solution isorhamnetin yields vanillic acid, Perkin[3] concludes that it has the methoxy group in position -3'- as given above.

Isorhamnetin occurs, as stated above, in the flowers of *cherianthus cheri* and of *Delphinium zali,* along with quercetin. It crystallizes in masses of fine, brilliant yellow, needle like crystals which are difficultly soluble in boiling alcohol and in acetic acid. With lead acetate it gives an orange red preciptate, with ferric chloride a greenish black coloration. Fused with alkalies it yields protocatechuic acid and phloroglucin.

Rhamnazin,—Quercetin-3, 3'-dimethyl ether, or Dihydroxy-1, 4'-dimethoxy-3, 3'-flavonol.

Rhamnazin was first found by Perkin[1] in "Persian berries," the fruit of various species of *Rhamnus,* while trying to purify rhamnetin, in 1895, and shown by him to be dimethyl-3, 3'-quercetin, as below.

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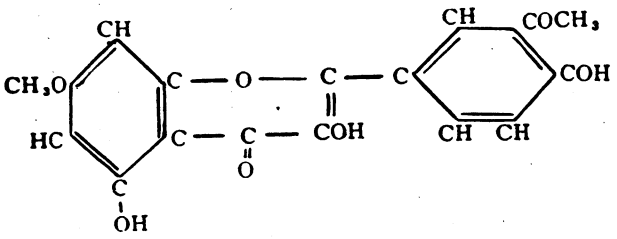
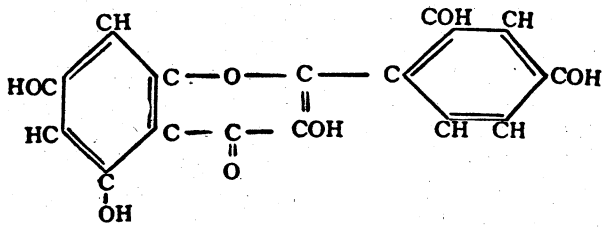
[1] Jr. Chem. Soc., 69, p. 1566.
[2] Jr. Chem. Soc., 73, p. 267.
[3] Proc. Chem. Soc., 14, p. 56.
[4] Jr. Chem. Soc., 67, p. 496; 71, p. 819.



Rhamnazin occurs in the fruit of *Rhamnus infectoria,*[1] and perhaps in other species of *Rhamnus.* Pure rhamnazin forms yellow needle like crystals which melt at 214°–215° and somewhat resemble anthraquinone in appearance. They are less soluble in acetic acid than are those of quercetin and very slightly soluble in alcohol. From acetic acid rhamnazin crystallizes with one molucule of water of crystallization which it loses at 100°. It dissolves easily in alkalies with an orange red color, with lime or baryta water it gives an insoluble orange red precipitate. The alcohol solution gives an olive green coloration with ferric chloride. It forms a triacetyl, also a trobenzoyl derivative.

Morin,—Penthydroxy- 1, 3, 2', 4', α-flavone, or Tetrahydroxy- 1, 3, 2', 4' -flavonol.



Morin was first found by Chevreul[1] in yellow wood, *Morus tinctoria,* in 1830, and later by Perkin and Cope[2] in the Indian dye stuff *Artocarpus tinctoria.* It closely resembles quercetin in appearance and reactions. Perkin[3] in his work on morin in 1896 assigned to it the constitutional formula of quercetin with the catechol nucleus replaced by a resorcinol group. This formula was verified by the work of Kostanecki[4] in 1904, and further

[1] Jr., Chim. Med., 6, p. 158.
[2] Jr. Chem. Soc., 67, p. 937.
[3] Jr. Chem. Soc., 69, p. 792; Chem. News., 73, p. 253.
[4] Ber., 37, p. 2350.

confirmed by his final synthesis[5] of morin from hydroxy-2′-tetramethoxy-4, 5, 2′, 4′ -chalkon in 1906.

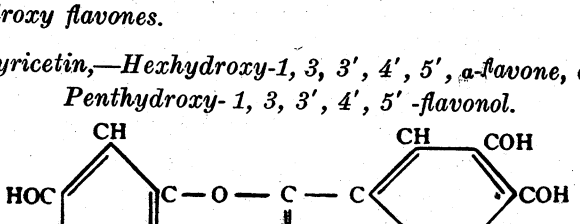
Morin occurs in fustic wood, *Morus tinctoria,*[1] the wood of *chlorophora tinctoria,*[6] and of *Artocarpus integrifolia,*[2] and *maclura tinctoria.*[6]

Morin crystallizes in long needle like crystals which are very slightly soluble in water, easily soluble in alcohol and less easily soluble in ether. It is not at all soluble in carbon disulphide, but soluble in alkalies with a yellow color. In alcoholic solutions it gives an olive green color with ferric chloride. It reduces an ammoniacal silver solution in the cold, Fehling's solution when warm. Treated with potassium salts a yellow precipitate is obtained which corresponds to the formula $C_{15}H_9O_7K$. With sodium acetate the corresponding sodium salt is obtained. Fused with alkalies morin yields phloroglucine and β- resorcyclic acid. To wools mordanted with aluminum morin imparts a yellowish olive color; with chromium, a deep brown; with tin, a bright yellow; and with iron, a dark olive brown color.

Besides those mentioned above, morin has been prepared and studied by the following chemists:
Wagner, — Jr. f. prakt. Chem., 50, p. 182.
Hlasiwetz and Pfaundler, — Ann., 127, p. 351.
Loewe, — Zeit, anal. Chem., 14, p. 119.
Benedikt, — B., 8, p. 606.
Benedikt and Hazura, — Monatsh., 5, p. 167.
Perkin, — Jr. Chem. Soc. 67, p. 649; 69, p. 792; 75, p. 433.
Herzig, — Monatsh., 18, p. 702.

Hexhydroxy flavones.

Myricetin,—Hexhydroxy-1, 3, 3′, 4′, 5′, a-flavone, or, Penthydroxy- 1, 3, 3′, 4′, 5′ -flavonol.



Myricetin was first isolated by Perkin,[1] in 1896, from *Myrica nagi,* an Indian dye stuff, and named by him from its source.

[6] Ber., 39, p. 81; 95, 627.

It was later isolated, by Perkin[2] and his associates, from a number of other dye stuffs and has been found to be a hydroxy quercetin.

Myricetin occurs as the glucoside myricitrin in the bark of *Myrica nagi*,[1] and *M. gale*.[2] In the leaves of *Rhus coriaria*,[2] *R. cotinus*,[2] and *R. metopium*.[2] It also occurs in *Pistachia lentiscus*,[2] *Haematoxylon campechianum*,[2] and in the leaves of *Arctostaphylos uva ursi*.[2]

Myricetin crystallizes in small clear yellow crystals which melt with decomposition above 300°. It dissolves with difficulty in boiling water, more easily in alcohol, and almost not at all in chloroform and acetic acid. It dissolves in potassium hydroxide solution with a yellow color which changes in the air to bluish, and becomes finally dull violet red in color. Concentrated alkali solutions give a permanent red color which goes through all the above changes upon dilution. Ammonia gives a more reddish color, lead acetate, a reddish orange color which becomes yellow upon boiling. Myricetin is dissolved with a red color in sulphuric acid and is precipitated upon the addition of water. Ferric chloride gives a black color in alcoholic solutions. Fused with alkalies myricetin rapidly becomes brown and yields principally gallic acid and phloroglucin. Myricetin dyes fabrics mordanted with aluminum a brownish orange; with chromium, a red brown; with tin, a deep orange red; and with iron, an olive black.

Gossypetin.

In 1899 Perkin[1] isolated from the yellow flowers of the Indian Cotton—*Gossypium herbaceum* a yellow pigment which he called gossypetin. This substance has the molecular composition $C_{15}H_{10}O_8$. It is isomeric with myricetin with six hydroxy groups, two of which are in relatively ortho-position. In its behavior it closely resembles the flavone derivatives. It is probably a member of the flavone group.

Gossypetin occurs principally in the form of the glucoside gossypitrin in the flowers of *Gossypium herbaceum*,[1] the Indian

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[1] Czapek, p. 521.
[1] Jr. Chem. Soc., 69, p. 1287.
[2] Jr. Chem. Soc., 81, p. 203; 77, p. 424, 427.
[1] Jr. Chem. Soc., 75, p. 825.
[2] Jr. Chem. Soc., 95, p. 1855.

cotton, and in the flowers of *Hibiscus sabdariffa.*[2] The Indian cotton flowers are used by the natives as a dye stuff. The seeds of the plant also contain a somewhat feeble yellow dyestuff, not identical with gossypetin, which by the action of acid is converted into the so called cotton seed blue.[3] Moreover, in the bark of the stem there exists a dye[4] which somewhat rsembles gossypetin.

Gossypetin crystallizes in glistening yellow needles. Its hexacteyl derivative melts at 222°–224°. Treated with sulphuric acid in acetic acid solution it forms a gossypetin sulphate consisting of glistening orange-red needles. This compound is decomposed by water into gossypetin and sulphuric acid. The hydrochloride prepared in the same way forms orange crystals and is very unstable. The hydriodide which forms orange red crystals is more stable. The hydrochloride could not be analyzed but the others are evidently formed by addition of one molecule of the acid to one of the pigment. This behavior suggests the oxonium formation.

Gossypetin is very soluble in alcohol and slightly soluble in water. It dissolves in alkalies with an orange red color. Fused with alkalies it yields phloroglucin and protocatechuic acid. To wools mordanted with aluminum it gives a pale orange brown color; with tin, an orange red color, with chromium, a dull brown and with iron a deep dull olive color.

The dyeing properties of the flowers of the Indian cotton are very distinct from those of gossypetin, due to the fact that they contain the glucoside and not the free coloring matter. With the ordinary mordants the following shades are obtained: Aluminum, dull yellow; tin, orange brown; chromium, dull brown-yellow; iron, dull olive.

f. B. p.) *Butein.*

While not a flavone derivative, butein is nevertheless referable to the same hydrocarbons as the flavone derivatives, being a tetrahydroxy-4, 5, 3′, 4′-diphenyl-1-3-propene-1-one-3, or tetrahy droxy chalkon.

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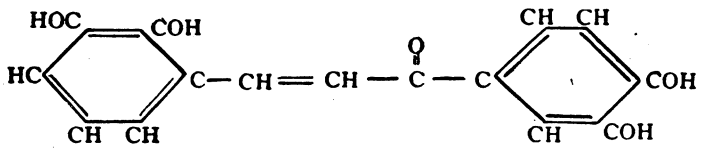
[3] C. r., 53, p. 444; Anzeiger der Akademie der Weissenschafter in Krakaw, Nov. 1897.



Butein[1] occurs in glucosidal formation, either as such or in the form of butin, in the flowers of *Butea frondosa*. Its synthesis from dimethylprotocatechuic aldehyde and monomethyl resacetophenone as well as its formation from butin[2] have been discussed in a previous chapter.

Butein crystallizes in needle like crystals which melt at 213°–215°. It is readily soluble in alcohol, somewhat soluble in ether, more sparingly soluble than butin in hot water. It dissolves in alkaline solutions with a deep orange red color. In alcoholic solutions with lead acetate it gives a deep red precipitate, with ferric chloride an olive brown coloration. In cold sulphuric acid it dissolves with an orange color, upon the addition of water the butein is precipitated unchanged.

Butein dyes wools mordanted with aluminum a beautiful orange color, with chromium a deep terra cotta, with tin a beautiful yellow, and with iron a brownish olive.

I. B. 3.) *The anthocyanin pigments.*

The so called anthocyanin pigments have long attracted the attention of both chemists and botanists, and have called forth considerable work from both classes of investigators. From time to time colored substances have been extracted from plant organs supposed to be colored by anthocyanin pigments and have been made the subject of special investigation. These colored substances, though sometimes crystalline, were probably seldom pure, so that little chemical knowledge was gained either of the special pigment studied or of the anthocyanins as a class. In addition to the above, many theories, all of which have been more or less unsatisfactory, have been advanced to account for both the appearance and the disappearance of color in flowers, fruits, and autumn foliage.

The recent exhaustive study, by Willstaetter, of anthocyanin

[1] Proc. Chem. Soc., 10, p. 11; 19, p. 133; 85, p. 1495.
[2] See Butin, Formula of saturation C_nH_{2n-14}.

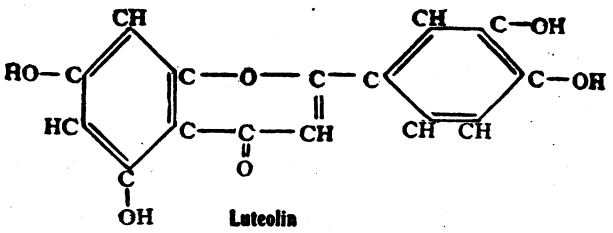
pigments in some plants, while not satisfactory in every detail, is a long step in advance. This work not only explains much concerning the chemistry of the anthocyanin pigments that has hitherto been unexplained; but it places the anthocyanins in a class, and shows the close chemical relationship between the various anthocyanin pigments studied, and also between the anthocyanins and other pigments occurring with them in the plant.

Anthocyanins are the red, blue and purple pigments extracted from flowers, fruits, and leaves by water and dilute alcohol. They are insoluble in ether, are turned red by acids, blue or green by alkalies, and give green, green-blue, gray-green, or yellow precipitates with lead acetate. It is commonly supposed that the purple color of flowers and fruits is due to the free pigment, the blue color to an alkaline combination, and the red color to an acid combination of the pigment.

The anthocyanins, according to Willstaetter, are present in the plants only as glucosides, sometimes as mono- and sometimes as diglucosides. The sugar molecule with which the pigment is combined is generally that of glucose, though in at least one instance galactose is present. The anthocyanins all exhibit a characteristic reaction, the anthocyanidin reaction. An anthocyanin dissolved in a normal or twice normal solution of sulphuric acid is unaffected by shaking with amyl alcohol. After hydrolysis, however, the colorod anthocyanidin is quantitatively extracted by the amyl alcohol, forming a reddish violet solution which slowly, more rapidly in the presence of sodium acetate, changes to a bluish violet.

All of the anthocyanins so far studied are very closely related, the color bases of the various glucosides being hydroxy and methoxy derivatives of pelargonidin, the least highly oxygenated of the known anthocyanins. They are also closely related to the flavone derivatives, so many of which constitute the yellow plant pigments, and many of which occur side by side with the anthocyanin pigments in the plants. According to Willstaetter the free anthocyanin pigments are isomers of some of the flavone pigments, the isomerism between them existing, not in the position of substitution in one or the other of the two benzene nuclei, but in the transformation, by the changing of valence of the ether oxygen from two to four, of the pyron to a pyrylium grouping, and of a difference in the posi-

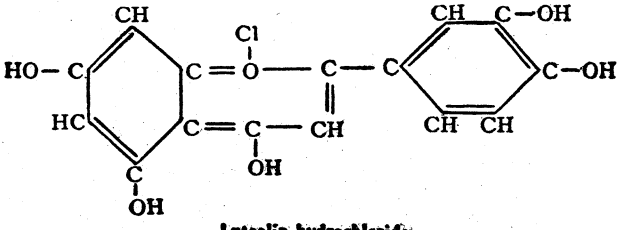
tion of the hydroxyl group in the pyrylium cycle, all of the anthocyanin pigments so far known possessing a hydroxyl group in the flavonol position. For example, luteolin and cyanidin are both represented by the formula $C_{15}H_{10}O_6$.



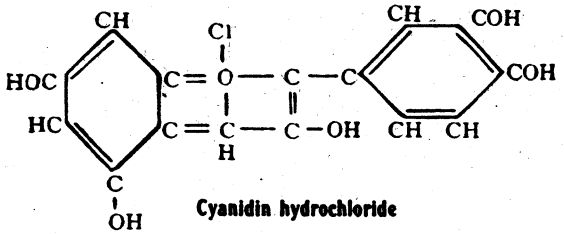
Luteolin

The formula for free cyanidin is not known.

The hydrochloride of luteolin Willstaetter represents by formula I, and that of cyanidin by formula II. below.



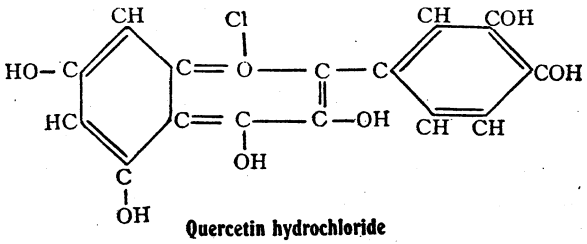
Luteolin hydrochloride



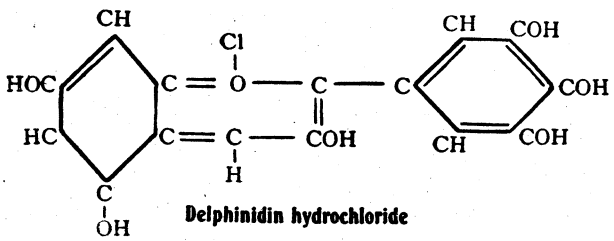
Cyanidin hydrochloride

While this difference of position of a hydroxy group may be sufficient to explain the difference in properties of the two groups of pigments in acid combination, something more seems to be required to explain this difference in the acid free form, since many of the flavone pigments as well as the anthocyanin pigments are flavonols. Moreover it does not appear to be sufficient to explain the difference between such isomers as,

for example, delphinidin hydrochloride and quercetin hydrochloride, quercetin having an hydroxy group in the flavonol position.

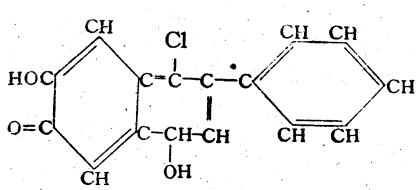


Quercetin hydrochloride



Delphinidin hydrochloride

The anthocyanin pigments have long been thought of as being of a quinoidal character. This supposition was encouraged by the oxidation of quercetin and chrysin, by Niernstein and Wheldale,[1] to quercetone and chrysone respectively, these oxidation products being "anthocyanin like." Willstaetter and Everest[2] sought to explain the constitution of cyanin by assuming a quinoidal arrangement and classifying the anthocyanins as paraquinoidal flavone derivatives, as below:



From such a molecule one would expect a hydroxy hydroquinone as one of the products of abbau. Since no such product, but phloroglucine just as with the majority of flavone

[1] Ber., 44, p. 3487; 45, p. 499.
[2] Amn., 408, p. 18.

pigments, is obtained, the quinoidal configuration as a possible explanation was abandoned and the arrangement of double bonds of the pyrylium grouping adopted instead. Whether or not the difficulties in the way of accepting Willstaetter's furmula are more easily explained away than are those in the way of accepting the quinoidal formula is still a matter of opinion.

Among the anthocyanins thus far studied pelargonidin is isomeric with apiginin and galangin. Cyanidin is isomeric with luteolin, lotoflavin, fisetin, and kaempherol. Paeonidin, a methyl ether of cyanidin is isomeric with kaempherid, a methyl ether of kaempherol. Delphinidin is isomeric with quercetin and morin, while myrtillidin, a methyl ether of delphinidin is isomeric with rhamnetin and isorhamnetin, both methyl ethers of quercetin, and malvinidin and oenidin, dimethyl ethers of delphinidin are isomeric with rhamnazin, a dimethyl ether of quercetin.

The isomerism of the above named compounds is probably not to be doubted. That this isomerism consists only in the different position of a hydroxyl group in the pyrylium ring, even in acid combination, is open to question, since no such marked difference in properties exists between the flavonols and the true flavones as is found to exist between the flavone derivatives and the anthocyanins.

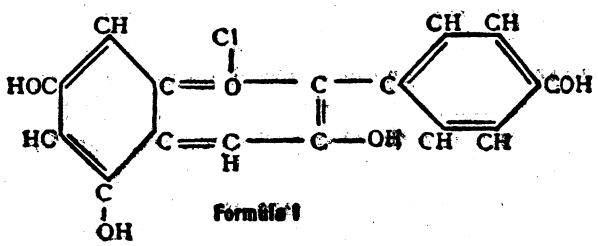
None of the neutral pigments, isomeric with the flavone pigments appear to have been isolated as such. They have been obtained as oxonium salts formed by the addition of a molecule of acid to a molecule of the pigment, as the colorless pseudo base, obtained by the elimination of the elements of hydrochloric acid and the addition of the elements of a molecule of water, and as the color base, a colored modification of the pseudo base into which it changes upon standing in concentrated solution.

According to Willstaetter red and pink colors in the organs examined are due to acid compounds of the pigment, oxonium salts; purple and violet colors to the free pigments; and blue colors to metallic derivatives of the pigment. The blue cornflower is probably colored by the potassium salt of the cyanin, and the scarlet geranium by the compound of pelargonin with tartaric acid, while the purple delphinum is supposed to be colored by the neutral delpninin.

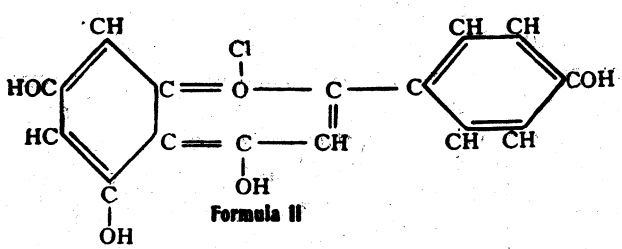
Tetrahydroxides.

Pelaogonidin.

According to Willstaetter pelargonidin chloride, the oxonium salt of pelargonidin, is probably represented by formula I. given below, though he also recognizes the possibility of its being represented by formula II. Willstaetter prefers the first fomula because he regards the second as the structural formula of a flavone derivative.



Formula I



Formula II

Pelargonidin exists in the blossoms of the red geranium, *pelargonium zonale,* combined with two molecules of glucose as the glucoside pelargonin.

The geranium pigment was isolated by Griffiths[1] from the blossoms of the red geranium in 1903. Griffiths decided that the pigment has the formula $C_{15}H_{10}O_6$ and that it forms a red diacetyl derivative.

In 1908 Wenzell[2] again isolated the crystalline red pigment from the flowers of *Pelargonium zonale,* but he made no chemical study of the compound.

During the winter of 1911–1912 the writer, having access to large quantities of geranium blossoms, again isolated the red crystalline pigment. The substance crystallized in fine needle

[1] Ber., 36, p. 3956.
[2] Pacific Pharmacist, 1908.

shaped crystals of a high melting point and a bright red color. The crystals dried in masses of a brownish color with a beautiful green reflection. This substance was no glucoside. If it existed as such in the plant, it was hydrolized in the process of preparation by the sulphuric acid used to decompose the lead precipitate. The crystalline substance was insoluble in ether, chloroform, hydrocarbon oils, and carbon disulphide, almost insoluble in hot water and in 95 per cent alcohol, but easily soluble in 60–70 per cent alcohol when heated. It was possibly the pelargonidin sulphate described by Willstaetter.

This compound gave a yellow acetyl derivative when heated with acetic acid anhydride and anhydrous sodium acetate. This acetyl derivative, computed upon Griffith's formula of $C_{15}H_{10}O_6$, contained five acetyl groups. This, interpreted in the light of Willstaetter's formula would probably mean that the sulphate, in the process of acetylization, was changed to the acetate and that all four of the hydroxy groups were acetylized. When heated in alcoholic solution with zinc and acetic acid the red color of the crystalline pigment disappeared leaving a colorless solution. This, after standing exposed to the air, gradually became deep red in color. No crystals could be induced to separate from this red solution.

Willstaetter criticises severely the old method of attempting to separate anthocyanin pigments by precipitation with lead acetate. However just this criticism may be when applied to anthocyanins in general the writer will not venture to say. The above described pigment, however, was easily obtained, apparently in a pure condition, by precipitating an aqueous extract of fresh geranium blossoms with lead acetate and decomposing the precipitate with sulphuric acid. The exact details of the process need not be given here.

In 1911 Grafe[3] isolated what he considered as two pigments from the scarlet geranium, one glucosidal and the other not glucosidal in character. Willstaetter says that as a matter of fact both of Grafe's pigments are glucosides, and that only one is present in the plant, the second being a mixture of the pigment with other substances.

Willstaetter's pelargonidin was separated in the form of the oxonium salt of the glucoside pelargonin. This upon hydroly-

[3] Sitzungsber. d. Wien Akad. Wiss. math. nat. kl., 120, p. 765.

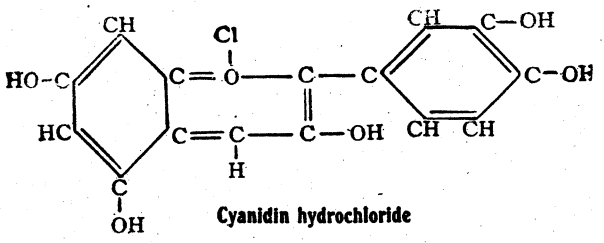
sis yields pelargonidin chloride which crystallizes in three different forms. The sulphate crystallizes in needles.

Willstaetter's idea of the structure of pelargonidin is obtained from its abbau with 50 per cent potassium hydroxide solution when phloroglucin, p-hydrohybenzoic acid, and small quantities of protocatechuic acid are obtained. It is isomeric with apiginin and galangin.

Willstaetter found pelargonin in the scarlet flowers of *pelargonium zonale*,[4] also in the scarlet red varieties of dahlia,[5] known as "*Rakete*" and "*Alt Heidelberg*," also in a violet red variety of dahlia.

Penthydroxides.

Cyanin.



Cyanidin hydrochloride

The above formula represents the constitution of cyanidin hydrochloride according to Willstaetter's more favored formula.

As early as 1854 Fremy and Cloez[1] isolated a blue pigment from the cornflower which they called cyanin. According to these investigators there are three kinds of pigments in plants, the green, called chlorophyll, the yellow known as xanthine and xantheine, and the red and blue, which they called cyanin. The red and rose colored flowers owe their color to the cyanin colored by acids in the juice of the plant.

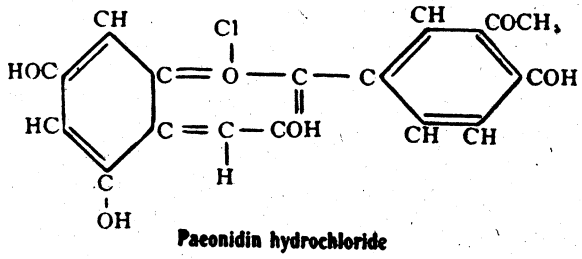
In 1913 Willstaetter and Everest[2] again isolated the blue pigment from cornflowers and made it the subject of an exhaustive investigation. They found that cyanin, the pigment, is a glucoside which they obtained as the hydrochloride. Upon hydrolysis this glucoside gave cyanidin chloride. To the hydro-

[4] Ann., 408, p. 42.
[5] Ann., 408, p. 151.
[1] Jr. de Pharm., 58, p. 249.
[2] Ann., 401, p. 189.

chloride of the glucoside they assigned the formula $C_{28}H_{33}O_{17}Cl.$ $3H_2O$, to that of the cyanidin $C_{16}H_{13}O_7Cl.$ As the result of a later determination these formulae were changed to $C_{27}H_{31}O_{16}Cl.$ $2\frac{1}{2}$ H_2O and $C_{15}H_{11}O_6Cl.$ respectively, with the structural formula as given above.

Cyanidin exists as the glucoside cyanin in *Centauria cyanus*, the corn flower, in the dark red varieties of the cactus dahlia[4] known as "J. H. Jackson," "Harold," "Matchless," "Othello," and "Night," in the petals of *Rosa gallica*, and in the fruit of the whortleberry, *Vaccinium vitis idaea*, as the glucoside idaein, a compound of one molecule of cyanidin with one of galactose. Cyanidin is isomeric with lotoflavin, luteolin, fisetin, and kaempherol.

Paeonidin,—a monomethyl ether of cyanin.



Paeonidin hydrochloride

Paeonidin[1] exists in the paeony blossoms as the glucoside paeonin, a compound of paeonidin with two molecules of glucose. Treated with hydriodic acid it yields cyanin and methyl iodide. The formula for paeonidin hydrochloride favored by Willstaetter is given above. Paeoninin is isomeric with luteolin methyl ether and kaempherid.

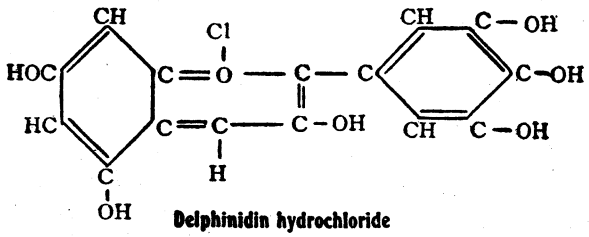
Hexhydroxides.

Delphinidin.

Delphinidin[1] occurs as the glucoside delphinin in the blossoms of *Delphinium consolida* where it exists along with the isomeric

[3] Ann., 408, p. 1.
[4] Ann., 408, p. 151.
[1] Ann., 408, p. 136.
[1] Ann., 408, p. 61.

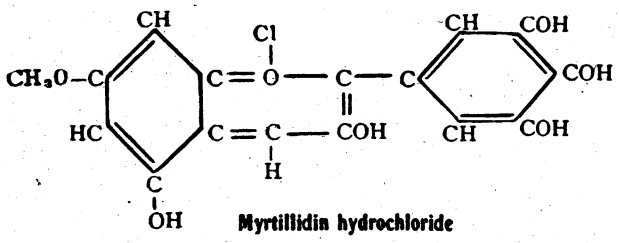
quercetin and the closely related kaempherol.[2] The glucoside delphinin is a compound of one molecule of delphinidin with two of glucose.



Delphinidin hydrochloride

Delphinidin is isomeric with quercetin and morin. The structural formula most favored by Willstaetter is given above.

Myrtillidin,—a monomethyl ether of delphinidin.



Myrtillidin hydrochloride

Myrtillidin was found by Willstaetter to exist in the form of the glucoside myrtillin in combination with one molecule of glucose in the fruit of the bilberry, *Vaccinium myrtillus;*[1] also as the glucoside althaein in the blossoms of *Althaea rosea,*[2] the wild mallow. Myrtillidin is isomeric with rhamnetin and isorhamnetin, monomethyl ethers of quercetin.

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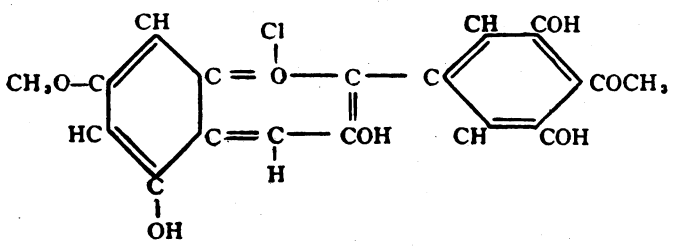
[2] Jr. Chem. Soc., 73, p. 275; 81, p. 585.
[1] Ann., 408, p. 103.
[2] Ann,, 408, p. 110.

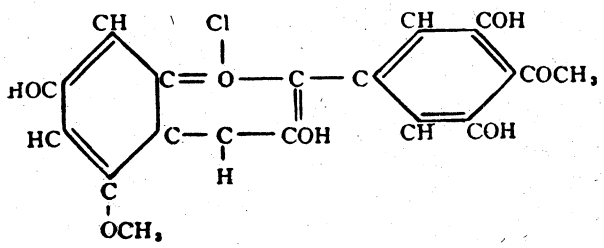
Oenidin,—a dimethyl ether of delphinidin.



The pigment from grapes had been separated in a more or less impure state many times before Willstaetter undertook his study of anthocyanin pigments. Mulder,[1] in 1856 obtained the pigment in the form of a bluish black mass, Mawmene,[2] in 1856, obtained the same substance and named it "oenocyanin." In 1858 Glenard obtained the pigment as an amorphous substance which he called "oenolin" and to which he assigned the formula $C_{20}H_{20}O_{10}$. Gautier[4] made several investigations of the coloring matter of grapes, continuing his studies for a number of years. Gautier traced a close relationship between the grape pigment and the tannins. Willstaetter,[5] in 1915, found the pigment to exist in the form of the glucoside oenin in *Vitis vinifera.* To the product of hydrolysis he gave the formula above.

Oenidin is an isomer of rhamnazin, a dimethyl ether of quercetin.

Malvinidin,—a dimethyl ether of delphinidin.



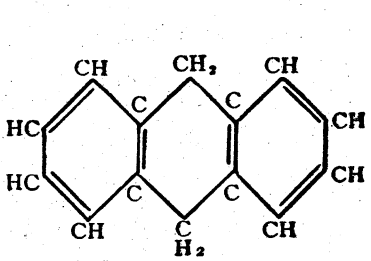
[1] Die Chemie des Wines, 44, p. 228.
[2] Le Travail des Vins.
[3] C. r., 47, p. 268; Ann. Chim. Phys., (3) 54, p. 366.
[4] C. r., 86., p. 1507; 87, p. 64; 114, p. 623.
[5] Ann. 408, p. 87.

Malvinidin was found by Willstaetter to exist in the violet flowers of the wild mallow, or wood mallow, *Malva sylvestris,* where it occurs in combination with two molecules of glucose as the diglucoside malvin. Malvinidin is isomeric with oenidin, also with rhamnazin, a dimethyl ether of quercetin.

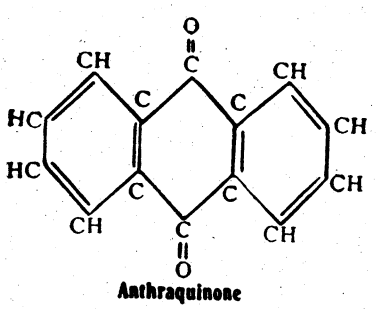
II. PIGMENTS REFERABLE TO DIHYDROANTHRACENE AND HOMOLOGUES.

A. Pigments referable to dihydroanthracene.
B. Pigments referable to homologues of dihydroanthracene.
   1. Pigments referable to methyl -1- dihydroanthracene.
   2. Pigments referable to methyl -2- dihydroanthracene.

Most if not all of the plant pigments referable to dihydroanthacene and its homologues, are derivatives of anthraquinone and its homologues, the quinones being tetrahydroxy derivatives of the underlying hydrocarbons.

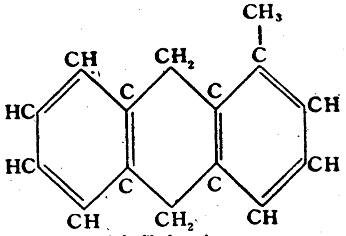


Dihydroanthracene

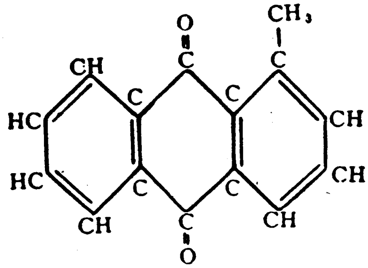


Anthraquinone

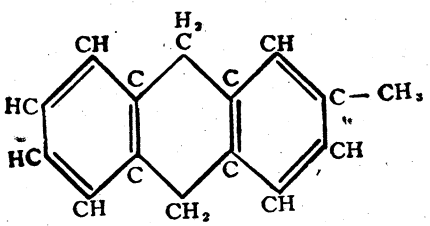
[1] Ann., 408, p. 122.



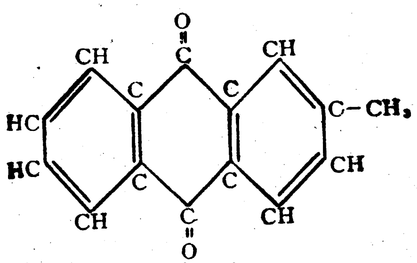
Methyl-1-dihydroanthracene



Methyl-1-anthraquinone



Methyl-2-dihydroanthracene



Methyl-2-anthraquinone

There exist in plants a large number of compounds, referable to these three hydrocarbons, most of which are used as dyestuffs. So far as is known, all except possibly the aloins, are hydroxy derivatives, and their alkyl or sugar ethers, of quinone oxidation products of these hydrocarbons, viz. anthraquinone and methyl anthraquinones. The aloins are possibly hydroxy derivatives of dihydro methyl anthracene.

Anthraquinone, which forms pale yellow crystals, is a quinone having its two carbonyl groups in p - position with reference to each other, a configuration which in itself is supposed to give color to a molecule. The intensity of the color, and especially the dyeing property, of the substance appears to depend upon the number and the position of free hydroxy groups introduced into the molecule.

As in the Xanthone and Flavone groups the compound appears to be more highly colored and to possess better dyeing properties when there is a hydroxy group in position -1- relatively ortho to the carbonyl group, so the anthraquinone pigments used particularly as dyes contain at least one hydroxy group in ortho position to one of the quinone oxygens. In 1887

Liebermann and Kostanecki[1] undertook a study of a large number of hydroxy anthraquinones in order to ascertain the relation between the number and position of the hydroxy groups and the dyeing properties of the compound. The result of their investigations may be summarized as follows: At least two hydroxy groups are necessary in order that the anthraquinones may become dye stuffs. This is shown by the fact that no monohydroxy anthraquinones have dyeing properties. Of the known dihydroxy anthraquinones, only alizarin with the hydroxy groups in positions 1 and 2 has strong dyeing properties. Hystazarin, which was not known at this time, 2, 3, dihydroxy anthraquinone, does, it is true, combine with mordants but its dyeing properties are weak and it is not satisfactory as a dye stuff. That the dyeing property of alizarin is not dependent on only one of the two hydroxy groups is shown by the fact that the monomethyl or mono ethyl ether of alizarin does not dye mordanted fabrics. From these facts Liebermann draws the conclusion that in order to have dyeing properties the polyhydroxy anthraquinones must have two of their hydroxy groups in positions 1 and 2.

All of the known trihydroxy anthraquinones which have the property of dyeing mordanted fabrics have two of their hydroxy groups in positions 1 and 2 or in similar positions. The same holds true for the tetrahydroxy derivatives. Those with hydroxy groups in positions 1, 4, 1′, 4′, have no dyeing properties whatever, and those with hydroxy groups in 1, 3, 2′, 4′, possess very weak dyeing properties. It is theoretically impossible to have pent- and hexhydroxy derivatives in which two of the hydroxy groups are not connected to carbon atoms in position 1 and 2. All of the known pent- and hexhydroxy anthraquinones are good dye stuffs.

An exception to Liebermann's rule seems to be found in chrysophanic acid. The formula for chrysophanic most favored at present represents the compound as possessing two hydroxy groups in positions 1′ - 4′, while none of the formulas considered have hydroxy groups in positions 1 - 2.

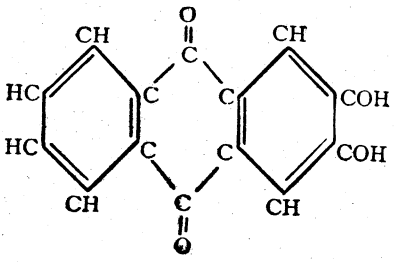
Although Liebermann has shown that all the hydroxyanthraquinones which have dyeing properties, with possibly a few exceptions, have hydroxy groups in positions 1 and 2, he has

[1] Ann., 240, p. 245.

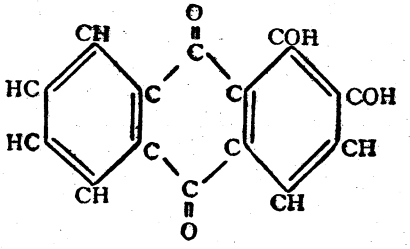
apparently made no attempt to explain why this is so. Brandel[1] in his monograph on Plant Pigments offers such an explanation.

"It is well known that the mordants which are used in dyeing with these substances are salts of aluminum, iron and chromium, in other words salts of trivalent metals. The process of dyeing with mordants depends upon the formation of the aluminum, iron or chromium derivative and its deposition in the fiber. This being true, one would possibly not expect the monohydroxyanthraquinones to have dyeing properties, inasmuch as the union of three molecules of the monohydroxyanthraquinone with one atom of aluminum, might hardly be expected to take place very readily.

"On the other hand, by the introduction of more OH groups into the same molecule the tendency to form these trivalent metallic derivatives would be increased and it would be expected to be the greatest in those cases in which the OH groups are connected to neighboring carbon atoms. The bonds of the aluminum atom would be subject to a less strain as it were, than when they united with bonds from different molecules or from widely separated bonds in the same molecule. From this standpoint, the 2, 3, dihydroxyanthraquinone



as well as the 1, 2, dihydroxyanthraquinone

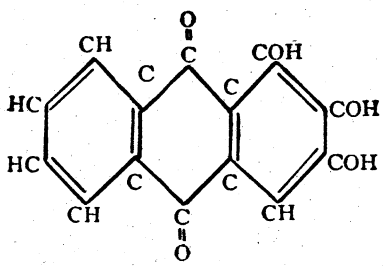


[1] Brandel—Plant Pigments, p. 29.

should have dyeing properties. Both of these compounds are dyestuffs, the former not agreeing with the rule as laid down by Liebermann.

"In those compounds in which the OH groups are not connected to neighboring carbons atoms as is the case in the dihydroxanthraquinones, 1, 3, 1, 4; 1, 5, etc., the separation of the OH groups from one another decreases the tendency to form metallic derivatives with trivalent metals and therefore these compounds have no dyeing properties.

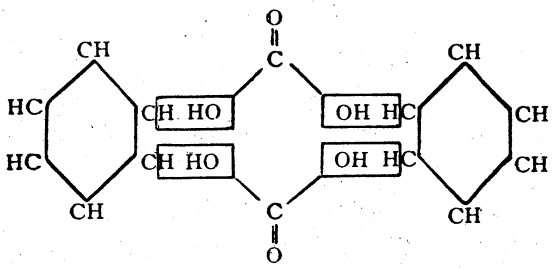
"On the basis of the same reasoning, the least strain of all would result and, therefore, an aluminum, iron or chromium derivative would be most readily formed in those cases in which there are three OH groups connected to neighboring carbon atoms. This is substantiated by the fact that anthragallol, 1, 2, 3, trihydroxanthraquinone.



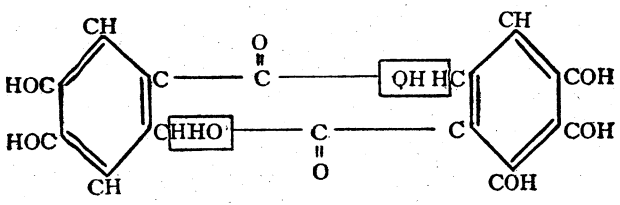
has more intense dyeing properties than alizarin, 1, 2, dihydroxy derivative."

Of greater interest to the biochemist, however, than the relation of number and position of hydroxy groups to the color and dyeing properties of the compound is the coexistence of a number of these closely related compounds in the same or closely related plants and the possibility of the formation of one from another, or of all of them from simpler products of plant metabolism. From the root of *Oldenlandia umbellata* there have been isolated monohydroxy -2- anthraquinone; alizarin, dihydroxy -1, 2--anthraquinone and its monomethyl ether; hystazarin, dihydroxy -2, 3- anthraquinone and its monomethyl ether; anthragallol, -1, 2, 3- trihydroxy anthraquinone and three of its dimethyl ethers (A. B. C.). From *Rubia tinctorium* there have been isolated alizarin, dihydroxy -1, 2- anthraquinone; xanthopurpurin dihydroxy -1, 3- anthraquinone; purpurin, trihydroxy

1-, 2, 4-anthraquinone; rubiadin, methyl -1-dihydroxy -2, 4-anthraquinone; and pseudo purpurin, trihydroxy -1, 2, 4-methyl -2- anthraquinone, all as glucosides. In several other instances several of these anthraquinone derivatives are known to exist side by side in the plant. In *Rheum officinale* are found emodin, isoemedin, rhein and chrysophanic acid, while in *Rhamnus purshiana* are found emodine, chrysophanic acid and chrysarobin, a reduction product of chrysophanic acid. Of how the plants build up any or all of these related compounds, or pass from one to the other, nothing appears to be known. By the aid of structural formulae it can be shown how the plant might be able to synthesize anthraquinone from two molecules of carbonic acid and two of benzene.



By the substitution of phenols or homologues of benzene for one or both of the benzene molecules the various anthraquinone pigments might be formed. Unfortunately for the probability of any such hypothesis little or nothing is known of the volatile constitutents of the anthraquinone producing plants. A large number of them contain tannic acid, gallic acid, and cinnamic acid however. By the condensation of two molecules of gallic acid a molecule of the anthraquinone configuration with six hydroxy groups would result.

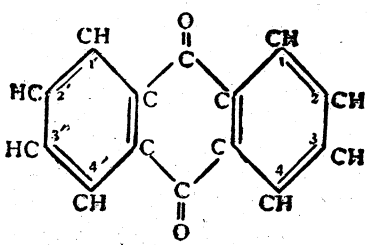


Unfortunately, again, such an anthraquinone derivative has not been isolated from plants. By the substitution of a mole-

cule of benzoic acid for one of gallic acid anthragallol results, and by substituting benzoic acid or its homologues, and various hydroxy benzoic acids for the gallic acid molecules any of the anthraquinone derivatives might be produced, just as any of the xanthone derivatives might be obtained by condensation of a molecule of benzoic acid or its derivatives with a phenol or of two molecules of phenols with one of carbonic acid.

II. A. *Pigments referable to dihydroanthracene.*

Six plant pigments of known constitution are referable to dihydroanthracene as the underlying hydrocarbon. These are all anthraquinone pigments, being mono- di- and tri- hydroxy derivatives of anthraquinone. The relation of anthraquinone to dihydroanthracene is shown on p. 870. The position of the hydroxy groups is here indicated by numbers in the usual way.



Anthraquinone

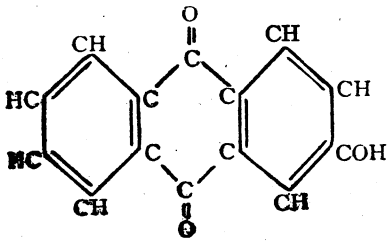
1.) Monohydroxyanthraquinones
Monohydroxy -2- anthraquinone

2.) Dihydroxyanthraquinones
a.) Alizarin
b.) Hystazarin
c.) Xanthopurpurin

3.) Trihydroxyanthraquinones.
a.) Anthragallol
b.) Purpurin

II. A. 1.) *Monohydroxy anthraquinone pigments.*

Of this group of dihydroanthracene derivatives only one representative, the monohydroxy -2- anthraquinone, is known.



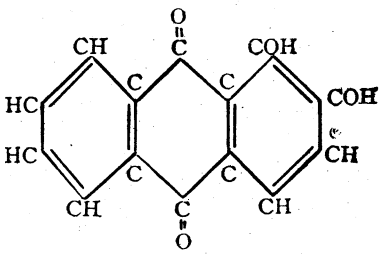
Monohydroxy -2- anthraquinone was first isolated from *Oldenlandia umbellata* by Perkin and Hummel[1] in 1893. It crystallizes in glistening yellow needles which melt at 302°. Solutions of the alkali hydrates dissolve it, forming a red liquid from which it separates, when very concentrated, in thin red plates of the corresponding salts. Sulphuric acid dissolves it with a red color.

Monohydroxy -2- anthraquinone does not combine with mordants to form a dye.

II. A. 2.) *Dihydroxy anthraquinone pigments.*

Of this group of dihydroanthracene derivatives three representatives have been isolated from plants. These are dihydroxy -1, 2- anthraquinone, alizarin; dihydroxy -2, 3- anthraquinone, hystazarin; and dihydroxy -1, 3- anthraquinone, xanthopurpurin.

Alizarin—Dihydroxy -1, 2- anthraquinone.



[1] Jr. Chem. Soc., 63, p. 1178; 67. 820.

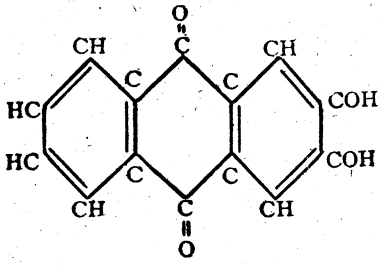
Alizarin, the first known pigment of this group was discovered by Colin and Robiquet[1] in 1826 in the rhizom of *Rubia tinctorium* where it exists principally as the glucoside ruberythric acid. This glucoside was isolated by Rochleder[2] and Schunk[3] almost simultaneously in 1851. The relationship of alizirin to anthracene was recognized by Graebe and Liebermann[4] when they obtained anthracene by the reduction of alizarin. After a further study of the properties of alizarin they were able to pronounce it a derivative of anthraquinone. In 1869 they effected a synthesis of the compound.

Alizarin occurs in the rhizom of *Oldenlandia umbellata,*[5] and *Rubia tinctorium.*[1]

Alizarin crystallizes in red needles which melt at 289°–290°. It sublimes in orange red needles. It is easily soluble in alcohol and carbon disulphide but difficultly soluble in water. It dissolves in alkaline solutions with a violet blue color. Sulphuric acid dissolves it unchanged: Alizarin combines with most mordants. To cotton mordanted with aluminum it gives a garnet red color; with tin, a light red; with iron, violet; with chromium, a brownish purple color.

o–*Methyl alizarin*—The methyl ether of alizarin occurs with alizarin in the root of *Oldenlandia-umbellata,*[6] and in *Morinda longiflora.*[7] It crystallizes in orange colored crystals which melt at 178°. It does not dye mordanted fabrics, but it dissolves in solutions of the alkalies, also barium and calcium hydroxide with a red color.

Hystazarin, 2, 3- Dihydroxyanthraquinone.



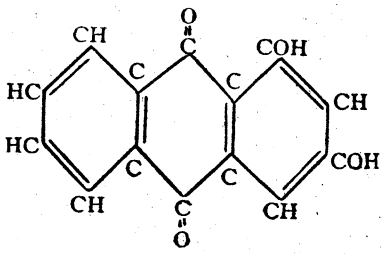
[1] Ann. chim. phys., (2) 34, p. 225.
[2] Ann., 80, p. 321.
[3] Ann., 81, p. 336.
[4] Ber., 2, p. 332.
[5] Proc. Chem. Soc., 23, p. 288.
[6] Jr. Chem. Soc., 64, p. 1160.
[7] Jr. Chem. Soc., 91. p. 1913; Proc. Chem. Soc., 23, p. 248.

Hystazarin exists in the Chay root, *Oldenlandia umbellata*,[1] in the form of its monomethyl ether.

Hystazarin crystallizes in orange yellow needles which melt at 260°. It is difficultly soluble in hot alcohol, ether, acetone, and acetic acid; insoluble in benzene and toluene; soluble in solutions of alkalies with a blue color, of ammonia with violet, and of acids with a red color. It forms a dark violet calcium[2] salt and a dark blue barium salt. It is not satisfactory as a dye.[3]

Hystazarin monomethyl ether crystallizes in orange yellow needles which melt at 232°. It is soluble in alkalies with a red color.

Xanthopurpurin or Purpuroxanthin.



Xanthopurpurin the dihydroxy -1, 3- anthraquinone exists in the rhizome of *Rubia tinctorium*.[1]

Xanthopurpurin crystallizes in yellow needles and sublimes in yellowish red needles. It melts at 262°–263°. It is easily soluble in alcohol, benzene, and acetic acid. By heating with alkali in contact with air it is transformed into purpurin, trihydroxy -1, 2, 4- anthraquinone. Xanthopurpurin imparts a rather fugitive yellow color to fabrics mordanted with aluminum.

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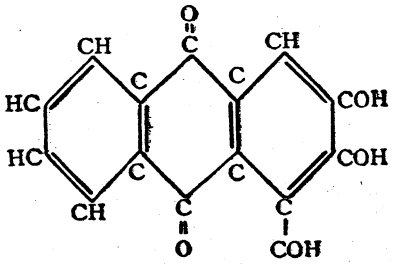
[1] Proc. Chem. Soc., 23, p. 228; Jr. Chem. Soc., 63, p. 1160.
[2] Ber., 28, p. 118.
[3] Ber., 35, p. 1778; 21, p. 2501.
[1] Bull. Soc., Chim., 4, p. 12.
[2] Ann. de Chim. et de Phys., (5) 18, p. 224.

II. A. 3.) *Trihydroxyanthraquinone pigments.*

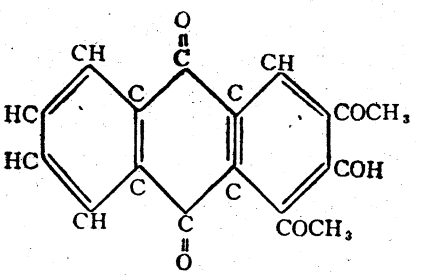
Of this group of dihydroanthracene derivatives two representatives are known in plants, trihydroxy -1, 2, 3- anthraquinone or anthragallol, and trihydroxy -1, 2, 4- anthraquinone or purpurin.

Anthragallol—Trihydroxy—1, 2, 3—anthraquinone.



Anthragallol exists in the Chay root, *Oldenlandia umbellata,*[1] in the form of three different dimethyl ethers, the dimethyl-1, 3-ether, known as the A ether, the dimethyl-1, 2-ether, known as the B ether, and the dimethyl-2, 3-ether, known as the C ether.[2]

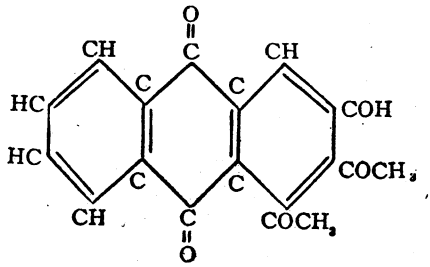
Anthragallol itself forms orange red crystals and is an excellent dye stuff, producing the anthracene brown of commerce. It's monomethyl-3-ether no longer colors mordanted fabrics brown, but in shades of red similar to those produced by alizarin. The two dimethyl ethers have no dyeing properties.



Anthragallol dimethyl ether A, Dimethyl-1,-3-hydroxy-2-anthraquinone.

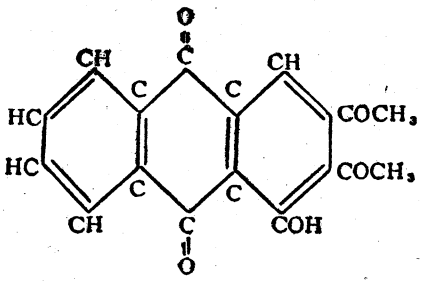
[1] Jr. Chem. Soc., 63, p. 1160; 91, p. 2066.
[2] Jr. Chem. Soc., 67, p. 826.

This compound is found in the root of *Oldenlandia umbellata.*[1] It crystallizes in yellow needles which melt at 209°. It is slightly soluble in alcohol and acetic acid, insoluble in chloroform and carbon disulphide. In solutions of alkaline carbonates it dissolves with a bright red color. It has no dyeing properties.



Anthragallol dimethyl ether B, Dimethyl-1, 2-hydroxy-3-anthraquinone.

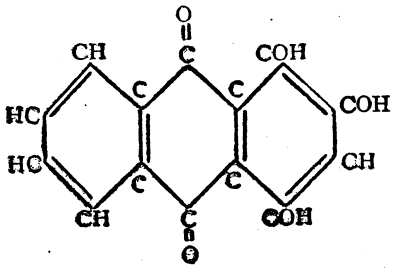
This compound occurs also in the root of *Oldenlandia umbellata.* It crystallizes in long pale straw colored crystals which melt at 230°–232° and are difficultly soluble in alcohol, acetic acid, and ether; but soluble in alkali solutions with a red color.



Anthragallol dimethyl ether C, Dimethyl-2, 3-hydroxy-1-anthraquinone.

This third dimethyl ether also occurs in the root of *Oldenlandia umbellata.* It forms a barium salt melting at 212°–213° and a lead salt.

Purpurin,—Trihydroxy-1, 2, 4-anthraquinone.



Purpurin exists in *Rubia tinctorium*[1] and other species of *Rubia*,[2] probably as a glucoside, along with alizarin. Purpurin crystallizes in long orange yellow crystals which melt at 253°. It is soluble in water with a deep yellow color, soluble in ether and carbon disulphide, acetic acid and hot benzene; but almost insoluble in alkaline solutions. It imparts to fabrics mordanted with aluminum a violet red color, with iron a violet blue, and with chromium a reddish brown color. These colors are not so permanent as those given by alizarin.

II. B.) *Pigments referable to the homologues of dihydroanthracene.*

The plant pigments referable to the homologues of dihydroanthracene are derivatives of two different monomethyl ethers of dihydroanthracene, the methyl-1-anthraquinone and the methyl-2-anthraquinone. Of the former five representatives and of the latter three representatives are found in plants.

1. Methyl-1-anthraquinone.
   - a.) Dihydroxy methyl-1-anthraquinones.
     - Rubiadin.
     - Chrysophanic acid.
   - b.) Trihydroxy methyl-1-anthraquinones.
     - Emodin.
     - Aloeemodin.
   - c.) Penthydroxy methyl-1-anthraquinones.
     - Rhein.

[1] Ann., 2, p. 34; Jr. prakt. Chem., 5, p. 366; Ann., 66, p. 351.
[2] Jr. Chem. Soc., 63, p. 1157.

2. Methyl-2-anthraquinone.
   a.) Trihydroxy methyl-2-anthraquinones.
   Morindon.
   b.) Hexhydroxy methyl-2-anthraquinones.
   Pseudo purpurin.

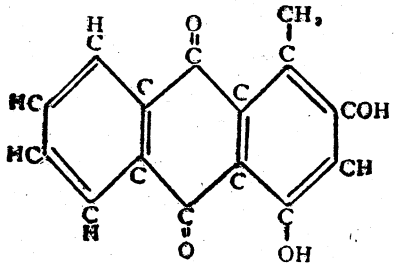
II. B. 1.) *Pigments referable to methyl-1-anthraquinone.*

Five pigments of known constitution are referable to methyl-1-anthraquinone. These are rubiadin, a dihydroxy-2, 4-methyl-1-anthraquinone; chrysophanic acid, a dihydroxy-1′, 4′-methyl-1-anthraquinone; emodin, a trihydroxy-3, 1′, 4′-methyl-1-anthraquinone; aloeemodin, a trihydroxy-3, 4′, 5-methyl-1-anthraquinone, and rhein, a dihydroxy-3, 4′-carboxy-1-anthraquinone.

Dihydroxides of methyl-1-anthraquinone.

Two pigments which are dihydroxides of methyl-1-anthraquinone are known to exist in plants. These are rubiadin, dihydroxy-2, 4-methyl-1-anthraquinone, and chrysophanic acid, dihydroxy-1′, 4′-methyl-1-anthraquinone. As would be expected from their similar constitutions the two compounds resemble each other quite closely in properties, though chrysophanic acid has the better dyeing properties. The fact that chrysophanic acid possesses dyeing properties appears to be an exception to Liebermann's rule regarding the relation between dyeing properties and the number and position of hydroxy groups, since of the three structural formulae assigned to it by different investigators none have the two hydroxy groups in relatively 1, 2, positions, while the formula which appears to be preferred at present has its hydroxy group in 1′, 4′, relatively para position, a position which is supposed to give no dyeing properties to anthraquinone derivatives.

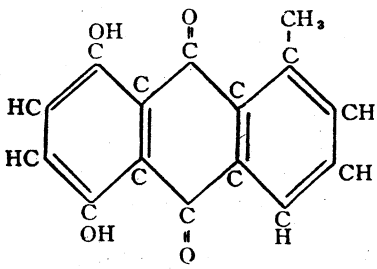
Rubiadin,—Dihydroxy-2, 4-mtehyl-1-anthraquinone.



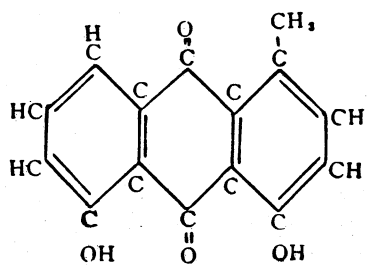
Rubiadin occurs as a glucoside in the root of *Rubia tinctorium.*[1] It crystallizes in yellow needles which melt at 290°. It is easily soluble in alcohol, ether, and benzene, but insoluble in water and carbon disulphide, also in lime water. In solutions of alkalies it dissolves with a red color.

Chrysophanic acid,—Dihydroxy-1', 4'-methyl-1-anthraquinone (?).

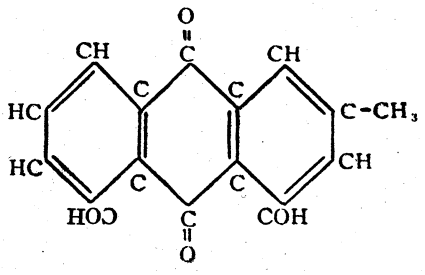
I.



II.



III.



Chrysophanic acid occurs in the root of *Rheum officinale,*[1] *Rheum rhaponticum,*[2] *Rumex obtusifolius,*[3] *Rumex ecklonianus,*[3] *Cassia angustifolia,*[5] *Cassia speciosa,*[4] *Rhamnus purshiana,*[7] *Rhamnus japonica,*[6] *Rhamnus frangula,*[6] and *Tecoma ochraceae.*[8]

Chrysophanic acid crystallizes in yellow leaflets and melts at 196°. It is insoluble in water, soluble in alcohol, ether, acetone, benzene, chloroform, and petroleum ether. These so-

[1] Jr. Chem. Soc., 63, p. 969, 1137; 65, p. 182; Chem. News, 67, p. 299.
[1] Ann., 309, p. 32; Arch. d. Pharm., 245, p. 680; Ann., 9, p. 85; 50, p. 196; 107, p. 324.
[2] Berl. Jahres., 23, p. 252; Jahresb. f. Pharm., 1882, p. 262.
[3] Jr. Chem. Soc., 97, p. 1.
[4] Arch. Pharm., 184, p. 37.
[5] Chem. Centralbl., 1864, p. 622; Jr. Pharm. Chim., 12, p. 505.
[6] Apoth. Ztg., 15, p. 537; 16, p. 257, 538; 17, p. 372.
[7] Proc. A. Ph. A., 52, p. 288.
[8] Z. oester. Apoth. Ver., 12, p. 31.

lutions color animal tissues deep yellow. It is soluble in solutions of alkaline hydroxides with a red color. Chrysophanic acid colors unmordanted silk and wool yellow. Wools mordanted with aluminum are colored orange red; with chromium, bright red; and with iron, bright brown.

The constitution[9] of chrysophanic acid is probably as indicated in formula I. above. This formula was first suggested by Hesse in 1899, and afterwards by Jowett and Potter in 1903. Attention should here be called to the fact that none of the above formulae are in accord with Liebermann's rule for a colored molecule.

The principal investigations of chrysophanic acid are mentioned in the following list:

Literature on Chrysophanic Acid.

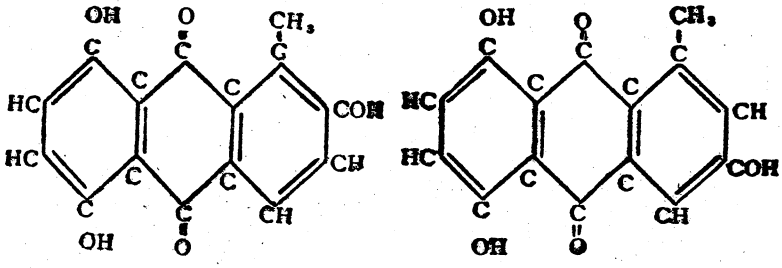
Aweng, — Pharm. Centralbl., 1898, p. 776; Apoth. Ztg., 15, p. 537; 17, p. 372.
Brandes, — Ann., 9, p. 85.
Dulk, — Arch. d. Pharm., 17, II. p. 26.
Geiger, — Ann., 9, p. 91.
Gilson, — Arch. internat. de Pharm et Therap., 11, p. 487.
Grandis, — Jahresb. d. Chem., 1892 p. 1654.
Grothe, — Chem. Centralbl., 1862, p. 107.
Hesse, — Ann., 291, p. 306; 309, p. 32.
Jowett and Potter, — Jr. Chem. Soc., 81, p. 1528; 83, p. 1327.
Le Prince, — C. r., 129, p. 60.
Liebermann, — Ann., 183, p. 169; 212, p. 36; Ber., 11, p. 1607.
Limousin, — Jr. de Pharm et de Chem., 1885, p. 80.
Marfori, — Chem. Centrlbl., 1900, I. p. 1292.
Oesterle, — Arch. d. Pharm., 243, p. 434.
Pelz, — Jahresber. d. Chem., 1861, p. 392.
Rochleder, — Ber., 2, p. 373.
Rue and Mueller, — Jahresber d. Chem., 1857, p. 516.
Rupe, — Chem. der nat. Farbs., 2, p. 117.
Schoeller, — Ber., 32, p. 683.
Scholzberger, — Ann., 50, p. 196.
Thann. — Ann., 107, p. 324.
Tschirch and Cristofoletti, — Arch. d. Pharm., 243, p. 434.

[9] Jr. Chem. Soc., 83, p. 1327; Ann., 309, p. 32.

Tschirch and Heuberger, — Arch. d. Pharm., 240, p. 605.
Tschirch, — B., 8, p. 189.
Tutin and Clewer, — Jr. Chem. Soc., 97, p. 1.
Vogel, — Arch. d. Pharm., 134, p. 37 (1868).

Trihydroxides of menthyl-1-anthraquinones.

Two trihydroxides of methyl-1-anthraquinone, emodin, and aloeemodin, are known to exist in plants. These two isomeric pigments occur together in various species of aloes and senna, along with chrysophanic acid, of which emodin is a hydroxy substitution product.



Emodin,[1] a methyl -1-trihydroxy-2, 1′, 4′-anthraquinone, or methyl-1-trihydroxy-3, 1′, 4′-anthraquinone is an hydroxy substitution product of chrysophanic acid.

Emodin occurs in various species of aloe,[2] including *Aloe ferox*,[3] *Aloe vulgaris*,[4] and *Aloe chinensis*;[5] in *Rheum officinale*,[6] *Rheum palmatum*,[7] *Polygonum cuspidatum*,[8] *Cassia occidentalis*,[9] *Cassia sophora*,[9] *Cassia tora*,[10] *Cassia angustifolia*,[9] *Xanthoxylon tingoassuiba*,[11] *Rhamnus cathartica*,[12] *Rhamnus japonica*,[13] *Rhamnus purshiana*,[14] and *Rhamnus frangula*,[15]

[1] Jr. Chem. Soc., 83, p. 1327.
[2] Jr. Pharm. Chim., 28, p. 529.
[3] B. Pharm. Ges., 1898, p. 174.
[4] Arch. Pharm., 236, p. 200.
[5] Arch. Pharm., 241, p. 340.
[6] C. r., 136, p. 385.
[7] Ber., 1882, p. 902; Pharm. Jr. Trans., 15, p. 136.
[8] Bull. Sci. Pharm., 14, p. 698.
[9] Apoth. Ztg., 1896, p. 537.
[10] Pharm. Jr. Trans., 3, p. 242.
[11] B. Pharm. Ges., 9,, p. 162.
[12] Jr. Russ. Phys. Chem. Ges., 40, p. 1502.
[13] Apoth. Ztg., 1896, p. 537.
[14] Arch. Pharm., 246, p. 315; Jr. Pharm. Chem., 246, p. 315.
[15] Ber., 9, p. 1775; Pharm. Jr., 20, p. 558; Arch. Pharm., 246, p. 315.

It will be seen from the above that emodin not only resembles chrysophanic acid in constitution but closely accompanies it in the plant as well. Emodin occurs both free and as a glucoside. It crystallizes in silky needles of an orange red color which melts at 250°. It is soluble in alcohol, amyl alcohol, and acetic acid, slightly soluble in benzene, and soluble in alkalies and ammonia with a red color. With sulphuric acid it gives an intense red solution which turns yellow, separates a flocculent precipitate, and becomes colorless upon standing.

A considerable number of derivatives of emodin have been prepared.

The principal investigators of emodin are listed below.

Combes, — Bull. de Soc. Chem., (4) 1, p. 800.
Hesse, — Ann., 284, p. 194; 309, p. 41.
Krassowski, — Jr. d. russ. phys. chem., Ges., 40, p. 510; Chem. Centrlbl. 1919, I. p. 773.
Le Prince, — C. r., 129, p. 60.
Liebermann, — B., 9, p. 1775; 21, p. 436.
Oesterle, — Arch. d. Pharm., 237, p. 699.
Oesterle and Tisza, — Arch. d. Pharm., 246, p. 112, 432; Chem. Centrlbl. 1908, I. p. 1548; II. p. 1441.
Tschirch and Pool, — Arch. d. Pharm., 246, p. 315.
Warren — Jr., Chem. Soc., 10, p. 100.
Rochleder, — B., 2, p. 373.

Frangulin.[1]—A glucoside of emodin, known as frangulin, occurs in the bark of *Rhamnus frangula.*[2] It crystallizes in lemon yellow crystals which melt at 226°. It is insoluble in water and in ether, soluble in alcohol and in benzene. Upon hydrolysis it yields emodin and rhamnose.[3]

Polygonin.—A second glucoside of emodin, known as polygonin, occurs in the root of *Polygonum cuspidatum.*[4] It crystallizes in fine orange yellow needles which melt at 202°–203°. It is insoluble in water, difficultly soluble in alcohol, also in hot water. When hydrolized it yields emodin and a sugar.

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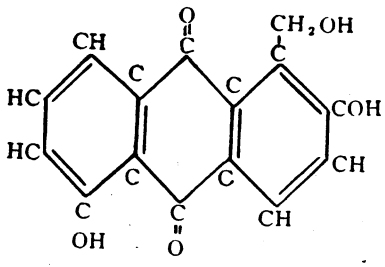
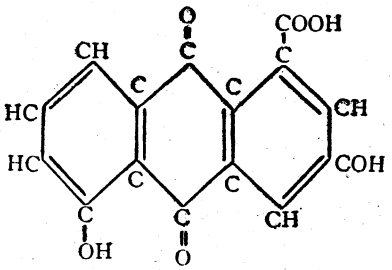
[1] C. r., 134.
[2] Rep. f. Pharm., 104, p. 151; Ann., 104, p. 77.
[3] Ann., 165, p. 230.
[4] Jr. Chem. Soc., 67, p. 1084.

Aloeemodin.—Trihydroxy-2, 4', 5-methyl-1-anthraquinone.



Aloeemodin, a primary alcohol, occurs with aloin in various species of aloes,[1] senna[2] and rhubarb.[3] It crystallizes in orange red needles which melt at 224°. It is easily soluble in ether, hot alcohol, and benzene, soluble in concentrated sulphuric acid with a cherry red color. Aloeemodin yields a triacetyl and a tribenzoyl derivative. Upon reduction it forms methyl anthracene. Oxidized with chromic acid mixture it yields rhein.



Since rhein is produced upon the oxidation of aloeemodin, one of its hydroxy groups must be in the side chain. The positions of the other hydroxy groups is uncertain. Robinson and Simonson[4] have suggested for it the formula given above.

Penthydroxides of methyl-1-anthraquinone.

Only one penthydroxide of methyl-1-anthraquinone is known to exist in plants. This is rhein, a dihydroxyanthraquinone carboxylic acid. Rhein is an oxidation product of aloeemodin which it accompanies in several species of aloes, and also of rhubarb.

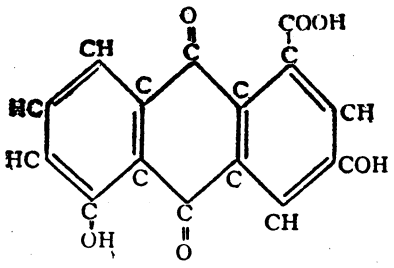
[1] Whemer, Die Pflanzenstoffe, p. 90; C.r., 150, p. 983; Arch. f. Pharm., 247, p. 413.

[2] Rupe, Natuerliche Farbstoffe, 2, p. 134.

[3] Arch. Pharm., 243, p. 443; 247, p. 413.

[4] Proc. Chem. Soc., 25, p. 76; Jr. Chem. Soc., 95, p. 1085.

Rhein.—Dihydroxy-3, 4′-carboxyl-1-anthraquinone.



Rhein occurs in *Rheum officinale;*[1] English rhubarb;[2] *Rheum rhaponticum;*[3] *Rheum palmatum;*[4] and *Aloe vulgaris.*[5] It may be formed by oxidation of Aloeemodin which occurs with it in several species of Rhubarb.

Rhein crystallizes in small yellow needles which melt at 321°–322°. It is difficultly soluble in most ordinary solvents. It is soluble in concentrated sulphuric acid with a red color, also soluble in ammonia with a red color, upon exposure to the air this color goes through violet into blue. In dilute alkaline solutions it is readily soluble. Acids precipitate it from these solutions as a yellow mass. It forms esters[6] and ethers,[6] the former with alcohol, the latter with dimethyl sulphate in the presence of potassium hydroxide. It dyes wools mordanted with chromium a yellow color.[7]

The structural formula given is that suggested by Robinson and Simonsen.[6]

II. B. 2.) *Pigments referable to methyl-b-anthracene.*

a. Trihydroxides of methyl-2-anthraquinone.

One pigment which is a trihydroxide of methyl-2-anthraquinone is known to exist in plants, this is morindon.

b. Hexhydroxides of methyl-2-anthraquinone.

One pigment, pseudo purpurin, which is a hexhydroxide of methyl-2-anthraquinone, is known to exist in plants.

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[1] Pharm. Post., 37, p. 233, Arch. Pharm., 245, p. 150.
[2] Arch. Pharm., 245, p. 141.
[3] Arch. Pharm., 243, p. 443.
[4] Schweiz. Wochenschs. Pharm., 1904. Nr. 40.
[5] Arch. Pharm., 247, p. 413.
[6] Jr. Chem. Soc., 95, p. 1085.
[7] Rupe. Natuerliche Farbstoffe, 2, p. 143.

Trihydroxides of methyl-2-anthraquinone.

Morindon,—A trihydroxy methyl-2-anthraquinone, isomeric with emodin, occurs in the rind of the root of *Morinda citrifolia,*[1] *Morinda umbellata,*[2] and *Morinda tinctoria,*[3] along with the glucoside morindin and other similar coloring principles. It was first isolated by Anderson[1] in 1849. It has sometimes been mistaken for alizarin which it resembles in many of its properties.

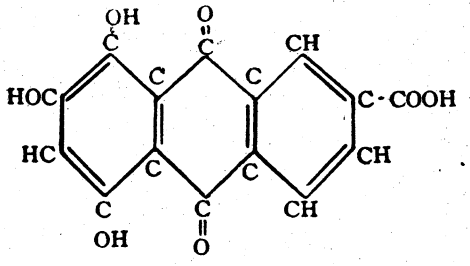
Morindon crystallizes in reddish brown crystals and sublimes in long orange red needles. It melts at 272°. It is easily soluble in alcohol ether, ethyl acetate, benzene and similar hydrocarbons. Ferric chloride colors a solution of morindon dark green, alkalies a violet blue. Morindin is soluble in concentrated sulphuric acid with a violet blue color.

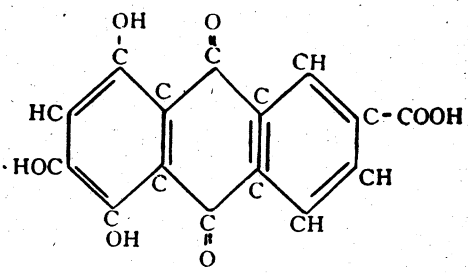
Literature on Morindin and Morindon.

Anderson, — Ann., 71, p. 216.
Oesterle and Tisza, — Arch. d. Pharm., 245, p. 534.
Perkin and Hummel, — Jr. Chem. Soc., 65, p. 851.
Rochleder, — Ann., 82, p. 205.
Steenhouse, — Jahresber. d. Chem., 97, p. 234.
Stockes, — Jahresber. d. Chem., 17,p. 543.
Stockes and Stein, — Jahresber. d. Chem., 19, p. 645.
Thorpe and Greenall, — Jahresber. d. Chem., 40, p. 2299.
Thorpe and Smith, — Jahresber. d. Chem., 40, p. 2363.
Tschirch, — Arch. d. Pharm., 222, p. 129.
Tunmann, — Chem. Centralbl., 1909. I. p. 199.

Hexhydroxides of methyl-2-anthraquinone.

Pseudopurpurin,—Trihydroxy-1, 2, 4-carboxyl-2′-anthraquinone, or Trihydroxy-1, 2, 4-carboxyl-3′-anthraquinone.





[1] Ann., 71, p. 216; 82, p. 205; Chem. News.,: 54, p. 293.
[2] Jr. Chem. Soc., 63, 1160; 65, 851.
[3] Whemer, Die Pflanzenstoffe, p. 737.

Pseudo purpurin occurs along with purpurin in the root of *Rubia tinctorium*,[1] It comprises a large part of the purpurin of commerce. The constitution of the molecule does not appear to have been yet definitely established. From the work of Rosenstiehl,[2] also that of Liebermann and Platt,[3] we learn the number and relative positions of the hydroxy groups. Perkin[4] has shown that the carboxyl group is in the second benzene nucleus, corresponding to one of the formulae given above.

Pseudo purpurin crystallizes in small red leaflets. It melts at 218°–219°. It is almost insoluble in water and in alcohol, difficultly soluble in chloroform and hot benzene, easily soluble in solutions of alkaline carbonates with an orange color. Its dyeing properties are almost identical with those of purpurin.

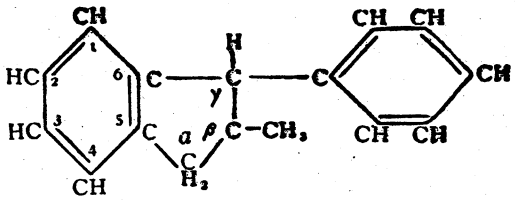
The Aloins.

Substances crystallizing in yellow needles and soluble in concentrated sulphuric acid with a red, in alkaline hydroxides and carbonates with an orange color, are found in various species of aloes. These are known as aloins. The formula of aloin has been variously given as $C_{16}H_{16}O_7$, $C_{16}H_{18}O_9$, $C_{17}H_{18}O_7$. According to Jowett and Potter who have performed some of the most recent work upon aloin, $C_{16}H_{18}O_7$ is probably correct.

The aloins, known as aloin, barbaloin, isobarbaloin, and nataloin are closely related to the anthraquinone pigments. Jowett and Potter think, however, that instead of the anthraquinone nucleus being present there is probably a reduced anthraquinone nucleus.

Upon treating aloin with sodium peroxide aloeemodin is produced.

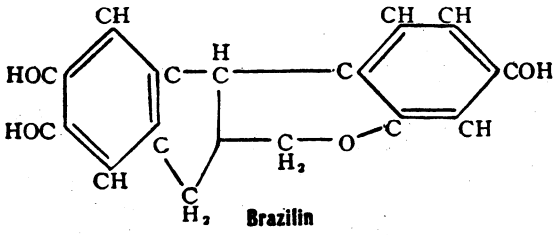
III. PIGMENTS REFERABLE TO PHENYL-HYDRINDINE AND HOMOLOGUES.



β -methyl- γ -phenyl hdyrindine

[1] Bull. Soc. Chim., 4, p. 12.
[2] C. r., 79, p. 680; 84, p. 559, 1902.
[3] Ber., 10, p. 1618.
[4] Jr. Chem. Soc, 65, p. 842.

No pigments referable to the above hydrocarbons are found in plants; but two pigment forming substances, brazilin and haematoxylon, which upon oxidation yield the pigments brazilein and haematein, are referable to it. The pigments themselves are referable to an isomer of methyl-phenyl hydrindine, falling under the same degree of saturation.



Brazilin

Brazilin was first discovred by Chevreul,[1] in 1808, in the heart wood of *Cisalpina echinata* where it exists in the form of a glucoside. It was not until one-hundred years later, however, that its constitution was definitely established when Perkin,[2] in 1908, after a long series of investigations, by the synthesis of brazilinic acid and other derivatives of brazilin, showed the formula to be that given above. Besides in *Cisalpina echinata,* brazilin occurs in another species of *Cisalpina, C. sappari.*[3] According to Rupe[4] a number of woods, known as red woods, employed as dyestuffs contain brazilin. These are all the products of varieties of *Cisalpinia* species and are known as Fernanabose or Brazil wood, Bahia red wood; St. Martha wood; Nicaragua wood, Sapan wood, Lima wood and Braziliette wood.

Brazilin crystallizes in colorless crystals which color readily upon exposure to the air. It is soluble in water, alcohol and ether, these solutions color quickly upon exposure to the air.

Brazilin and its derivatives have been the subject of a large number of chemical investigations, the principal ones of which are listed below.

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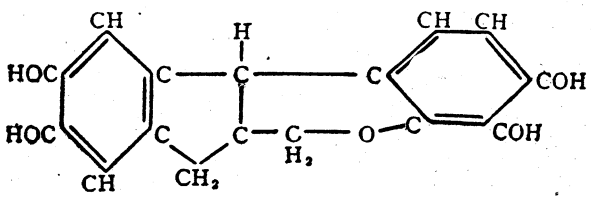
[1] Ann. Chim. et Phys., 66, p. 225.
[2] Proc. Chem. Soc., 79, p. 1396; 81, p. 221, 235, 1008; 91, 1073; 93, p. 489.
[3] Ber., 5, p. 572.
[4] Chemie der natuerlichen Farbestoffe., 1, p. 224.

Literature on Brazilin.

Benedict, — Ann., 178, p. 100.
Bolley, — Schweiz polytech. Zeit., 9, p. 267.
Buchka, — Ber., 17, p. 685; 18, p. 1140.
Chevreul, — Ann. Chem. Phys., 66, p. 225.
Dralle,—Ber., 17, p. 375; 20, p. 3365; 21, p. 3009; 22, p. 1547; 23, p. 1430; 25, p. 3670; 27, p. 527.
Herzig, — Montsh. f. Chem., 19, p. 738; 23, p. 241; 25, p. 871.
Herzig and Pollak, — B. 36, p. 398.
Kostanecki, — Ber., 35, p. 1674; 36, p. 2202.
Liebermann and Burz, — B. 9, p. 1885.
Perkin, — Jr. Chem. Soc. 79, p. 1396; 81, p. 225, 1008, 1057; 91, p. 1073; 93, p. 489, 1115; 95, p. 385.
Rein, — Ber., 4, p. 334.
Schall, — B. 27, p. 529; 35, p. 2306.

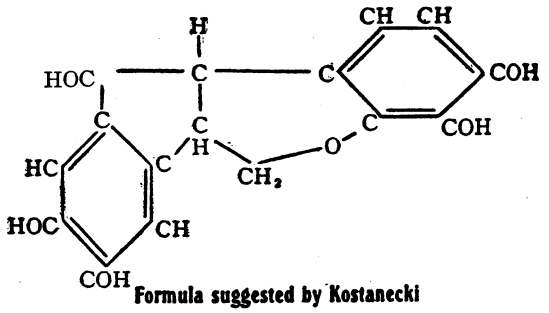


Haematoxlyn

Haematoxlyn was discovered by Chevreul,[1] in 1812, in the heart of *Haematoxylon campechianum.* It has also been reported in the bark of *Sancta indica,*[2] another leguminous plant.

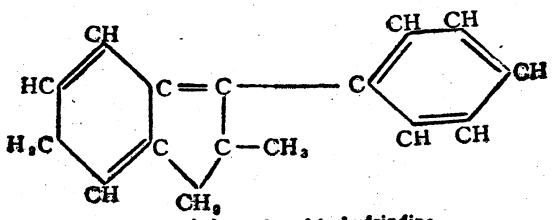
Haematoxylon, being a hydroxy brazilin, resembles it closely in physical and chemical properties. Its history also has been almost identical with that of brazilin since the work which proved the constitution of one compound proved also that of the other. According to Perkin[3] the formula of haematoxylon is as given above. Kostanecki and Lampe[4] have suggested another formula which differs only in the position of one benzene nucleus and one hydroxy group. The later formula of Perkin and his associates is probably to be preferred.

[1] Ann. Chim et Phys., 66, p. 225 (2) 82, p. 53, 126.
[2] Pharm. Post., 1887, p. 778.
[3] Jr. Chem. Soc., 93, p. 496.
[4] Ber., 35, p. 1674.



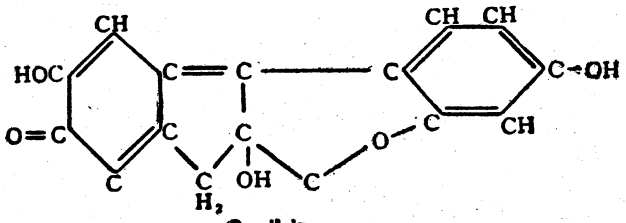
Formula suggested by Kostanecki

PIGMENTS REFERABLE TO β-METHYL-γ-PHENYL-ISOHYDRADINDINE.

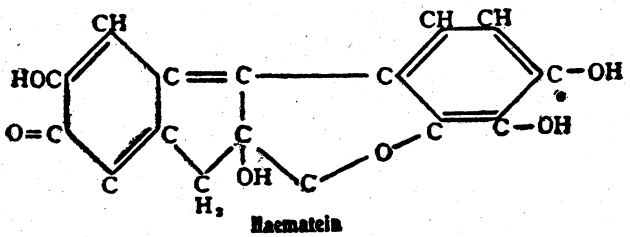


β - methyl-γ -phenyl isohydrindine

Two pigments, brazilein and haematein, oxidation products of brazilin and haematoxylon are referrable to the above hydrocarbon.



Brazilein



Haematein

Brazilein occurs along with brazilin in various species of "red wood," *Cisalpinia.* It crystallizes in microscopic reddish brown crystals with a metallic reflection. It is very slightly soluble in cold water, better in hot water. The solution is bright red with an orange fluorescence. It is soluble in alkaline solutions with a bright red color which turns brown upon standing in contact with the air.

Brazilein dyes fabrics mordanted with aluminum a blueish red; with chromium, grayish brown to violet gray; with tin, orange red; with iron and aluminum mixed, a dark purplish red.

Brazilein has been the subject of a large number of chemical investigations and a large number of derivatives have been prepared.

Literature on Brazilein.

Herzig, — Monatsh. f. Chem., 19, p. 739; 20, p. 461; 22, p. 207; 23, p. 165; 25, p. 734; 27, p. 743.
Kostanecki, — B., 32, p. 1042; 41, p. 2373.
Perkin, — Proc. Chem. Soc., 22, p. 132; 23, p. 291; 24, p. 54, 148; 93,, p. 489, 1115; 95, p. 381.
Liebermann, — Ber., 9, p. 1866.
Scholl and Dralle, — Ber., 17, p. 375; 20, p. 3365; 21, p. 3009; 22, p. 1547; 23, p. 1430; 25, p. 18; 27, p. 524.

Haematein.

Haematein occurs in the "blue wood" of *Haematoxylon campechianum,* forming the characteristic pigment of the logwood dye stuffs. It crystallizes in microscopic crystals of a reddish brown color with a yellowish green metallic reflection. It is very slightly soluble in water, difficultly soluble in alcohol, ether, and acetic acid, insoluble in chloroform and benzene. It is soluble in alkaline solutions, in sodium hydroxide with a bright red and in ammonia with a violet red color. It dyes fabrics mordanted with aluminum a grayish blue to black color; with iron, black; with chromium, blue black; with copper, greenish black and with tin, a violet color.

Literature on Haematein.

Baeyer, — Ber., 4, p. 457.
Erdmann, — Ann., 44, p. 294; 216, p. 236.
Halberstadt, — B., 14, p. 611.
Hesse, — Ann., 109, p. 337.
Mayer, — Chem. Centralbl., 1904, I. p. 228.
Perkin, — Ber., 15, p. 2337; Jr. Chem. Soc., 41, p. 368; 93, p. 1115; 95, p. 381.

PIGMENTS REFERABLE TO HYDROCARBONS OF THE DEGREE OF SATURATION C_nH_{2n-18}.

There occur in plants pigments referable to four hydrocarbons of four distinct structural configurations, falling under this degree of saturation, as follows:

I. Nine double bonds and one cycle.
The chlorophylls.
II. Eight double bonds and two cycles.
Pigments referable to diphenyl-1, 7-heptadiene-1, 6.
III. Seven double bonds and three cycles.
Pigments referable to phenyl-dihydronaphthalene.
IV. Six double bonds and four cycles.
Pigments referable to anthracene.

With the exception of the first configuration under which we find the two chlorophylls chlorophyll-a and chlorophyll-b, so far as is known only one pigment under each structural configuration has been isolated. These are II. Curcumin, III. Trifoletin, IV. Chrysarobin.

I. PIGMENTS REFERABLE TO HYDROCARBONS OF THE CONFIGURATION NINE DOUBLE BONDS AND ONCE CYCLE.

The Chlorophylls.

The chemistry of chlorophyll has attracted more attention and has been the subject of more investigations than that of any other plant pigment. This is due not only to the extremely wide distribution and abundant occurrence of chlorophyll in plants but also to its physiological importance and the role

which it appears to play in photosynthesis. Notwithstanding, however, the abundance of material available, the importance of the problem and the attention paid to it, up until quite recently, but little light has been thrown upon the subject and even now the chemistry of chlorophyll is far from being elucidated.

It is not at all the purpose of this paper to discuss the complex chemistry of chlorophyll, nor is this necessary in view of the very thorough revision of the subject by Marchlewski,[1] published in 1909, and the yet more recent one by Willstaetter[2] in 1913. A brief mention of the subject, however, is not out of place and seems desirable in order to place chlorophyll in its class among the plant pigments.

Chlorophyll, according to Willstaetter, is a complex magnesium compound, or rather, a mixture of at least two such complex compounds, the blue green chlorophyll-a, $C_{55}H_{72}O_5N_4Mg$, and the yellow green chlorophyll-b, $C_{55}H_{70}O_6N_4Mg$.

Both of these chlorophylls are esters of phytol, which is a constant constituent of chlorophyll. Phytol is an open chain primary alcohol of the formula of saturation C_nH_{2n}. The following structural formula has been suggested for phytol:

$$\begin{array}{l} CH_3—CH—CH—CH—CH—CH—CH—CH—C=C—CH_2\,O\,H \\ \quad\;\; |\quad\;\; |\quad\;\; |\quad\;\; |\quad\;\; |\quad\;\; |\quad\;\; |\quad\;\; |\quad\;\; | \\ \quad CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3 \end{array}$$

Making it, according to the Geneva Congress system of nomenclature, nonmethyl-2, 3, 4, 5, 6, 7, 8, 9, 10-undecene-2-ol-1.

By the action of an anzyme, chlorophyllase, chlorophyll-a is hydrolised yielding phytol and chlorophyllid-a

$C_{20}H_{39}OH$	$MgN_4C_{32}H_{30}OCOOCH_3COOH$
Phytol	Chlorophyllid-a

while chlorophyll-b yields phytol and chlorophyllid-b.

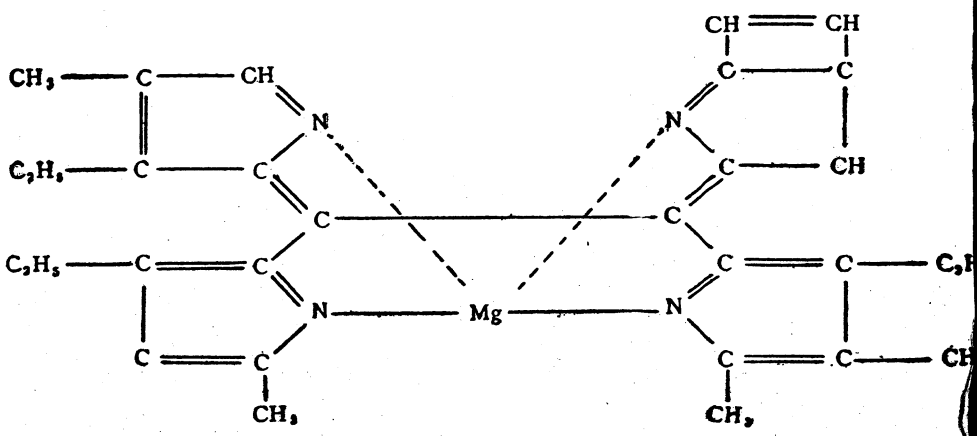
$C_{20}H_{39}OH$	$MgN_4C_{32}H\,O\,COOCH_3COOH$
Phytol	Chlorophyllid-b

Phytol is a non-colored substance and appears to play no direct part in pigmentation.

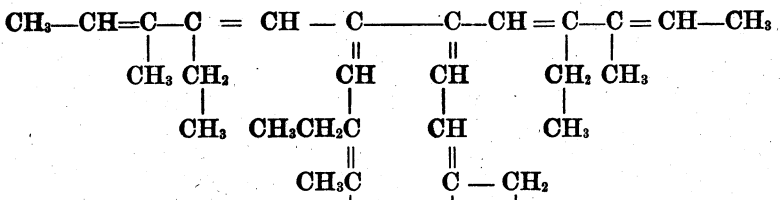
---

[1] Die Chemie der Chlorophyll, Braunschweig, 1909.
[2] Untersuchungen ueber Chlorophyll, Berlin, 1913.

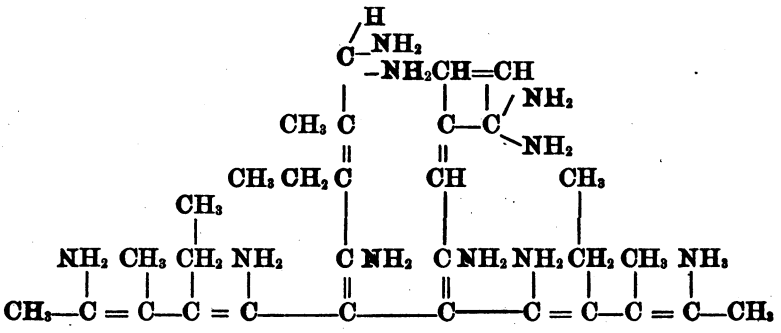
Chlorophyllid-a and chlorophyllid-b, heated with caustic alkalies, after passing through a number of intermediate stages, both yield aetiophyllin, $C_{31}H_{34}N_4Mg$, which appears to be the basic colored portion of the molecule. For aetiophyllin Willstaetter has suggested the following structural formula:



Referring aetiophyllin, as represented above, to the underlying hydrocarbon it is found to be derived from $C_{31}H_{44}$, a duodeactetrene derivative with six side chains, and falling under the formula of saturation C_nH_{n2-18} as below.



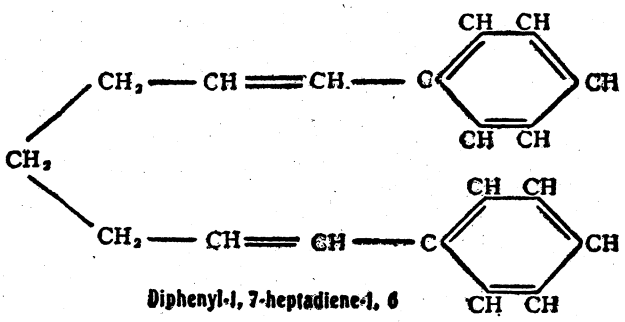
Aetiophyll itself is the product of the deammoniation and combination with magnesium, of a decamido substitution product of the above hydrocarbon.



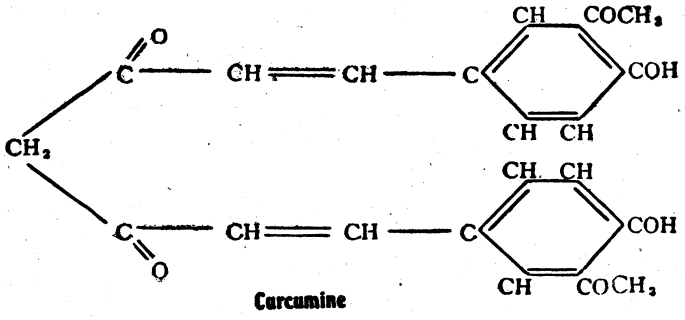
By deammoniation of the above compound and subsequent substitution of magnesium for imdio hydrogen aetiophyllin would be formed.

II. PIGMENTS REFERABLE TO HYDROCARBONS OF THE CONFIGURATION EIGHT DOUBLE BONDS AND TWO CYCLES.

Pigments referable to diphenyl- 1, 7 -heptadiene- 1, 6.

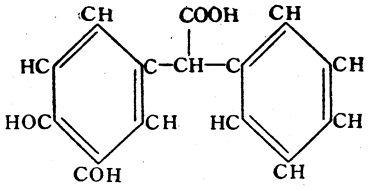


Diphenyl-1, 7-heptadiene-1, 6



Curcumine

Curcumine occurs in the rhizom of *Curcuma longa, C. viridiflora,* and probably in other species of *Zingiberaceae.* It was first obtained in the crystalline form by Daube,[1] in 1870, though it had been studied by Vogel and Pelletier[2] as early as 1815. In 1881 Jackson and Menke[3] made the first correct analysis of curcumine and ascribed to it the formula $C_{14}H_{14}O_4$. After making an extended study of its reactions they ascribed to it the structural formula,-



This formula was accepted until 1897 when Ciamician and Silber[4] concluded that the molecule contained two hydroxy and two methoxy groups and should be represented by the formula $C_{21}H_{30}O_6$ instead of $C_{14}H_{14}O_4$. Molecular weight determinations made by Perkin[5] and his associates in 1904 sustained the conclusion of Ciamician and Silber. Jackson and Clarke,[6] in 1905–1908, made another examination which they interpreted as proving the correctness of Jackson's earlier formula. In 1910 Milobendzki, Kostanecki, and Lampe[7] by a series of synthesis proved the correctness of Camician and Silber's formula, assigning to the molecule the structural formula given above. In 1914 Jackson and Clarke[8] by further work confirmed this formula.

Curcumine crystallizes in orange yellow crystals with a bluish reflection, and a melting point of 178°. It is insoluble in water and ligroin, almost insoluble in benzene, somewhat soluble

[1] Ber., 3, p. 609.
[2] Jr. de Pharm., 50, p. 259.
[3] Ber., 14, p. 485; 15, p. 1761; 17, (Ref.) p. 332.
[4] Ber., 30, p. 192; Gazz. chim. ital., 27, I. p. 561.
[5] Jr. Chem. Soc., 85, p. 63.
[6] Ber., 38, p. 2712; 39, p. 3269; Am. Chem. Jr., 39, p. 699.
[7] Ber., 43, p. 2163.
[8] Am. Chem. Jr., 45, p. 48.

in cold alcohol, ether and carbon disulphide. The solution in ether gives a green fluorescence. Its reddish brown reaction is well known.

In the course of the various investigations of which curcumine has been the subject a large number of derivatives have been formed.

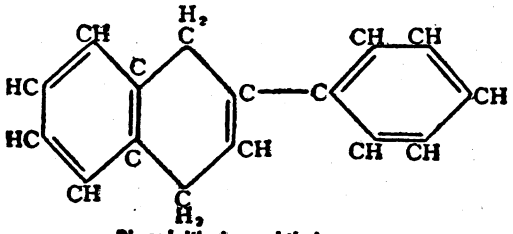
The more important of the many investigations of curcumine are given in the following list:

Literature on Curcumine.

Bolley, Suida and Daube, — Jr. prakt. Chem., 103, p. 474.
Ciamician and Silber, — B., 30, p. 192; Gazz. chim. ital., 27, I. p. 561.
Daube and Claus, — Jr. prakt. Chem., (2) 2, p. 86; B., 3, p. 609.
Iwanon, — Ber., 3, 624.
Jackson, — Ber., 14, 485.
Jackson and Mencke, — B., 15, 1761; Am. Jr. Chem., 4, 368.
Jackson and Mencke, — Pharm. Jr. Trans. III. 13, 839.
Jackson and Mencke, — Ber., (Ref.) 17, 332.
Jackson and Warren, — Am. Chem. Jr., 18, 111.
Jackson and Clarke, — Ber., 38, 2712.
Jackson and Clarke, — Ber., 39, 2269.
Jackson and Clarke, — Am. Chem. Jr., 39, 699.
Jackson and Clarke, — Am. Chem. Jr., 45, 48.
Kachler, — B., 3, 713.
Leach, — Jr. Chem. Soc., 26, 1210.
LePage, — Arch. Pharm. (2) 97, 240.
Milobendzki, Kostanecki and Lampe, — Ber., 43, 2163.
Perkin, — Jr. Chem. Soc., 85, 63.
Rupe, — Ber., 40, 4909.
Thompson, — Pharm. Jr. Trans. 23.
Vogel and Pelletier, — Jr. de Pharm., 50, 259.
Vogel, — Ann., 44, 297, B. Repert. Pharm., 27, 274.

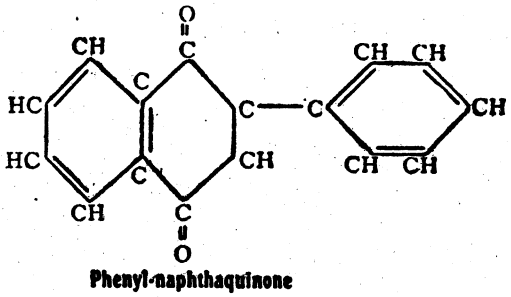
III. PIGMENTS REFERABLE TO HYDROCARBONS OF THE CONFIGURATION SEVEN DOUBLE BONDS AND THREE CYCLES.

Pigments referable to phenyl- dihydronaphthalene.



Phenyl-dihydronaphthalene

One pigment apparently a derivative of the above hydrocarbon has been isolated from plants. This is trifolitin, probably a tetrahydroxy derivative of phenyl-naphthaquinone.



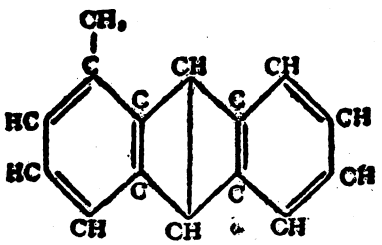
Phenyl-naphthaquinone

Trifoletin was first isolated by Power and Solway,[1] in 1910, from the flowers of red clover, *Trifolium pratense.* Trifolitin, which crystallizes in yellow crystals, is apparently a tetrahydroxy derivative of phenyl-naphthaquinone. It is readily soluble in alcohol and glacial acetic acid, sparingly soluble in chloroform, ether, and benzene. In alkaline solutions it dissolves with a bright yellow color, in sulphuric acid with a yellow color. It dyes mordanted cotton a bright yellow.

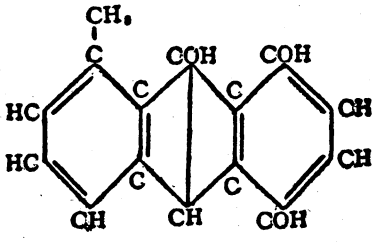
[1] Jr. Chem. Soc., 97, 241.

IV. Pigments referable to hydrocarbons of the configuration six double bonds and four cycles.

Pigments referable to methyl-anthracene.



Methyl Anthracene



Chrysarobin

Chrysarobin[1]—a trihydroxy derivative of methyl-anthracene occurs along with dichrysarobin, dichrysarobin methyl ether and another similar substance $C_{17}H_{18}O_4$ in Goa powder, obtained from *Andira araroba,*[2] also along with chrysophanic acid and emodin in *Rhamnus purshiana.*[3]

Chrysarobin and chrysophanic acid were formerly thought to be identical but the work of Hesse[4] and later that of Jowett and Potter[5] have shown the latter to be a derivative of methyl anthraquinone, while the former is a derivative of methyl anthracene. Chrysorobin is however readily oxidized to chrysophanic acid.

Chrysorobin crystallizes in small yellow tabular crystals and needles. It melts at 177°. It is easily soluble in chloroform, acetic acid and benzene, more difficultly soluble in alcohol and ether. It is insoluble in water and ammonia but soluble in sulphuric acid with a yellow color, insoluble in very dilute potassium hydroxide but soluble in a stronger solution with a yellow color. Upon exposure to the air in alkaline solution it goes to chrysophanic acid.

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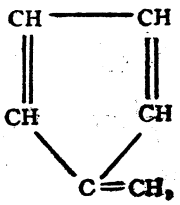
[1] Jr. Chem. Soc., 81, p. 1575.
[2] Ber., 11, p. 1603; Ann., 309, p. 32, Pharm. Jr., 5, p. 721.
[3] Am. Pharm. Assoc., 52, p. 288 (1904).
[4] Ann., 309, p. 32.
[5] Jr. Chem. Soc., 81, p. 1573; 83, p. 1327.

PIGMENTS REFERABLE TO HYDROCARBONS OF THE FORMULA OF SATURATION C_nH_{2n-24}.

Falling under this degree of saturation are the isomeric carotin and lycopin, hydrocarbons of unknown constitution, probably derivatives of fulvene.[1]



Fulvene

Carotin is the yellow pigment of the carrot, *Dauces carota.* It is supposed to be very widely distributed in the plant kingdom, being the yellow pigment which almost universally accompanies chlorophyll. Carotin was probably isolated by Fremy[2] as early as 1865 and later by others investigators who obtained red crystalline substances from green leaves. It is probable that the chrysophyll of Hartsen,[3] the erythrophyll of Bougarel[4] and the xanthin of Dippel[5] were identical with carotin.

In 1885 Armand[6] easily obtained from dried green leaves, by extraction with petroleum ether a yellow pigment which, purified with ether, formed orange red crystals. To this substance which corresponded with the pigment from carrots Armand assigned the formula $C_{26}H_{38}$. Later investigations by Willstaetter[7] have shown the true formula to be $C_{40}H_{56}$.

Lycopin, isomeric with carotin is the red pigment of the tomato, *Lycopersicum esculentum.* This pigment was formerly supposed to be identical with caroten. In 1904, Montanari[8] recognized its difference from carotin and called it dicarotin, $C_{52}H_{74}$ (Carotin $C_{26}H_{37}$). According to the later work of

[1] Zeit. Physiol. Chem., 64, p. 47.
[2] C. r., 61, p. 189.
[3] Arch., Pharm., 207, p. 136.
[4] Ber., 10, p. 1173.
[5] Flora, 1878, p. 18.
[6] C. r., 100, p. 751; 102, p. 1119, 1319.
[7] Ann., 355, 1.
[8] Staz. sperim. arga. ital., 37, p. 909. Wehmer, p. 686.

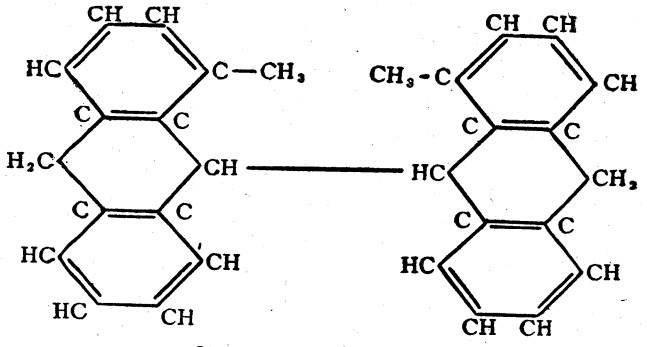
Willstaetter[2] it is isomeric with carotin, the formula of each being $C_{40}H_{56}$.

Xanthophyll[3] ($C_{40}H_{56}O_2$) is probably referable to a hydrocarbon of this degree of saturation. It appears to accompany chlorophyll and carotin. Xanthophyl crystallizes in yellow crystals. It is easily soluble in acetone but difficultly soluble in petroleum ether and easily affected by light.[4] Whereas carotin is easily soluble in petroleum ether, difficulty in acetone, and is unaffected by light.

Other studies of Carotin have been made by Hansen,[8] Tschirch,[9] Schunck [10] and Tsweth.[11]

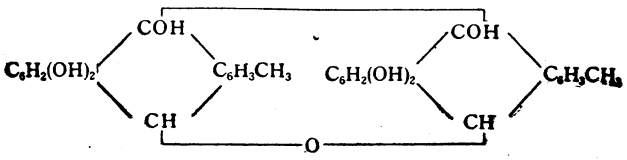
PIGMENTS REFERABLE TO HYDROCARBONS OF THE DEGREE OF SATURATION C_nH_{2n-34}.

One pigment of known constitution falling under this degree of saturation has been isolated. This pigment is dichrysorobin, referable to di (methyl-dihydroanthracyl.)



Di (methyl-dihydroanthracyl)

[2] Zeit. Physiol. Chem., 64, p. 47.
[3] Czapek, Biochemie der Pflanzen (1913) I. p. 583.
[4] B., Bot. Ges., 22, p. 414.
[8] Sitz, ber. phys. med. Ges., Wuerzburg (1883).
[9] B. Bot. Ges., 14, p. 76; Bot. Zentr., 67, p. 78.
[10] Proc. Roy. Soc., 63, p. 389; 65, p. 177; 72, p. 165.
[11] B. Bot. Ges., 24, p. 384.



Dichrysarobin is a partial dehydration product of octahydroxy di (methyldihydroanthracyl).

Dichrysarobin[1] and the methyl ether of dichrysarobin occur along with chrysarobin in goa powder obtained from *Andira araroba.*[2]

Dichrysarobin crystallizes in orange colored tabular crystals which are soluble in ethyl acetate and acetic acid but insoluble in benzene (distinction from chrysarobin) it is more readily oxidized to chrysophanic acid, in alkaline solution than chrysarobin.

NOTE. The writer wishes to acknowledge her indebtedness to Mills College, the woods and gardens of which were generously opened to her for the collection of plant material. She also desires to extend thanks to her colleagues at Wisconsin, who have freely criticised her work, especially to Professor E. R. Miller, who has kindly placed at her disposal the results of his observations along this line, and to Professor L. R. Ingersoll of the Department of Physics, who read and criticised part of the introductory chapter. Above all she wishes to express her gratitude to Professor Edward Kremers, who first introduced her to the study of plant pigments, and whose enthusiastic interest and patient supervision have been her inspiration and guide.

[1] Jr. Chem. Soc., 81, p. 1575.
[2] B., 11, p. 1603; Ann., 309, p. 32.

A CENTURY OF THE UNITED STATES PHARMACOPOEIA, 1820-1920.

I — The Galenical Oleoresins.

By Andrew G. Du Mez.

CONTENTS

PART I—GENERAL

PART II—INDIVIDUAL OLEORESINS

Abbreviations Used for the Titles of Pharmacopoeias and Treatises on Pharmacy.

Allg. P.—Strump, *Allgemeine Pharmakopöe.*
Argent. P. — Argentine Pharmacopœia — *Farmacopœa Nacional Argentina.*
Aust. P. — Austrian Pharmacopœia — *Pharmacopœa Austriaca.*
Bad. P. — Baden Pharmacopœia — *Pharmacopœa Badensis.*
Belg. P. — Belgian Pharmacopœia — *Pharmacopœa Belgica.*
Bern. P. — Bernese Pharmacopœia — *Pharmacopœa Bernensis.*
B. P. — *British Pharmacopœa.*
B. P. C. — *British Pharmaceutical Codex.*
Comp. to the U. S. P. — *Companion to the United States Pharmacopœia.*
Dan. P. — Danish Pharmacopoeia — *Pharmacopoea Danica.*
Dan. Mil. P. — Danish Military Pharmacopœia.
Dict. of Pharm. Sc. — Schweringer, *Dictionary of Pharmaceutical Science.*
Fin. P. — Finnish Pharmacopœia — *Pharmacopœa Fennica.*
Fr. P. — French Pharmacopœia — *Pharmacopoée Francaise.*
G. P.—German Pharmacopœia—*Pharmacopœa Germanica.*
Geiger's P. — Geiger's *Pharmacopöe.*
Han. P. — Hannoverian Pharmacopœia — *Pharmakopöe für das Koenigreich Hannover.*
Hess. P. — Hessian Pharmacopœia — *Pharmakopöe für das Kurfuerstenthum Hessen.*
Hung. P. — Hungarian Pharmacopœia — *Pharmacopœa Hungarica.*
Ital. P. — Italian Pharmacopœia — *Farmacopœa Ufficiale del Regno d'Italia.*
Jap. P. — Japanese Pharmacouœia — *The Pharmacopœia of Japan.*
King's Am. Disp. — King's *American Dispensatory.*
Mex. P. — Mexican Pharmacopoeia — *Pharmacopoea Mexicana.*
Nat. Stand. Disp. — *National Standard Dispensatory.*
Neth. P. — Netherlands Pharmacopœia — *Pharmacopœa Nederlandica—Nederlandische Apotheek.*
Nor. P. — Norwegian Pharmacopœia — *Pharmacopœa Norvegica.*

Port. P. — Portuguese Pharmacopœia — *Pharmacopœa Portugueza.*

Pruss. P. — Prussian Pharmacopœia — *Pharmacopœa Borussica.*

Roum. P. — Roumanian Pharmacopœia — *Pharmacopœa Romana.*

Russ. P. — Russian Pharmacopœia — *Pharmacopœa Russica.*

Schlesw. Holt. P. — Schleswig-Holstein Pharmacopœia — *Pharmakopöe für Schleswig und Holstein.*

Sp. P. — Spanish Pharmacopœia — *Farmacopœa Oficial Española.*

Swed. P. — Swedish Pharmacopœia — *Pharmacopœa Suecica.*

Swiss P. — Swiss Pharmacopœia — *Pharmacopœa Helvetica.*

U. S. Disp. — *United States Dispensatory.*

U. S. P. — *United States Pharmacopœia.*

Univ. P. — Hirsch, *Universal-Pharmacopöe.*

Ver. P. der Lond., Edinb. und Dub. Med. Coll. — *Vereinigte Pharmacopœen der Londoner, Edinburgher, und Dubliner Medicinæ Collegien.*

PART I — GENERAL

Historical Introduction

The type of galenical preparation now known as an oleoresin has been official in the *United States Pharmacopoeia* since 1850, the oleoresins of cubeb and pepper being the first members of this class of preparations to receive recognition, however, under the title of fluid extract.

The suggestion for the name oleoresin appears to have come from Buchner though first applied as the name of a galenical by Peschier. The latter, in 1825, had prepared an ethereal extract of male fern which he designated *Huile de Fougère Mâle.* To this name, Buchner objected, suggesting the title *Extractum resinosum.* In reporting Peschier's work, however, Buchner speaks of the constituents of the ethereal extract as the *oelharzige Bestandtheile* of male fern, and later in his account, he refers to the finished preparation as the *oelharziges Extract, i. e.* an oleoresinous extract. It would appear, therefore, that when Peschier, in his second account (1828), speaks of an *oléorésine,* our English oleoresin, he evidently took his suggestion from Buchner's use of the German attribute, *oelharzig.*

The suggestion of Buchner, that the above mentioned preparation of male fern be called an extract, appears to have met with general favor throughout Europe as is indicated by its title in the various European pharmacopoeias, past and present. Likewise, such other members of this class of preparations as have received recognition in the European countries are to be found in the respective pharmacopoeias of these countries under the heading, *extracta.* In the United States, a latinized form of Peschier's title, *oléorésine,* has been adopted and these preparations are officially known as *oleorsinae.*

The following table of titles will give a fair idea of the early development of the synonymy of these preparations:

Table I. *Early titles of oleoresins*

1825. *Huile de Fougère Mâle* — Peschier.
1826. *Extractum Filicis maris resinosum* — Buchner.
1827. *Extractum oleo-resinosum Filicis* — Brandes.
Oleum Filicis Maris — Van Dyk.
Oleo-Resina Filicis, Peschier — Ver. P. d. Lond., Edinb. and Dub. Med. Coll.
1828. *Oléorésine de Fougère Mâle* — Peschier.
Extrait oléorésineux de Cubebe — Dublanc.
1829. *Extractum Filicis aethereum* — App. to Pruss. P.
Aetherisches Farnkrautextract — App. to Pruss. P.
1832. *Extractum Filicis oleo-resinosum* — Jourdan, Univ. P.
1834. *Piperoide du Gingembre* — Béral.
1841. *Extractum Radicis Filicis Maris aethereum* — Bad. P.
Aetherisches Farrnkrautwurzel Extract — Bad. P.
Extractum Cubebarum aethereum — Bad. P.
Aetherisches Cubeben Extract — Bad. P.
1845. *Extractum Filicis Maris aethereum* — Geiger's P.
Farrnwurzelextract — Geiger's P.
Extrait éthéré de Cubebe — Geiger's P.
Oleoresinous Extract of Cubebs — Bell
Ethereal Extract of Cubebs — Procter.
1849. *Oleoresinous Ethereal Extracts* — Procter.
1852. *Extractum Filicis Maris aethereum* — Swiss P.
Extrait oléo-résineux de Fougère — Swiss P.
Huile de Fougére de Peschier — Swiss P.
1854. *Extractum Stipitum Aspidii* — Nor. P.
1857. *Oléo-Résineux de Cubebe* — Garot and Schaeuffele.
1859. *Oleoresina (ae)* — Procter.
1863. *Oleoresina Capsici* — U. S. P.
Oleoresina Cubebae — U. S. P.
Oleoresina Lupulinae — U. S. P.
Oleoresina Piperis — U. S. P.
Oleoresina Zingiberis — U. S. P.

As becomes apparent from the preceding table, oleoresins became a recognized class of galenical preparations with their introduction into the *United States Pharmacopœia* of 1860. The name, as applied to a class of galenicals, appears to have been suggested by Procter in 1846. Although this term thereby acquired a dual meaning,[1]) it was not only shorter, but in other respects more convenient than *extracta aetherea,* previously in use in some of the European pharmacopoeias. The disadvan-

[1] As a class of natural plant products and as a class of galenicals.

tage accruing from the substitution of *oleoresina* for *extracta aetherea* lay in the fact that as a sub-class they were removed from the other sub-classes of extracts: *e. g.*, the *extracta (solida)*, *extracta fluida, etc.* With the substitution of acetone for ether as an extracting medium, in the eighth revision of the *United States Pharmacopœia,* it is possibly fortunate that the designation *extracta aetherea* never gained a footing in this country.

The preparation of this particular class of galenicals was dependent upon the use of ether. Although, a number of chemists before the eighteenth century had obtained some ether as an ingredient of a mixture resulting from the action of sulphuric acid upon alcohol, it appears that the first commercial ether was prepared in 1730 by Frobenius,[1]) who, however, kept his process a secret. The use of the distillation residues for the preparation of more ether, known to Frobenius, was emphasized by several German chemists, and caused a considerable reduction in the price of this article. Thus Cadet, in 1774, pointed out that he could sell an ounce of ether at 40 sous,[2]) whereas Baumé had sold it at 12 livres. But even with this reduction in price, ether does not appear to have been a common pharmaceutical commodity at that time. Thus, *e. g.*, Hermbstaedt[3]) in 1792, mentions ether and enumerates its properties evidently for the reason that it is of pharmaceutical interest primarily because it is an ingredient of *Liquor anodynus mineralis Hoffmanni.* However, it should be remarked that Baumé mentioned it in 1762 as a solvent in the preparation of resin of Jalap,[4]) and in 1790,[5]) he described its use in the preparation of ethereal tinctures.

The first positive reference concerning the use of ether as a solvent in the preparation of a galenical of the type of our present oleoresins, that appears in the literature, is to be found in Peschier's report (in 1825) on the preparation of the *Huile de Fougère Mâle,* the present oleoresin of aspidium. As a result of the almost immediate popularity of this preparation, other pharmacists were induced to experiment with ether in attempting duplicate or modify Peschier's process. However, none of the

[1] Kopp. Geschicht. d. Chem., vol. 4, p. 302.
[2] Ibid.
[3] Grundriss d. exp. Pharm., part 2. p. 161.
[4] Éléments de Pharm. (1872), p. 284.
[5] Ibid. (1790), p. 262.

early workers attempted to employ it in the extraction of other plant drugs, and it was not until 1834, when Béral again called attention to the use of ether as a solvent in his preparation of *Piperoide du Gingembre,* our present oleoresin of ginger, that its value in the extraction of oleoresinous drugs appears to have been recognized. From then on, however, its use seems to have widened rapidly as the French Pharmacopœia of 1839 contained no less than nineteen ethereal tinctures. The increase in the number of oleoresins was not as rapid as might be expected in view of the statement concerning the ethereal tinctures. Only two other members of this class of preparations made their appearance before 1850, namely, the *Extractum Cubebarum aethereum* and the *Extractum Seminis Cinae aethereum.*

Some idea of the rate at which the *Extracta aetherae,* our present oleoresins, came into existence and were given official recognition will become evident from the following:

In the Prussian Pharmacopœia of 1829, but one such preparation was official, namely,

Extractum Filicis aethereum.

The Baden Pharmacopœia of 1841 contained three preparations of this class, *viz:*

1.) *Extractum Radicis Filicis Maris aethereum.*
2.) *Extractum Cubebarum aethereum.*
3.) *Extractum Seminis Cinae aethereum.*

The Danish Pharmacopoeia of 1850 contained two preparations of this class, *viz:*

1.) *Extractum Cubebarum aethereum.*
2.) *Extractum Filicis Maris aethereum.*

The third edition of the *United States Pharmacopoeia,* which appeared in 1851, included two fluid extracts prepared with ether as a menstrum, *viz:*

1.) *Extractum Cubebae fluidum.*
2.) *Extractum Piperis fluidum.*

The Belgian Pharmacopœia of 1854 recognized no less than seven ethereal extracts, *viz:*

1) *Extrait éthéré de Fougère*
2) *Extrait éthéré de Cantharides*
3) *Extrait éthéré de Croton*
4) *Extrait éthéré de Cubebe*
5) *Extrait éthéré de d'Aunee*
6) *Extrait éthéré de Bois garu*
7) *Extrait éthéré de Semen-contra*

It will be seen from the above array of ethereal extracts official in European pharmacopœias that the introduction of oleoresins into the fifth edition of the *United States Pharmacopœia,* which appeared in 1863, was well prepared.

Procter is commonly given credit for having introduced oleoresins into American materia medica. That he was instrumental in bringing them to the attention of the representatives of the regular medical school, and that he obtained a place for them in the *United States Pharmacopœia,* possibly no one has reason to doubt. A review of the early American literature on this subject not only reveals this fact, but it also brings out the fact that Procter appears to have been ignorant in large part of the use of this class of preparations in Europe,[1]) for nowhere does he mention it. It is note-worthy that it was a medical practitioner (Goddard) who first drew Procter's attention (1846), not to a typical representative of this class, but to the preparation of Dublanc which was a representative of the *extracta oleoresina* made by a very cumbersome process, now long discarded as being as unscientific as it is impractical. In the same year, the English pharmacist, Bell, had his attention drawn to this same preparation by Vore, thus showing that valuable preparations not advertised were ignored, while a quasi scientific preparation heralded about apparently attracted general attention.

To what extent the Eclectic school of medical practioners contributed to the popularization of this class of galenicals before 1860 cannot be definitely stated from the scanty informa-

[1] That Proctor did know of Mohr's work on this class of preparations becomes apparent when the fact is recalled that he adapted Redwood's translation of Mohr's *Pharmaceutische Technik* to American pharmacy under the title of Mohr, Redwood & Procter's *Pharmacy* in 1849, and that he had previously reviewed Redwood's translation in the Am. Jour. of Pharm.

tion at hand. However, it is interesting to note that the *American Dispensatory* of 1854, gives the formula of Robinson for preparing the *ethereal oil of xanthoxylum,* the present Eclectic oleoresin of xanthoxylum. The same is directed to be prepared by extracting the bark with ether and subsequently removing the ether by evaporation—a process similar to the one now employed in preparing the official oleoresins. Of but slightly lesser interest is the advertisement of Wm. S. Merrel which appeared in the *Eclectic Medical Journal* in 1855. Under the heading, Class II.—Soft resinoids and oleo-resins, etc., the following preparations were listed:

Apocynin	(from Dogsbane).
Ascelepedin	(from Pleurisy Root).
Aletrin	(from Star Root).
Eupurpurin	(from Queen of the Meadow).
Iridin[1]	(from Blue Flag).
Ptelein, or Oil of Ptelea	(from Water Ash).
Oil of Lobelia	(from Lobelia Seed).
Oil of Xanthoxylum	(from Prickley Ash).
Oil of Capsicum	(from African Cayenne).
Oil of Stillingia	
Oil of Male Fern	

In view of the fact that these preparations were already being manufactured and advertised commercially in 1855, there can be but little doubt that the Pharmacopoeial Revision Committee of 1860 must have been aware of their existence and have been influenced to some extent thereby.

Definition

Oleoresins, as a class of galencials, are extracts prepared, as a rule, with the aid of a highly selective solvent. Ether is the solvent usually employed for this purpose at the present time, whereas, acetone was directed to be used in the eighth revised edition of the *United States Pharmacopœia.* Other solvents of this nature, namely: petroleum ether, benzene, chloroform, carbon tetrachloride, et cetera, have been used, but have not been officially recognized. The oleoresin of cubeb is an exception

[1] Prof. John King is said to have prepared and used *Irisin* (identical with *Iridin*) in 1844. Letter from J. U. Lloyd to Edward Kremers (1906).

to the rule as alcohol is the menstruum directed to be used in its preparation.

These preparations derive their name from the fact that the drugs from which they were originally prepared contained appreciable amounts of fatty or volatile oil and resin, substances, for which ether and acetone were recognized to be good solvents. They do not by any means necessarily correspond to the so-called natural oleoresins, which consist for the main part of volatile oil and resin; but, in some cases, are products relatively poor in one or both of these constituents. Thus, for example, the oleoresin of capsicum contains little or no volatile oil and only a small amount of resin, while the oleoresin of parsely is practically free from resin. Furthermore, these preparations are not always liquid as is generally stated. The oleoresin of lupulin, for instance, is of the consistence of a soft extract when prepared according to pharmacopoeial directions, and tends to become firmer with age owing to the transformation of the so-called soft into hard resin.

The manner in which the oleoresins have been defined in the various text books and treatises on pharmacy is brought out by the following examples, which are representative of the periods corresponding to the different decennial revisions of the *United States Pharmacopoeia:*

"*Oleoresinae* — Their peculiarity is that they consist of principles which when extracted by means of ether, retain a liquid or semi-liquid state upon the evaporation of the menstruum, and at the same time have the property of self-preservation, differing in this respect from the fluid extracts which require the presence of alcohol to prevent decomposition. They consist chiefly, as their name implies, of oil, whether fixed or volatile, holding resin and sometimes other active matter in solution." *U. S. Disp.* (1870), p. 1315.

"*Oleoresinae,* Oleoresins — Mixtures of volatile oils with resins prepared by exhausting certain drugs containing both together, the menstruum being usually ether which extracts both. The menstruum or solvent is evaporated off, and the usually semi-liquid extract which remains constitutes the oleo-resin." Oldberg and Wall, *Comp. to the U. S. P.* (1884), p. 721.

"The oleoresins are official liquid preparations, consisting principally of natural oils and resins extracted from vegetable substances by percolation with ethylic ether. The oleoresins were formerly classed with the fluid extracts, but they differ essentially from the latter:

1. They do not bear any uniform relation to the drug as fluid extracts do, of gramme to cubic centimeter,—the yield of oleoresin obtained

from the drug varying according to the proportion of oil and resin naturally present:

2. The menstruum used, ethylic ether, extracts principles which are often insoluble in alcohol or diluted alcohol, and vice versa. Oleoresin of Cubeb, for instance, is not identical with Fluid Extract of Cubeb:

3. They are without exception the most concentrated liquid preparations of the drugs that are produced." Remington, *Pract. of Pharm.* (1894), p. 433.

"Oleoresins are those substances obtained from vegetable medicines by means of ether (sometimes alcohol, *etc.*,) which consist principally of a fixed or volatile oil and a resin. In some cases the resin will be held in solution by the oil, while in other cases, it will be precipitated upon standing and will require agitation to diffuse and suspend it in the oil. A third case occurs in which the oil and resin form a more or less permanent mixture, having the consistency of a very soft extract." King's *Am. Disp.* (1900), p. 1330.

"Oleoresins are ethereal extracts of an oleoresinous nature, obtained from vegetable drugs by percolation with ether." Coblenz's *Handbook of Pharm.* (1902), p. 290.

"Oleoresins, *Oleoresinae* (Oleoresins, L. *oleum*, oil and *resina*, resin)—Natural solutions of resin in volatile oils, extracted by ether, acetone or alcohol." Culbreth, *Mat. Med.* (1906), p. 20.

"The pharmaceutical oleoresins are liquid preparations of drugs containing volatile oil and resin, obtained by percolation of such drug with acetone, ether, or alcohol, and subsequent distillation of the solvent from the dissolved oleoresins." Arny, *Prin. of Pharm.* (1909), p. 259.

"Solutions of this class represent the medicinal virtues of the drugs from which they are made, in a more concentrated form than is possible in any other. They possess the power of self-preservation, and in this respect are superior to fluidextracts. Oleoresins consist chiefly of fixed or volatile oils associated with resin and other constituents; those officially recognized, with one exception, are all prepared, " et cetera. Caspari, *Treat. on Pharm.* (1916). p. 354.

Drugs Used, Their Collection, Preservation, Etc.

Since the oleoresins are characterized chiefly by their content of oil and resin (see definition above), it is evident that they may be prepared from many of the numerous vegetable drugs) of which these substances constitute an appreciable part. The number of such drugs, however, which has actually been used for this purpose, is comparatively small as is shown in the table which follows. The table also reveals the fact that nearly all of these drugs are derived from phenogamous plants and that they consist, as a rule, of those organs in which oils and resins are usually present in the greatest abundance.

TABLE 2—*Drugs from which oleoresins have been prepared.*

Phenogams

Alkanet (root)	Cypripedium (rhizome)	Pepo (seed)
Anacardium (fruit)	Eucalyptus (leaf)	Pepper (fruit)
Annatto (seed)	Galangal (rhizome)	Pomegranate (root)
Asarum (root)	Ginger (rhizome)	Ptelea (bark)
Capsicum (fruit)	Helenium (flower)	Pyrethrum (root)
Cardamon (seed)	Iris (rhizome)	Sabal (fruit)
Chenopodium (fruit)	Kousso (flower)	Santonica (unexp. flower)
Clove (unexp. flower)	Lobelia (seed)	Savine (leaf)
Conium (leaf)	Lupulin (strobile)	Senecio (root & herb)
Croton (seed)	Matico (leaf)	Spiraea (herb)
Cubeb (fruit)	Mezereum (bark)	Taxus (leaves)
	Parsley (fruit)	Xanthoxylum (bark)

Cryptogams

Aspidium (rhizome) Ergot (sclerotium of *Claviceps purpurea*)

Of the total number of drugs enumerated above, seven have been utilized in the preparation of the oleoresins official in the *United States Pharmacopoeia,* namely:

Aspidium	Ginger	Parsley
Capsicum	Lupulin	Pepper
Cubeb		

With respect to the collection (harvesting) of the foregoing and their preparation for use, there is little of a general nature to be said as the plants from which these drugs are obtained differ so widely in their habits. This subject will, therefore, not be given consideration here, but will be discussed in Part II under the treatment of the individual preparations.

SOLVENTS USED.

At the present time, ether is the solvent directed to be employed in the preparation of the official oleoresins with the exception of the oleoresin of cubeb which is prepared with alcohol. It will be recalled that the first of this class of preparations to make its appearance, namely, the *Huile de Fougère* of Peschier, was also prepared with ether. In fact, ether appears to have been the only solvent[2]) given consideration in this connection by the early European investigators.

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[1] One animal drug, cantharides, has been utilized for the preparation of an ethereal extract. This preparation, which was official in the Belgian Pharmacopoeia of 1854, cannot properly be classed with the oleoresins since it contained no resin—the animal organism being free from constituents of this nature.

[2] Buchner in 1826 experimented with alcohol in preparing the *Extractum Filicis resinosum,* while Brandes, in 1827, made use of a menstrum containing both alcohol and ether, namely the *Liquor anodynus,* for the same purpose. Later, 1828, Dublanc and Oberdoerffer employed alcohol in the preparation of the oleoresinous extract of cubeb.

With the introduction of the oleoresins into the *United States Pharmacopœia* of 1860, and their extensive use in this country, a number of American pharmacists were lead to the conducting of experiments, which had for their main object the discovery of a solvent less expensive and less dangerous to handle than ether. We must, however, note that prior to this time (1860) an attempt was made by Berjot, a Frenchman, to use carbon disulphide for the purpose of preparing the *Extrait oléo-résineux de Cubebe.* Garot and Schaeuffele, in 1857, in a paper on Berjot's preparation showed that nothing was gained by its use, as two and one-half times as much carbon disulphide as ether was required to extract the drug. Furthermore, the removal of the last traces of this solvent was a matter of considerable difficulty.

The solvent which first appears to have suggested itself to American investigators was benzin as is indicated in the publications of Procter, Maish, Trimble and others. The first account of its use in this connection appeared in 1866, when Procter published his results on the preparation of the oleoresin of cubeb. The following table shows the relative value of alcohol, benzin and ether for the extraction of cubeb as found by Procter.

TABLE 3.—*Yield of oleoresin of cubeb.*

Quantity of drug	Menstruum	Total Yield
grains		grains
1000	Alcohol	250
1000	Benzin	170
1000	Ether	219

While Procter could find no objection to the use of alcohol as a solvent in the preparation of this oleoresin, he advised against the use of benzin as he stated that it did not extract the cubebin completely.

Simultaneously with the above publication of Procter, there appeared an account of a general method for preparing the oleoresins by Rittenhouse. The latter also worked with benzin, but employed it as a "follow up" solvent after percolation had been partially completed with ether. He also experimented with glycerin and fusel oil, employing them in a similar manner.

In 1872 Maish published a review of the experiments of A. H.

Bolton and M. Roth. The latter of these two men conducted an investigation on the extraction of ginger and cubebs with benzin, the former also included capsicum in his series of experiments. These workers found that ether still extracted some non-volatile matter after the drugs had presumably been exhausted with benzin. Further, that, while the benzin oleoresins were all soluble in ether, the ethereal oleoresins of cubeb and ginger were only partially soluble in benzin, thus confirming Procter's work in 1866 on the oleoresin of cubeb.

Henry Trimble was the next investigator[1]) to experiment to any considerable extent with benzin as a solvent. In his report to the Pennsylvania Pharmaceutical Association, in 1888, on commercial oleoresins, he stated that while benzin was in his opinion preferable to concentrated ether for the extraction of capsicum, it would not answer for the other official oleoresins. Following is a table showing the comparative extractive powers of ether and benzin as compiled by Trimble:

TABLE 4—*Relative extractive power of benzin and ether.*

Drug	Yield with ether	Yield with benzin
	Per cent.	Per cent
Aspidium	6.51	5.8
Capsicum	19.5	18.1a
Cubeb	21.26	16.65
Lupulin	60.59	7.04
Pepper	8.79	2.80
Ginger	3.97	2.48

Results similar to the above with respect to the oleoresins of ginger were reported by Samuel J. Riegel in 1891.

About this time George M. Beringer became interested in the preparation of the oleoresins, and in 1892, he published an account of his researches, in which he had employed not only ether and benzin as extracting menstrua, but also the heretofore little used solvent, acetone. With respect to benzin, he arrived at the same conclusions as did Trimble, *viz:* that its use

[1] In 1877, L. Wolff in an article entitled: *The use of Petroleum Benzine in Pharmacy,* stated that benzin extracted none of the pungent resin from ginger, no cubebic acid from cubeb, no piperin from pepper and no santonin or resin from wormseed.

is not permissable in the preparation of the official oleoresins[1]), except, perhaps in the case of capsicum, and then only under certain restrictions, namely: that percolation be terminated after 2 cubic centimeters of percolate are obtained for every gram of the drug, as upon further percolation, the oleoresin becomes almost solid owing to the large increase of palmitin extracted. In his experiments with acetone[2]) he found that, as with ether, the first portion of the percolate contained nearly all the medicinal ingredients of the drug. He, however, continued percolation until the drug was exhausted. The marc was then removed from the percolator, dried and re-percolated with stronger ether; but except in the case of capsicum no further extractive matter was obtained. The oleoresins were stated to be of excellent quality and the yield and properties were nearly the same as when ether was used. He especially recommended the use of acetone in preparing the oleoresin of ginger, as he claimed that it was in every way equal to the preparation made with ether. Following is a table showing Beringer's results with acetone as compared with ether and benzin:

TABLE 5—*Relative extractive values of acetone, ether and benzin.*

Drug	Yield to acetone		Yield to ether		Yield to benzin	
	U. S. P. method	Complete exhaustion	U. S. P. method	Complete exhaustion	U. S. P. method	Complete exhaustion
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Aspidium	.....	18.00	.....	.....	16.18	.....
Capsicum	18.00[1]	25.00	17.32	.....	21.00[1]	25.00
Cubeb	.....	25.00	.....	.....	.....	.....
Cubeb	.....	24.10	.....	.....	.....	.....
Cubeb	.....	21.75	.....	.....	.....	.....
Ginger	.....	5.57	.....	.....	.....	.....
Lupulin	.....	71.00	.....	70.80	.....	.....
Parsley	.....	24.00[2]	.....	.....	.....	22.30
Pepper	.....	5.93to9.97[3]	.....	5.00 to 6.70	.....	.....

[1] Two cubic centimeters of percolate were collected for each gram of drug.
[2] Represents total extract from which 3 per cent. of wax precipitated, leaving 21.00 per cent. of oleoresin.
[3] Represents total extract which yielded 5.93 per cent. of oleoresin.

[1] Pile (1867) confirms this statement in so far as it concerns the oleoresin of ginger. He states that neither benzin nor ether completely extract ginger, but that alcohol is the best solvent for this purpose.

[2] The acetone used by Beringer was procured from manufacturers of chloroform as the product obtained from the distillation of wood was found to consist largely of methyl alcohol and even higher boiling fractions.

From a comparison of the above data with those obtained by Trimble (See table 4), it would appear that acetone is equally as serviceable as ether in the preparation of the official oleoresins. Such appears, also, to have been the opinion of the Revision Committee of the United States Pharmacopœia of 1900, as the edition, which became official in 1905, directed that acetone be employed in the manufacture of those oleoresins which were formerly required to be prepared with ether.[1] That this change was unsatisfactory is evidenced by the numerous comments on the subject occurring in the literature, and by the fact that ether is again directed to be used for this purpose in the ninth revised edition of the *United States Pharmacopœia.*

To those unacquainted with the situation, the above action of the Revision Committee of 1910, might be taken to indicate that the matter of the proper solvent to be employed in the manufacture of these preparations has been definitely settled and the superiority of ether in this respect firmly established. A close inspection of the preceding reports, along with other information of a similar nature occuring in the literature, would,, however, appear to point out, that, as in the case of the oleoresin of cubeb, other solvents might be advantageously employed in the preparation of certain of these individuals. In this connection the use of benzin,[2]) or better, petroleum ether,[3]) in the preparation of the oleoresins of capsicum and parsley fruit might be mentioned, or the employment of acetone in the preparation of the oleoresin of ginger..[4]) As further evidence of the possibilities along this line, attention is also called to the experiments of Wollenweber (1906) on the extraction of aspidium with benzene, and to the mention of chloroform[5]) and carbon tetrachloride[6]) as solvents for the preparation of the oleoresins in general.

The manner in which these solvents have been employed in

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[1] The most important factor in determining this change was probably the difference in cost of the two solvents at the time (1900), acetone being the cheaper. This statement is confirmed in a measure by the fact that now, since the price of ether has been reduced, owing to its preparation from denatured alcohol, it is again the solvent officially recognized.

[2] See preceding reports by Trimble, Beringer and others.

[3] Hyers (1895) also made use of petroleum ether in extracting cubeb.

[4] Idris (1898) stated that he found acetone, b. p. 65° C, to be the most suitable solvent for extracting ginger.

[5] Dorvault, *L'Officine* (1898), p. 591.

[6] Lucas, *Practical Pharmacy* (1908), p. 149.

the preparation of the various oleoresins will be discussed in a general way under methods of preparation and in detail under individual oleoresins.

Methods of Preparation

The methods of preparing the oleoresins as outlined in the present edition of the *United States Pharmacopœia* may be stated in the following general way: extract the drug completely[1]) by percolation, expose the percolate in a warm place until the solvent has completely evaporated and separate the remaining liquid portion from any deposited material. This is essentially the method of procedure given in most of the late editions of the foreign pharmacopœias as well, notable exceptions being the German and Japanese. In the two latter, the drug is directed to be exhausted by maceration instead of percolation. In detail, the methods described in the United States Pharmacopœia, as well as the foreign pharmacopœias, differ somewhat with the particular oleoresin as will be brought out to some extent in the following discussion and more minutely under the separate treatment of each individual. It is perhaps needless to state that these methods are not of recent invention but have been gradually evolved from the numerous experiments conducted both in this country and abroad.

The first of these experiments dates back to the year 1825, when Peschier prepared the *Huile de Fougère Mâle,* our present oleoresin of aspidium. In his description of the method of preparation, he directs that the male fern rhizomes be extracted with successive portions of ether, the decanted ethereal solutions mixed and evaporated at a gentle heat, and the remaining oily residue collected and preserved as the finished product. This is essentially the method which appeared in the early European pharmacopœias as is shown in the following typical example taken from the Prussian Pharmacopoeia of 1830:

> Agitate one ounce of powdered male fern root with successive portions of eight ounces of ether until the ether decants clear. Then mix the several portions and strain. Distill down to one-fourth of the volume and evaporate the remainder on a water bath to a thin yellowish-brown extract.

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[1] Percolation, in the extraction of capsicum is directed to be discontinued when eight hundred mills of percolate have been obtained.

An inspection of the above method brings out the fact that the decanted menstruum was directed to be clarified by the process of straining. Not only was a great deal of the solvent lost by evaporation in this procedure, but a very considerable amount remained adhering to the marc. While some of the latter was, in actual practice, removed by pressing the drug on the strainer with the hands, Mohr[1]) in commenting on the method stated that, inasmuch as three-fourths of the ether were often lost in these operations, it was useless to recover the remainder by evaporation. To overcome this loss to some extent, he suggested making these preparations in the winter when the low temperature would be less favorable for the volatilization of the solvent. As ether, at this time and for many years later, was a comparatively expensive solvent, it will become apparent that a change in the method was to be desired.

The first decided departure[2] from the above method of procedure, which appears to have been given official recognition, is to be noticed in the Baden Pharmacopœia of 1841. The method briefly stated is as follows:

Mix the powdered male fern root with a sufficient quantity of ether to thoroughly moisten it. Then extract it in a *Real'sche Presse* so connected with a receiving flask that none of the menstruum will be lost by evaporation.

A few years later, in 1846, there appeared a method in the Swedish Pharmacopœia which likewise included the process of displacement, *viz:*

Macerate the male fern root, cut in small pieces, with ether and extract in a displacement apparatus.[3] Then distill the ethereal solution to one-fourth of its volume and evaporate the remainder on a water bath to the consistence of a thin extract.

Even with the use of a pressure percolator, so much ether was still lost through spontaneous evaporation and through ab-

[1] Mohr, Redwood and Procter's *Pharmacy* (1849), p. 263.

[2] Geiger, in 1827, employed the *Real'sche Presse* in the preparation of the *Oleum Filicis Maris,* our present oleoresin of aspidium.

[3] The apparatus employed for this purpose was most probably the *Filtre-presse* of Count Real or the *Luft-presse* of Dr. Romershausen, as both of these so-called presses were in general use at that time. In fact, both are mentioned in connection with the preparation of the *extracta* by the Prussian Pharmacopœia as early as 1834.

sorption by the bag,[1]) that, in operating with small quantities of the drug, the recovery of the remainder was scarcely worth the trouble. The recognition of these defects by Mohr lead him to construct (in 1847) a special form of apparatus for continuous extraction with volatile solvents. However, while Mohr's apparatus was a success from an economical standpoint, there is no evidence to show that it was ever employed to any extent by the American pharmacist, although, Procter, the American editor of Redwood's translation of Mohr's treatise on pharmacy, advocated its use in this connection in 1849.

About this time (1846) Procter caused the American pharmacists to become interested in this class of preparations by calling attention to his improvement upon Soubeiran's method (as suggested by Dublanc)[2]) for preparing the *Extrait oléo-résineux de Cubebe*, a preparation similar to our present oleoresin of cubeb. The following is the method as devised by Procter.

"Take cubebs, in powder, one pound avoirdupois, and sulphuric ether a sufficient quantity, which is two and one-half to three pounds; introduce the powder into a displacer, insert the lower end into a bottle that fits it, add the ether carefully, and cover the top of the filter with a piece of wet bladder through which several pin holes have been made.[3] The flow should be very gradual and if too rapid, the filter should be partially closed with a cork. By attention to this point, much less ether will be required. The ethereal tincture should be introduced into a large retort, heated by a water bath, and the receiver well refrigerated. The distillation should not be hurried toward the last. When five-sixths of the ether have passed, it should be separated for use, and the evaporation be continued in the retort, observing to keep the temperature below 120°F, so as not to volatilize the volatile oil."

A few years later (1850), this method (in essential detail) was given recognition by the *United States Pharmacopœia* in connection with the preparation of the fluid extracts of cubeb and pepper, later known as the oleoresins of cubeb and pepper, respectively. For the purpose of better bringing out this

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[1] Mohr, Redwood and Procter's *Pharmacy* (1849), p. 263.

[2] Although Dublanc described a method for preparing the oleoresinous extract of cubeb, similar to that of Soubeiran, in 1828, neither method is given consideration here as both differed to such an extent from the usual procedure that they had little or no influence on the development of the present process.

[3] From the above description, it appears that the form of displacer used by Procter was the one described in Mohr, Redwood and Procter's *Pharmacy*, (1849), p. 270.

similarity, the following general statement of the pharmacopoeial methods is also given:

Take of the Drug, in powder, a pound;
Ether a sufficient quantity.
Put the drug into a percolator, and having packed it carefully, pour the ether gradually upon it until two pints of filtered liquid are obtained, then distill off by means of a water-bath, at a gentle heat, a pint and a half of the ether, and expose the residue in a shallow vessel, until the whole of the ether has evaporated.

The methods in general as they were given in the *United States Pharmacopœia* of 1860 differ from the above only in the quantity of drug and menstruum directed to be taken. Thus, twelve troy ounces of drug were directed to be subjected to percolation with ether until twenty-four fluidounces of filtered liquid were obtained, when eighteen fluidounces of the ether were to be removed by distillation. In the preparation of the oleoresin of ginger, however, the following method of procedure was given:

"Take of Ginger, in fine powder, twelve troyounces;
"Stronger ether twelve fluidounces;
"Alcohol a sufficient quantity.
"Put the ginger into a cylindrical percolator, press it firmly, and pour upon it the stronger ether. When this has been absorbed by the powder, add alcohol until twelve fluidounces of filtered liquid have passed. Recover from this, by distillation on a water-bath, nine fluid-ounces of ether, and expose the residue in a capsule until the volatile part has evaporated."

That the Pharmacopœial Revision Committee was informed of the work of Béral in this connection appears to be clearly evident, as it was he, who first suggested this procedure (1834), also, in the preparation of the oleoresin of ginger, then known as the *Piperoide du Gingembre.*

In 1866, Rittenhouse, commenting on the methods in general, which were given in the *United States Pharmacopœia* of 1860, stated that about thirty-six fluid ounces of ether were required to extract the drug when proceeding as officially directed. He, however, conceived the idea of reducing the amount of ether by a procedure similar to that employed in extracting the ginger rhizomes. Alcohol did not appeal to him as the proper "follow up" solvent for this purpose and he, therefore, conducted a series of experiments, in which he made use of benzin,

glycerin and fusel oil. The following is the working formula finally devised by him:

"Take any convenient quantity of the drug; for each ounce thus employed, 1½ fluid ounces of ether, and 1 fluid ounce or q. s. of benzin. Pack the drug in a suitable apparatus, add the ether, and when it has ceased to pass, pour on the benzin in the proportion of one fluid ounce for each ounce of the drug employed or until as much percolate has been obtained as equals the amount of ether employed. Recover the ether by distillation in the usual manner."

The process of Rittenhouse does not appear to have received much attention as there is no subsequent mention of it to be found in the literature.

During the meantime Procter continued his work on the oleoresins and in the same year (1866), he pointed out that practically all of the oleoresinous material was to be found in the first portions of the percolate, and that a considerable quantity of menstruum could be saved by discontinuing the operation before the drug was completely exhausted. The following table compiled by Procter clearly brings out this point:

TABLE 6—*Yield of oleoresin of cubeb to ether, alcohol and benzin.*

Quantity of cubeb	Solvent	Quantity of 1st percolate	Yield of oleoresin	Quantity of 2nd percolate	Yield of oleoresin	Total yield
grains 1000	Ether	grains 1000	grains 205	grains 1000	grains 14	grains 219
"	Alcohol	"	240	"	30	250
"	Benzin	"	140	2000	25	170

The effect of Procter's work is noticed in the 1870 and 1880 editions of the *United States Pharmacopœia.* Thus, the Pharmacopoeia of 1870 directed that twenty instead of twenty-four fluidounces (as formerly required) of percolate be collected for every twelve troyounces of drug, while the Pharmacopœia of 1880 required that only 150 parts of percolate be obtained for every 100 parts of drug taken. It should also be noted, that in the 1880 edition, the method of preparing the oleoresin of ginger was made to conform with that given for the other oleoresins.

The *United States Pharmacopœia* of 1890, directed, that, in the preparation of all of the official oleoresins, the drug be completely exhausted by percolation with ether. The following directions for the preparation of the oleoresin of cubeb are typical of the methods given:

"Cubeb, in No. 30 powder, 500 Gm.; ether a sufficient quantity.

"Put the cubeb into a cylindrical glass percolator provided with a stop-cock, and arranged with a cover and receptacle suitable for volatile liquids. Press the drug firmly and percolate slowly with ether, added in successive portions, until the drug is exhausted. Recover the greater part of the ether, etc."

The next edition of the *United States Pharmacopœia* (1900) contained a number of changes with respect to the methods of preparing this class of galenicals. Two new solvents were introduced, namely, acetone and alcohol; the method of procedure was modified in the case of the oleoresin of capsicum, and an ordinary percolator was directed to be used in the preparation of the oleoresin of cubeb. The following is a general statement of the manner in which the oleoresins of aspidium, ginger, lupulin and pepper were directed to be extracted.

Introduce the powdered drug (degree of fineness specified) into a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and receptacle suitable for volatile liquids. Pack the powder firmly, and percolate slowly with acetone, added in successive portions, until the drug is exhausted.

The method of extracting the cubeb was stated as follows:

Introduce the powdered cubeb (degree of fineness specified) into a cylindrical glass percolator, pack the powder firmly, and percolate slowly with alcohol, added in successive portions, until the cubeb is exhausted.

The method described for the extraction of capsicum was similar in all respects to the first of the methods given above, except that percolation was directed to be discontinued when eight hundred cubic centimeters of percolate had been obtained.

The above changes, except in the case of the oleoresin of cubeb[1]) must be attributed to the work of Beringer, an account of which was published in 1892. Not only did he advocate the use of acetone in these preparations, but he also pointed out

[1] It will be recalled that Procter in 1866 suggested the use of alcohol in preparing the oleoresin of cubeb. See table 3, page 922.

the advantage of discontinuing percolation short of exhaustion in the case of capsicum.

The ninth revised edition of the *United States Pharmacopœia* shows but one change in the method of preparing the oleoresins, *viz:* ether is directed to be used in those cases where acetone was employed in the preceding edition.

From the foregoing discussion, it becomes apparent that the *United States Pharmacopœia,* even to the present edition, has consistently adhered to the process of simple percolation in extracting the oleoresinous drugs. This condition not only appears strange, in view of the fact that modern methods of operating with the volatile solvents, such as ether, make use of some form of continuous extraction apparatus; but is thought to show a lack of progress as well. Maish, in 1900, suggested the use of Soxhlet's apparatus for this purpose and pointed out its advantage, especially when operating with small quantities of drug. Reference is also made in this connection to similar forms of apparatus in most of the present day text-books on pharmacy.

With reference to the preparation of the oleoresins on a commercial scale, there is good reason to doubt the employment of any of the heretofore mentioned methods. The method most likely in use at the present time is one similar to that official in the *British Pharmacopœia* of 1867. The latter, briefly stated, is as follows:

> Exhaust the powdered drug by percolation with alcohol, and distill the percolate until a soft extract is obtained. Treat this extract with successive portions of ether, mix the ethereal solutions and again distill off the solvent, when the residue will constitute the oleoresin.

The advantage of this method lies in the fact that it requires the handling of comparatively small amounts of ether, and thereby lessens the danger incurred in working with large quantities of this highly inflammable solvent. The disadvantage is that alcohol may not extract all of the eother-soluble material from the drug.

In the preceding survey, only the official oleoresins and their methods of preparation have been considered. There is, however, a number of preparations which have been classed as oleoresin, in Parrish's *Treatise on Pharmacy,* and King's *American Dispensatory,* although, they have never received of-

ficial recognition. They are the so-called Eclectic oleoresins and are in general directed to be prepared in the following manner:

Extract the drug by percolation with alcohol or ether and precipitate the oil and resin by pouring the alcoholic or ethereal tincture into water. Lastly, separate the product from the water by filtration.

Among the preparations which have been made in this way are the following: oleoresin of iris (iridin), oleoresin of xanthoxylum, oleoresin of cardamon (oil of cardamon), oleoresin of ergot, (oil of ergot) and oleoresin of parsley,[1]) (oil of parsley).

In this connection, it should be pointed out that the foregoing are liquid preparations and do not constitute the so-called resinoids, which are solids, although prepared in a similar way.

Apparatus Employed.

Under the two preceding headlines, the preparation of the oleoresins has been discussed from the standpoint of the solvent employed in extracting the drug, and with respect to the method of procedure. There, is however, still another factor of interest which deserves consideration in this connection, namely: the form of apparatus made use of.

It will be recalled that the first of this class of preparations to make its appearance, the oleoresin of aspidium, as originally prepared, required the use of nothing but a macerating jar, a cloth strainer and some sort of container, in which the colated liquid could be collected and exposed to the air to permit the evaporation of the solvent. Likewise, these were the utensils generally employed in the experimental stages of the preparation of the other members of this class which became known at an early date. As soon, however, as the oleoresins became recognized as regular pharmaceutical commodities, the method of preparation as outlined above was found to be impractical owing to the complete loss of the solvent by evaporation. In adapting the same to commercial use, steps were, therefore, taken to recover as much of the latter as possible. For this purpose, some form of distilling apparatus was employed, pre-

[1] This preparation should not be confused with the oleoresin of parsley as official in the present edition of the *United States Pharmacopœia.*

sumably, the retort and condenser. Even with this modification, however, a large part of the solvent was still lost in the operation of straining.

About this time (1820 to 1840), the extraction of drugs by the process of downward displacement was attracting considerable attention, and, as the pharmacist saw in this procedure a means of eliminating the operation of straining, it is not at all surprising that it should have received early application in the preparation of the oleoresins. In explanation of the method of procedure as followed at the time, it should be stated that it was in reality a process of percolation under pressure, and, as such, required the use of a special form of apparatus. Two such forms were already available at the time when the oleoresins became a subject for investigation, namely: the *Filtre-Presse* of Réal and the *Luft-Presse* of Romershausen. In fact, Geiger made use of the former in the preparation of the oleoresin of male fern as early as 1827. While these forms of pressure percolators eliminated the process of straining, their use, nevertheless, appears to have been disadvantageous in certain other respects. For instance, the method of operation was rather cumbersome, and a considerable amount of solvent was absorbed by the cloth bag containing the powdered drug, thus rendering the apparatus of little value in working with small quantities of the latter.

As a result of the early work with the pressure percolators, experimentation along this line was stimulated and it was soon shown that drugs could be completely extracted by simple percolation under ordinary atmospheric pressures. The first evidence of the use of a simple percolator in the preparation of the oleoresins appears in Béral's account of his preparation of the *Piperoide du Gingembre* in 1834. Fifteen years later (1849), Procter, in an article on the oleoresinous ethereal extracts, mentioned two forms of simple percolators, a conical percolator made of tin, and Gilbertson's displacement apparatus constructed of glass. Both of these were similar in essential detail to the percolators in general use at the present time. In fact, the *United States Pharmacopœia* still directs that these preparations be made by simple percolation, a modified form of Gilbertson's displacement apparatus being specified for use in this connection. This condition seems strange, indeed, in view of the fact that modern methods of operating with volatile

solvents, such as ether, make use of some form of continuous extraction apparatus.

Such an apparatus was invented by Mohr in 1847 and its advantages in the preparation of the oleoresins pointed out by him at this time, and later, by Procter. An apparatus operating on similar principles was described by Parrish in 1884 in his *Treatise on Pharmacy*. More recently Maish (1900) has suggested the use of the Soxhlet apparatus for the preparation of small quantities of oleoresins, while a number of other forms of continuous extraction apparatus have been mentioned in this connection in the various periodicals and text-books on pharmacy.

The different forms of apparatus, which have been mentioned at various times in connection with the preparations of the oleoresins, and the methods of operating with the same are described in detail in the following chronological list:

Cadet, C. L.

Filtre-presse de M. Réal.

Jour. de Pharm., 2, pp. 165 and 192; Repert. der Pharm. 2, p. 356.

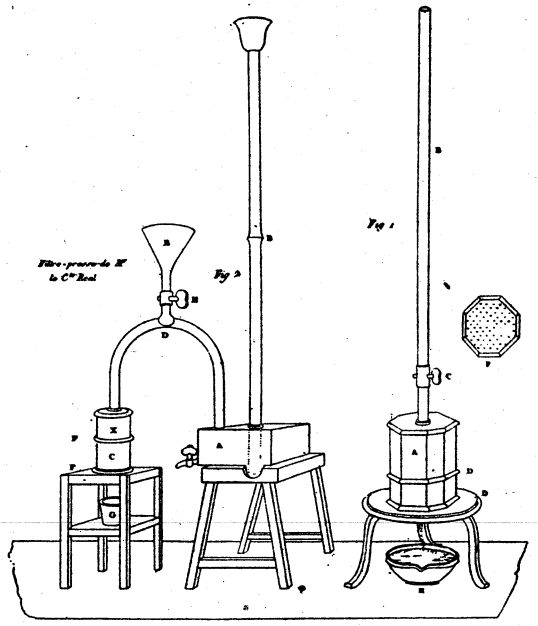


Fig. 1.) The body of the extraction apparatus A is made of tin, the top of which, being screwed on, can be removed. It is

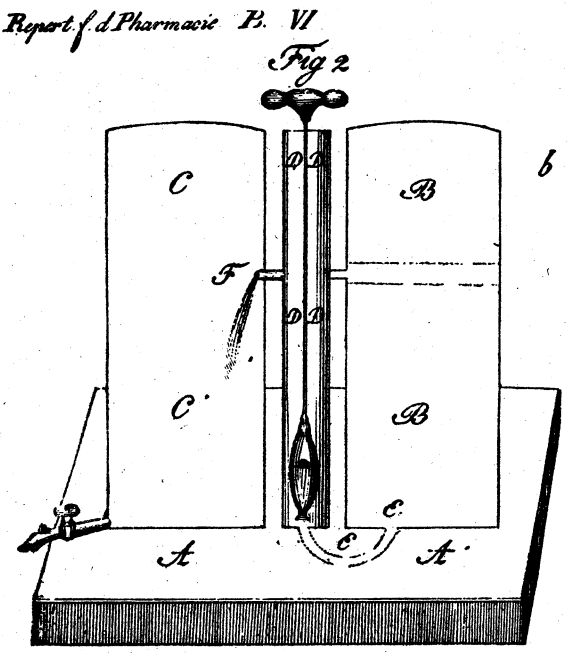
supported on a tripod. At D and D are two false bottoms between which the material to be extracted is packed. Into the cover of the apparatus, the pipe B, which may be 50 to 60 feet high, is fitted. The communication between B and A may be stopped by means of the stop cock C. The dish E under the tripod receives the percolate.

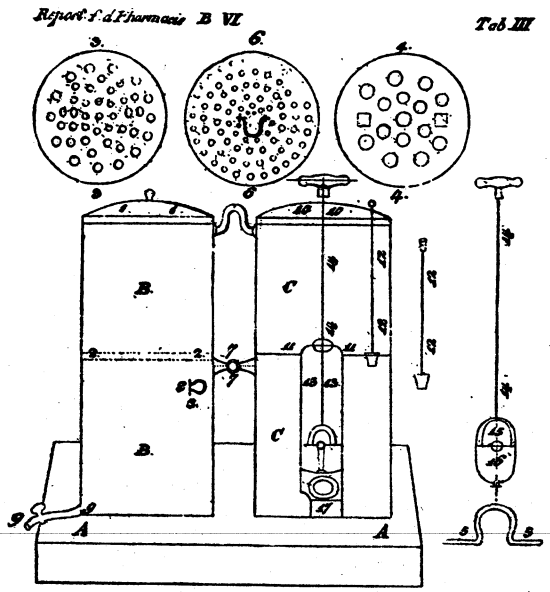
Fig. 2.) The second figure is a modification of the first doing away with the long tube. The solvent is admitted to the space X by pouring it into the funnel E. The percolate is collected in the container G. The pressure is secured by filling the cast iron container A with mercury. After the apparatus C is charged with drug and solvent, the stop-cock H is closed and the pipe B also filled with mercury which then forces the menstruum through the firmly packed drug.

Buchner, J. A. 1819

Beschreibung und Abbildung der von Herrn Dr. Romershausen erfundenen Luft-presse.

Repert. der Pharm., 6, p. 316.





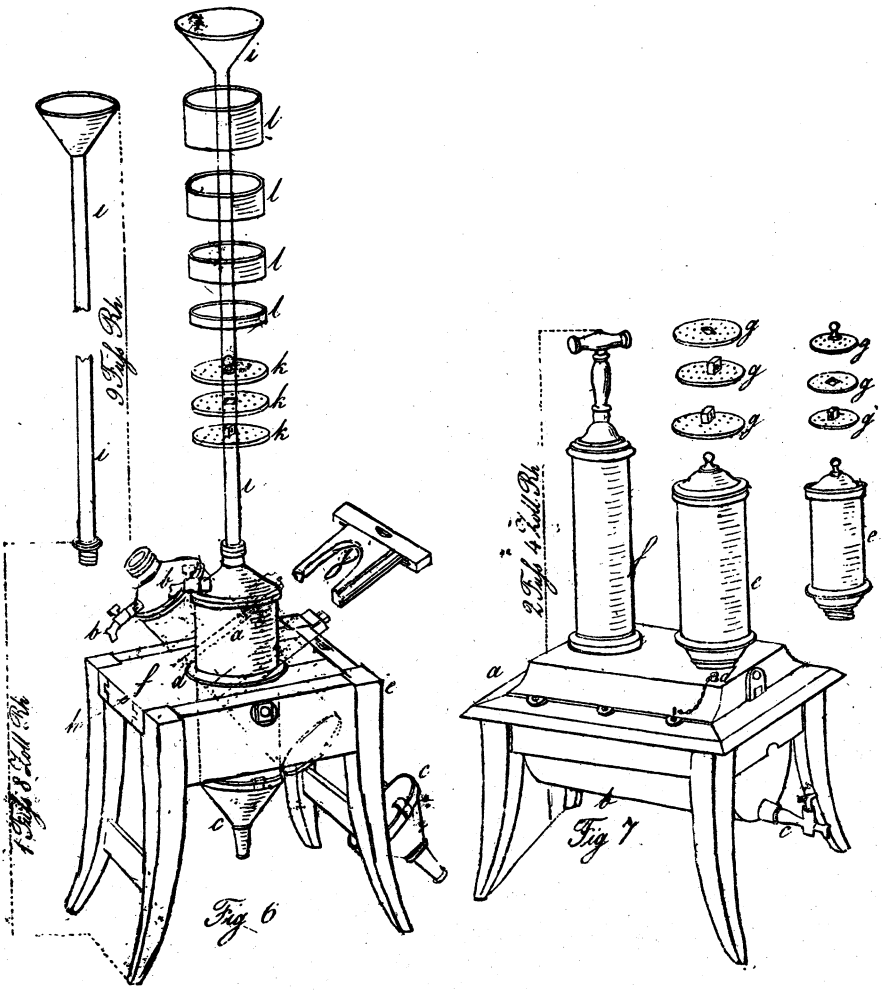
The two twin cylinders B and C are mounted on the support A and are provided with covers 1 and 10. On the support, the diaphragm 3 is placed, covered with a straining cloth which is held in position by the diaphragm 4 which in turn is fastened by the clamp 5. A third diaphragm 6 is used to cover the substance to be extracted. The two cylinders are united by the tube 7 provided with a stop-cock. The lower part of B is also provided with a stop-cock at S in order to allow the percolate to flow out at 9. The lower section of C is converted into an air tight compartment by the cover 11, which is provided with an opening and stopper at 12. The parts indicated by 13, 14, 15, 16, and 17 belong to the suction pump necessary to create a vacuum. The suction pump is outside the cylinder and the percolate is not allowed to collect underneath the percolator B, but is at once pumped in the reservoir C.

Beindorff 1826

Mag. f. d. Pharm., 9, p. 185. [Geiger, Hanbuch d. Pharm. (1830), p. 142].

The cuts represent Beindorff's modification of the Réal and Romershausen extraction apparatus. It will be noticed that the apparatus in figure 6 is so mounted that it can be tipped

at a convenient angle for filling and emptying. In figure 7, a more compact form of the apparatus is shown. In the latter, the long tube is replaced by an air pump.



These forms of pressure percolators were mentioned in connection with the preparation of the oleoresins by Mohr (1854) in his *Commentary on the Prussian Pharmacopoeia.*

Simonin 1834

Journ. de Pharm. et de Chim., 20, p. 128.

Fig. 1.

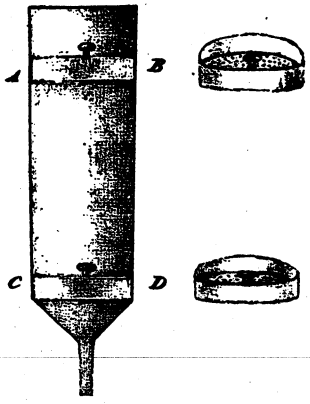


Fig. 2.

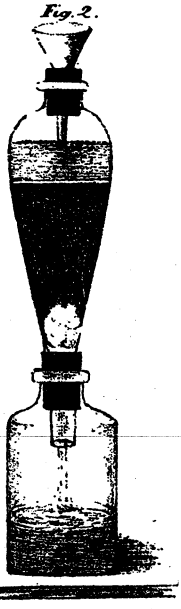
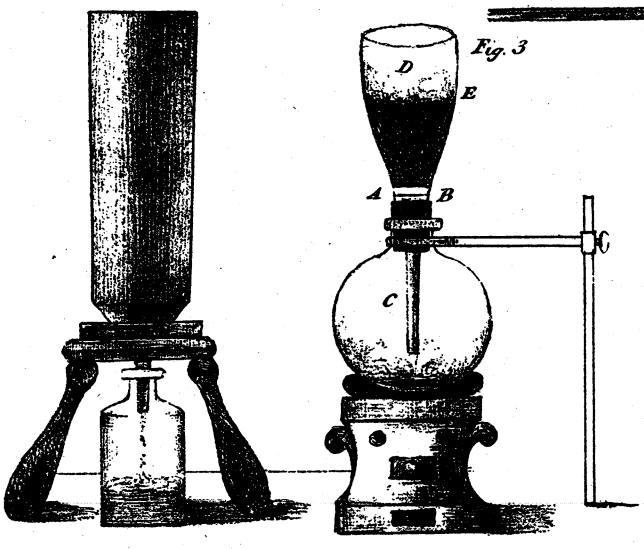


Fig. 3



It is thought that one of the above represents the form of percolator made use of by Béral (1834) in his preparation of the *Piperoide due Gingembre.*

Mohr 1847

Neuer Extractions Apparat fuer Weingeist und Aether. Arch. der Pharm., 100, p. 305. [Am. Journ. Pharm., 21, p. 117].

Fig. 78.

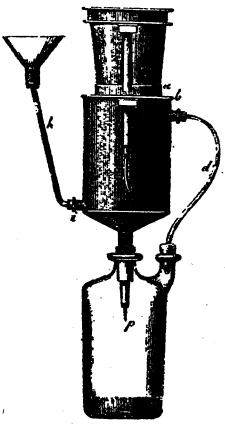
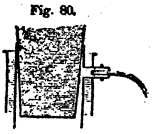


Fig. 79.

Fig. 80.

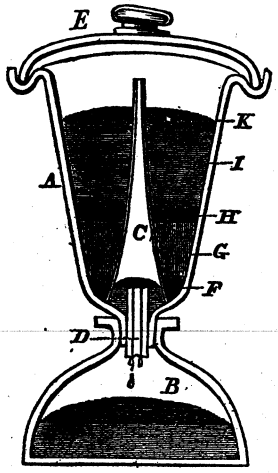


The apparatus consists of a two-necked Woulf's bottle, figure 78 p, into the central mouth of which the metallic vessel R, figure 79, is fitted by means of a cork. The vessel R consists of a metallic cylinder *a* having a perforated strainer *k* near the bottom and terminating with a funnel neck, to admit of its being fitted into the Woulf's bottle. This cylinder is surrounded by a second cylinder *b*, the space between them being intended to contain either hot or cold water. In the top of the inner cylinder *a*, a slightly conical vessel *c* is made to fit air tight, as shown in the drawing. This vessel *c* is intended to be used as a condensing apparatus, and for this purpose it is filled with cold water. From the second or lateral opening of the Woulf's bottle, a glass or tin tube *d*, figure 78, is carried to the upper part of the cylinder *a*, where it is inserted as shown in figure 80. The cold water in the vessel *c* is renewed through the pipe *e* which conducts it to the bottom, while the warm water runs off from the top through the pipe *f*, figure 79. Hot or cold water is renewed to the space between the two cylinders R by the tube funnel *h*, figure 78, and the water from this space overflows into *g* and is carried off together with that from *f*. The tube *h* is inserted through a perforated cork at *i* so that by turning the

tube downwards, the water from the space between the cylinders can be run off.

1849

Mohr, Redwood and Procter's Pharmacy, p. 270.



This consists of a conical vessel A with a water joint rim at the top into which the cover fits. A tube D is ground to fit into the opening in the bottom, and over the end of this tube is placed a conical tube C, the lower end of which has several notches cut in it, so that the liquid can pass under when placed as shown in the drawing. The lower extremity of the vessel A is ground to fit into the mouth of the receiver B.

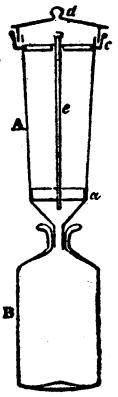
The above apparatus was mentioned by Procter, in 1849, in his article on "the preparation of the oleoresinous ethereal extracts."

1849

Mohr, Redwood and Procter's *Pharmacy*, p. 272.

A is an ordinary tin displacer, except that the rim *c* is soldered around the mouth, in such a manner as to form a water joint when the rim of the cover *d* is placed in it; *a* is a perforated diaphragm, *e* a tin tube open below and above. The latter is soldered to the lower diaphragm, through which it passes, while the upper diaphragm slips over it loosely. In using the dis-

placer, the ingredients are introduced around the tube to a suitable height, the upper diaphragm put in its place, and menstruum poured on, the joint half filled with water and the lid inserted. The atmosphere of the bottle B communicates with that of A through the tube *e*.

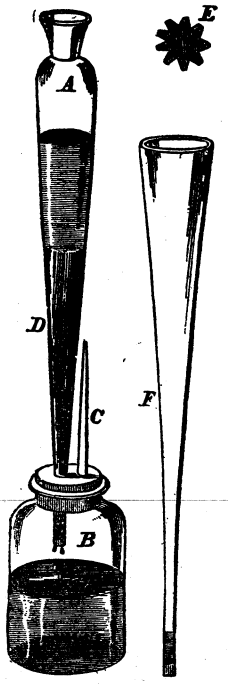


This form of percolator was mentioned by Procter (1849) in his article on "The oleoresinous ethereal extracts."

1849

Mohr, Redwood and Procter's *Pharmacy*, p. 270.

Figure A is a glass adapter, which is selected of suitable size. The lower extremity of this is partially stopped with a cork cut as represented in F. A layer of coarsely pounded glass is put over the cork, and above this a layer of clean sand, thus forming a strainer for arresting the passage of the solid particles of material to be acted upon. The end of the adapter is fitted, by means of a perforated cork, into the mouth of a bottle. A glass tube, one end of which is drawn to a capillary opening, is also fixed in the cork as shown at C so as to allow the air to escape out of the bottle as the liquid drops in. A piece of bladder may be tied over the mouth of the vessel at A to prevent the evaporation of the solvent, but a few pin holes must be made in the bladder to admit of the ingress of air as the liquid passes into the receiver below.



The above form of percolator was mentioned by Procter (1849) in his article entitled *The Preparation of the Oleoresinous Ethereal Extracts.*

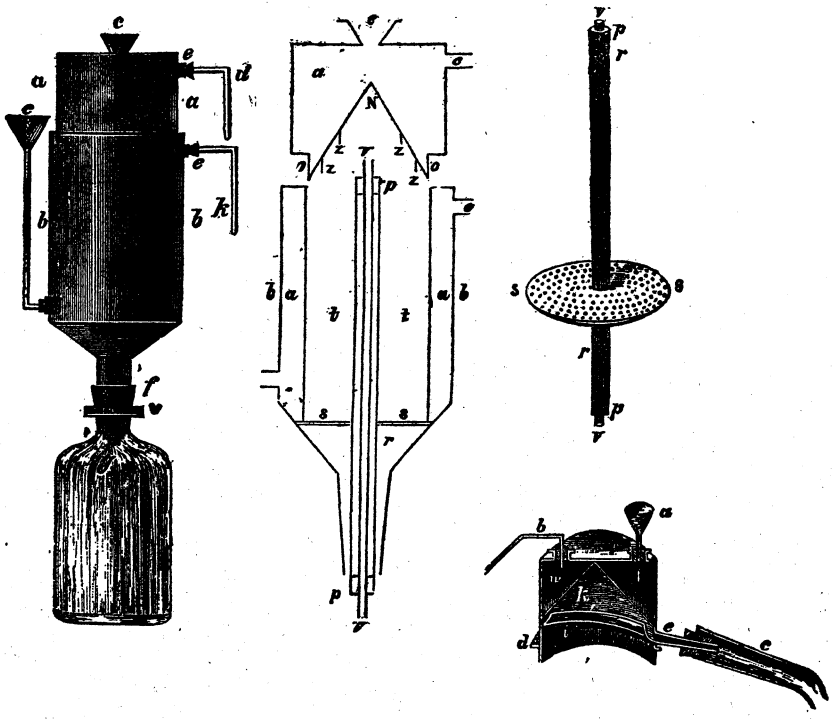
1873

Utensilien zur Bereitung der aetherischen und weingeistig-aetherischen Extracte.

Hager's *Commentar zur Pharmakopœa Germanica,* 1, p. 620.

This consists of a cylinder *bb* fitted into a cork *f* which is inserted into the neck of a flask or bottle *g, aa* is a cover which serves as a condenser. In the lower end of the cylinder *bb* is a tin sieve plate *ss* in the center of which is a tin tube *rr* enclosed in a glass tube *vv*. The glass tube is held firmly in place by a cork at each end *pp*. The condenser *aa* has a conical shaped bottom N around the interior of which run two corrugated rings *zz* of tin. The space *a,* Fig. B, contains cold water which enters from the openings *cc* and flows out through

less, the top *aa* is taken off and D put on in its place. It is also a condenser. The water flows in at *a* and off through *b*. The conical bottom K is so arranged that the condensed solvent the tubes *ee*. As soon as the menstruum drops through color-



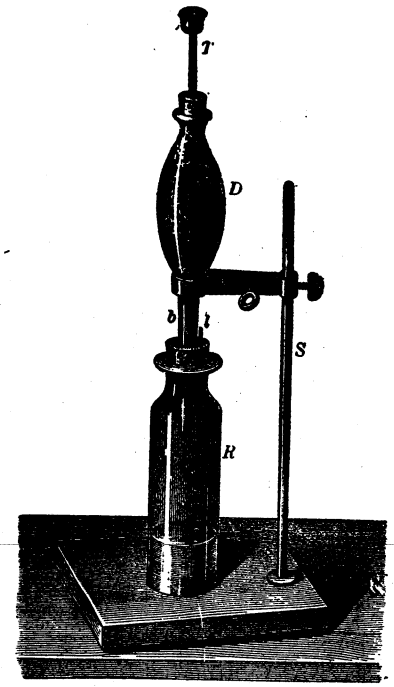
drops from off the receiver *i* and is carried off into a flask through the outlet *e*. The space between *vv* and *aa* is filled with either hot or cold water.

1873

Utensilien zur Bereitung der aetherischen und weingeistig-aetherischen Extracte.

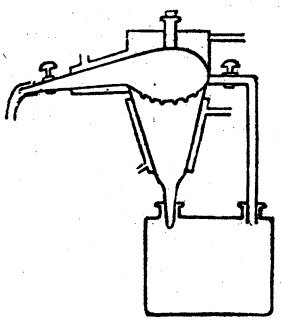
Hager's *Commentar zur Pharmakopoea Germanica*, 1, p. 622.

A displacement tube D with a wide mouth at its upper end is closed with a cork through which runs a thistle tube T. The lower end is pushed through a cork which fits tightly in a receiving bottle R. The small glass tube *l* is for the purpose of letting the air escape from the receiver R.



1884

Parish's *Treatise on Pharmacy*, p. 755.



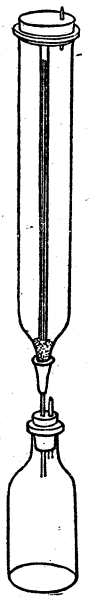
A percolator of tinned copper is surrounded by a jacket of the same material; the receiver is a copper vessel with two necks into one of which the percolator is secured, the other is connected with a pipe leading to the closed head of the percolator which is also jacketed; on the other side of the head is a perforated plate

of tinned copper, which distributes the ether over the surface of the drug when it has been volatilized by placing the receiver in hot water. After the exhaustion of the drug, the receiver is removed, the lower orifice of the percolator closed, and the head well refrigerated; a stream of hot water is then passed into the jacket around the percolator, by which means the contained ether may be recovered.

1886

Remington's *Practice of Pharmacy* 1886, p. 366.

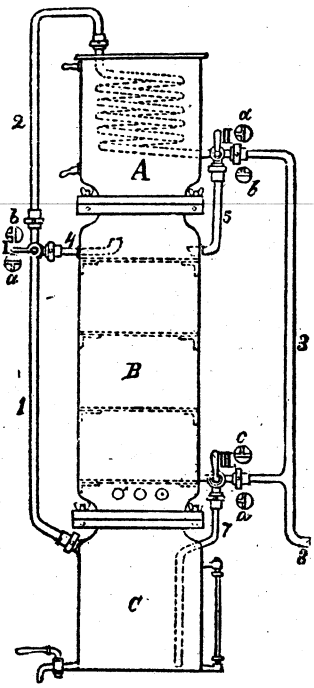
The apparatus consists of a cylindrical percolator fitted into the mouth of a receiving bottle with the aid of a cork. The upper part of the percolator being closed and a small opening left in the cork to allow the escape of air from the receiving bottle.



A continuous extraction apparatus can be made of this percolator by enclosing the upper part in a suitable case and passing cold water between, arranging the apparatus like a Liebig's condenser. A glass tube is connected with the top of the percolator and the mouth of the bottle by rubber tube connections,

and if the receiving bottle be placed in a water bath and the water gently heated, the ether will evaporate from the percolate, the vapors rising in the tube and condensing in the upper part of the percolator.

Lewin R. 1887
Ein neuer Extractions Apparat,
Arch. der Pharm., 215, p. 74. [Proc. A. Ph., 35, p. 12.]



This apparatus is adapted for 1) continuous extraction with hot menstrua, 2) continuous extraction with cooled menstrua, 3) recovery of the menstrua from the finished extract by direct distillation.

It is composed of three easily separable principle parts: C, the tinned copper still, B, the copper percolator, which is provided with three movable sieve bottoms for the reception of

1) For continuous extraction with hot solvents, the vapors pass from the still C, in the tube 1, and enter through the tri-

faucet I, when in position *a*, through tube 4, into the percolator, the substance to be extracted. A is the condenser.
B, penetrate the substance to be extracted, and condense. The percolate passes into the receiver and from this flows through the tri-faucet III in its position *a*, through the tube 7, again into the still, to repeat this course as long as it may be desirable. To prevent pressure in the apparatus, the tube 2, is removed during this operation, and the tri-faucet II is placed in position *a*. This admits the vapor into the cooling worm, A, which thus forms a safety valve.

2) For the continuous extraction with cooled solvents, the vapors pass from the still C, into tube 1, and enter through the tri-faucet I, in its position *b*, through tube 2, into the cooling worm A, from this as a liquid through the tri-faucet II, in its position *a*, into the percolator, and so through the substance to be extracted into the still as before.

3) For the recovery of the solvent from the extract by direct distillation, the vapors pass from the still C, through tube 1, through the tri-faucet I, in its position *b*, through tube 2, into the cooler, A, through the tri-faucet II in its position *b*, into the exit tube 3, which latter may be lengthened at pleasure.

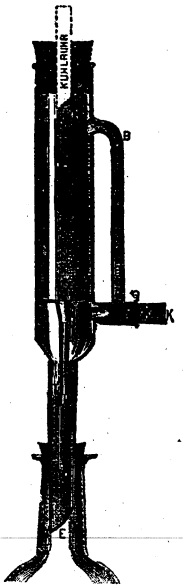
Portions of the percolator may be removed from the receiver at pleasure through the tri-faucet III, in its position *c*, by the tubes 2 and 3. All of the tubes are connected or disconnected by good screw joints.

Flueckiger, F. A. 1889

Ein zweckmaessiger Extraktionsapparat.

Arch. d. Pharm., 227, p. 162. [Proc. Am. Pharm. Assoc., 37, p. 338.]

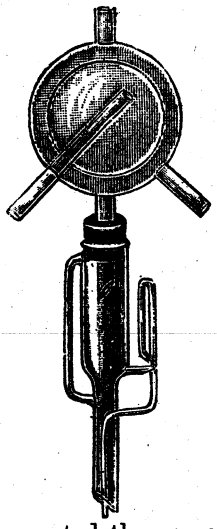
The extraction tube A is provided at C with a diaphgram from the center of which a small tube or neck extends into the funnel D. The tube B F attached to the side, passes into a tubulure G, which is provided with an ordinary cork K by means of which communication through the tube B F, between the upper, and the lower portions of the apparatus may be cut off or established. Thus causing the condensed liquid to return through the drug when the communication is closed or allowing the liquid to be distilled off when it is open.



Caspari in his *Treatise on Pharmacy* (1916) describes the use of this apparatus in connection with the preparation of the oleoresins.

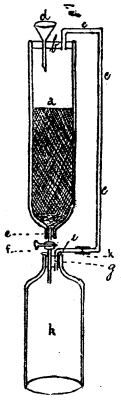
1890

Szombazi Soxhlet's Extraction Apparatus.
Dingler's Pol. Journ., 256, p. 461. [Zeitschrift f Anal. Chem., 19, p. 365.]



Maish (1900) first suggested the use of this apparatus in the preparation of the oleoresins.

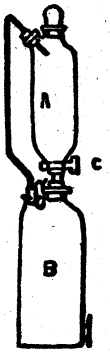
Alpers, William C. 1896
Oleoresinae.
Merck's Rep., 5, p. 593. [Proc. Am. Pharm. Assoc., 45, p. 435.]



The apparatus consists of a cylindrical percolator *a*. The upper end of the percolator is closed with a large cork *b* through which two holes have been bored—the one for receiving a bent glass tube *c*, the other for a small glass funnel *d*. The lower narrow end of the percolator is closed by a cork *e* through which a straight connecting glass cock *f* passes into another perforated cork *g* that closes the receiving bottle *h*. This cork contains a second perforation with a small bent glass tube *i*. The glass tubes *c* and *i* are joined by means of a small piece of rubber tubing at *k*.

1902

Coblentz's *Handbook of Pharmacy*, p. 290.

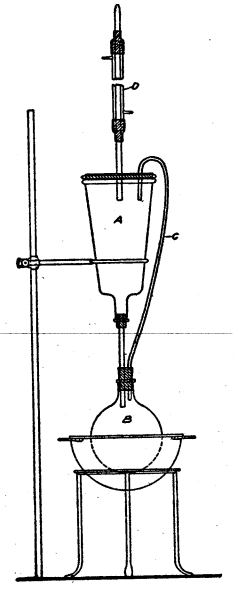


A is a percolator with a stop cock C. It is inserted into a receiver B. The receiver B and percolator A are connected

with a tube as shown in the figure for the purpose of equalizing the pressure as the apparatus is closed throughout.

1908

Brandel and Kremers, Percolation, p. 52.



A is an ordinary conical percolator of such a size that it will not be more than two-thirds filled with the drug to be extracted. B is a round-bottom flask, containing a twice perforated stopper, through one hole of which a glass tube connects the flask to the percolator. Through the second hole is inserted the glass tube C which also passes through the cork stopper in the top of the percolator. The end of the condenser D is also inserted through this cork. All cork connections should be tightly sealed with gelatine.

The above is the form of apparatus which was used in the laboratory in the preparation of the oleoresins when 500 grams or more of the drug were extracted.

Yield

The yield of oleoresin is a variable quantity depending, first of all, upon the oleoresin content of the particular drug from which it is prepared. Thus, the oleoresin content of ginger is only about one-half that of the aspidium and one-fourth that of cubeb. Not, only, however, does the oleoresin content vary with the different drugs, but the drug, when of the same genus and species, may show a variation due to a number of influences, such as the climate in which grown, time of harvesting, conditions under which stored, *et cetera.* As an illustration of these influences, aspidium may be taken. The maximum yield of oleoresin, in this case, is obtained from the freshly dried Russian rhizomes collected in the month of September.[1]) Or, the case of ginger may be cited. In this instance, the African rhizomes harvested at maturity (usually in February)[1]) give the largest amount of oleoresin. This characteristic will be taken up in detail under the treatment of the individual oleoresins. The other important factors in determining the amount of oleoresin obtained, in general, are two in number, *viz:* the solvent employed in extracting the drug, and the method employed in operating with the same. Both of these factors have been dealt with in a general way under the two preceding headings. They will also be discussed more fully in connection with the individual preparations.

Chemistry

The Chemistry of the oleoresins *per se* has apparently received but little attention, except in the case of the oleoresin of aspidum. The latter has been the subject of numerous investigations and its chemistry is now understood fairly well. Some work has also been done toward determining the composition of the oleoresins of cubeb and lupulin, but our present knowledge of the chemistry of these preparations is still very indefinite.

A very considerable amount of work has been done toward clearing up the chemistry of the drugs from which the oleoresins are prepared, and it is from this source that we are

[1] See tables of yield under the oleoresins of aspidium and ginger, respectively.

obliged to obtain what information we have concerning the composition of most of these preparations. It is for this reason that the chemistry of the drugs from which the oleoresins are prepared is given consideration in this monograph. See "Chemistry of the drug and its oleoresin" under the treatment of each individual oleoresin.

Physical and Chemical Properties

The determination of the physical and chemical properties of the galenical oleoresins in general does not appear to have been undertaken systematically in the past. While there are numerous references in the literature concerning color, odor, taste and consistence, there is no mention, except in connection with the oleoresins of aspidium and cubeb, of the properties which we should naturally expect to find under a description of a class of preparations of this nature, *viz:* specific gravity, refractive index, acid number, saponification value, *et cetera.* This condition is surprising in view of the work which has been done along this line in connection with the natural products of the same name. That cognizance is, however, being taken of the subject at the present time is evidenced in the comparatively recent work which has been done abroad on the oleoresin of aspidium. In the latter case, the methods usually employed in fixing the standards of similar natural products were applied, and with considerable success. A brief general discussion of these properties as well as other characteristics, which have been mentioned in this connection, follows.

Physical Properties

Color:

The color is a characteristic property of the individual members of this class of preparations. Considered with respect to a single member, it serves in some cases as a measure whereby the quality of the product may be roughly determined. Thus, a brown color in the oleoresin of aspidium indicates an inferior preparation, in the making of which old deteriorated rhizomes have been used, whereas, a deep green color is said to indicate adulteration with salts of copper. Likewise, a brown color in the oleoresin of cubeb warrants the opinion that ripe instead of unripe fruits have entered into its preparation. How-

ever, as the color of the individual preparations, when properly made, varies to a considerable extent, and as the description of exact shades is a difficult matter, this property as described in the literature is naturally somewhat indefinite. This subject will receive further consideration of the treatment of the individual oleoresins.

Odor:

The oleoresins without exception possess distinct odors resembling in an intensified degree those of the drugs from which they are prepared. In general, this property offers a ready means of identifying these preparations. In specific instances, it may also serve as an indication of the quality of the product. For example, a rancid odor in the case of the oleoresin of aspidium is evidence of the use of old deteriorated rhizomes in its preparation or of undue exposure to the air while kept in storage. For similar reasons, the oleoresin of lupulin may have a disagreeable cheesy odor. Furthermore, unevaporated solvent, even when present in comparatively small amounts, may be most easily detected by this means. This property will be discussed in greater detail under the individual oleoresins.

Taste:

The taste of the individual oleoresins, like the odor, is a property acquired in an intensified degree from the drugs from which they are prepared. Likewise, this property also serves as an aid in the identification of these preparations. In addition, however, it has been made the basis of a quantitative physiological test(1) for the determination of the quality of the oleoresins of capsicum and ginger. For a further discussion of this property, see the individual oleoresins.

Consistence:

The U. S. P. oleoresins, with the exception of the one prepared from lupulin, are liquids. The degree of fluidity, however, varies with the individual under consideration, with the temperature and with certain other conditions, which will be discussed in detail under the separate treatment of each individual. The oleoresin of lupulin is usually of the consistence of a very soft extract.

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[1] See under the oleoresins of capsicum and giner respectively.

Solubility:

The solubility of the different oleoresins naturally depends to a large extent on the solvent which was employed in their preparation. It does not, however, follow from this statement that, because an oleoresin was prepared with ether, it will always be completely soluble in the same. Some of these preparations on standing undergo chemical changes with a resulting change in solubility. For example, the oleoresin of aspidium forms a deposit on ageing, and the deposited material is practically insoluble in ether. As a rule, the oleoresins, when prepared with ether, form clear or slightly cloudy solutions with absolute alcohol, acetone and chloroform, whereas, they are only partially soluble in petroleum ether and carbon tetrachloride.

In the case of certain members of this class of preparations, this property has been of considerable value in detecting adulterations or in the identification of the solvent which was employed in their manufacture. For specific instances of the application in this connection, see under the oleoresins of aspidium and ginger.

Specific gravity:

The value of determining the specific gravity as an aid to standardizing the oleoresins appears to have been first noted by Procter. In 1866, he published data showing how this constant, in the case of the oleoresin of cubeb, varied with the solvent employed in its preparation, and further pointed out that a low specific gravity observed in the commercial product was, in one instance at least, an indication of the incomplete removal of the solvent, ether. Procter's observations were as follows:

TABLE 7.—*The specific gravity of the oleoresin of cubeb.*

Drug	Solvent	Specific gravity	Remarks
		at 76° F.	
Cubeb.....	Alcohol.....	0.9850	Prepared by Procter.
"	Ether	0.9675	" " "
"	Benzin	0.9325	" " "
"	Ether.....	0.9000	Commercial sample containing ether.

This work, however, appears to have received but little attention as there is no further mention of the determination of this

constant in this connection in the literature until 1903. In that year, the English firm of Southall Brothers and Barclay published a statement in their *Laboratory Reports,* in which a standard range for the specific gravity of the oleoresin of aspidum was given. Interest in the matter again seems to have waned and it was not until 1911, when Parry showed that the last named preparation was being extensively adulterated with castor oil, that the necessity for standardizing this preparation became apparent. The subject was then taken up in earnest, however, and in 1913, no less than four articles on the determination of the physical and chemical constants of the oleoresin of aspidium made their appearance. In each of these, the determination of the specific gravity was given some consideration.

From the foregoing brief résumé of the literature on this subject, it becomes apparent that the determination of the specific gravity as a factor in evaluating the oleoresins has received consideration in connection with but two of the official preparations. Furthermore, that practical use has been made of this constant only in the case of the oleoresin of aspidium. The results obtained with respect to these two preparations, however, are deemed to be of sufficient importance to warrant the determination of this constant in the case of the other members of this class of preparations.

The manner in which this constant was determined by the above mentionel investigators does not become apparent from their work as reported in the literature. It is thought, however, that an ordinary glass pycnometer and chemical balance were employed for this purpose. In the determinations made in the laboratory, a 10 cubic centimeter pycnometer was used, except in the case of the oleoresin of lupulin which was usually too thick to handle in this manner. For the determination of the specific gravity of the latter, a Nicholson's hydrometer was employed. All determinations were made at 25° C.

The results as obtained in the laboratory and those reported elsewhere will be discussed in detail under the treatment of the individual oleoresins.

Refractive index:

The determination of the refractive index has received consideration only in connection with the standardization of the

oleoresin of aspidium. In this case, it has proven to be of particular value in detecting adulteration with castor oil as was first pointed out by Parry in 1911. Subsequent work by other investigators has not only confirmed Parry's observations, but has shown that in some instances a low refractive index may be an indication of a low filicin content due to natural causes[1]) as well.

Since most of the other official oleoresins are sufficiently transparent to permit of the direct determination of this constant, it was thought that such determination might likewise prove to be of some aid in standardizing these preparations. That such an opinion has proven to be correct will be shown in connection with the discussion of this topic under the individual cases.

For the determination of this constant in the laboratory, the Abbe refractometer was employed, all observations being made at 25° C. In those cases (the oleoresins of ginger and lupulin) where the color was too intense to permit of a direct determination being made, the oleoresin was dissolved in an equal volume of castor oil and the refractive index computed from the following formula:

$$n_D\,(b) = 2n_D\,(a + b) - n_D\,(a)$$

a = refractive index of castor oil.
b = " " " oleoresin.

Chemical Properties

Loss on Heating:

The oleoresins without exception lose weight on drying. This loss is usually referred to in the literature as the moisture content. It has been determined by heating the preparation at 100 to 105° C. for a definite period of time, or until of constant weight. The falacy of designating the loss of weight thus obtained as the moisture content becomes evident when we take into consideration the fact that these preparations contain volatile substances other than water, which would also be removed by heating to a temperature of 100° C. Indeed, the oily

[1] The male fern rhizomes have been shown to vary in filicin content due to the climatic conditions under which they were grown, time of harvesting, *et cetera*. See under "Drug used, its collection, preservation, *etc.*"

nature of these preparations exclude the presence of any great quantity of moisture. This statement has been borne out by laboratory experiments. Attempts to determine the moisture by means of the xylene[1]) method failed to reveal the presence of a measurable amount of water in any of the samples examined. The loss in weight is, therefore, due, ordinarily, to the removal of volatile oil and in exceptional cases to the removal of unevaporated solvent. Such being the case, the determination of this constant serves as a means of measuring the amount of volatile oil naturally occurring in these preparations and as a means of detecting the presence of unevaporated solvent.

The amount of weight lost by the oleoresins when determined as stated above varies greatly with the individual members comprising this class of preparations. The oleoresin of cubeb which contains a comparatively large amount of volatile oil naturally sustains a comparatively great loss, while the oleoresin of capsicum which contains a small amount of volatile matter shows but a slight loss. There is noted a further variation in the case of each individual due to a variation in the amount of volatile matter naturally occurring in the drug from which the oleoresin was obtained, or to a variation in the conditions under which the individual was prepared. As an illustration, the oleoresin of cubeb may be cited. The volatile oil content of cubeb is stated to be 10 to 18 per cent. A much greater variation is, therefore, to be expected in the oleoresin which represents only the alcohol soluble portion of the drug. With respect to the conditions under which the oleoresin of cubeb is prepared, observations in the laboratory have shown that the preparation will contain a larger amount of volatile oil when the solvent is allowed to evaporate spontaneously at room temperature, than when the same is removed by evaporation on a water bath. In most cases, the variation, due to the difference in solvent used in extracting the oleoresins, appears to be so slight as to be almost negligible. In the case of the oleoresin of pepper, however, there is a very noticeable difference. This is very likley due to the nature of the preparation, its viscosity making it difficult to remove the last traces of the less volatile solvents without the application of heat.

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[1]U. S. Dept. Agric., Forest Service, Circ. 134.

In the determinations of this nature made in the laboratory, a weighed amount of the oleoresin (about 2 grams) was heated in an electric oven at 100° C for 3 hours, cooled in a desiccator and weighed, the difference in the two weights being taken as the loss.

A more detailed consideration of this subject will be found under the treatment of the individual oleoresins.

Ash Content:

The determination of the ash content of the oleoresins is of special value in identifying the solvents which have been used in their preparation. Such determinations, made in this laboratory, also by the firm of Dieterich[1]) in Helfenberg, have shown that, while there is as a rule comparatively little difference in the ash content of these preparations, when prepared with the same solvent, there is a marked variation in the case of each individual when different solvents are employed. The oleoresin of lupulin is an exception to this rule. Its ash content varies to a considerable extent even when prepared with the same solvent.

In addition to the above, the qualitative examination of the ash of commercial samples has revealed the fact that nearly all of them contain copper, due in most cases to the action of the free fatty acids on the utensils employed in their preparation. In some instances, the presence of the metal must be attributed to the addition of copper salts for the purpose of imparting the desired green color to preparations of inferior quality. See under the adulteration of the oleoresins of aspidium and cubeb, respectively.

The ash content of the oleoresins examined in the laboratory was determined as directed by the last edition of the *United States Pharmacopœia* under "Determination of Ash or Non-volatile Matter," p. 589.

Copper, when present, was identified by the blue color of the solution formed when the ash was dissolved in a few drops of hydrochloric acid, diluted with water, and ammonium hydroxide solution added.

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[1] The firm of Dieterich has for a number of years determined the ash content of the oleoresins of aspidium and cubeb. A tabulation of the results as obtained by this firm will be found under the separate treatment of these oleoresins.

For a more detailed discussion of this subject, see under individual oleoresins.

Acid Number:

Kremel in 1887 determined the acid numbers of the oleoresins of aspidium and cubeb. Inasmuch. however, as he made but one determination in each case, no conclusions can be drawn from his work. Similar determinations made in this laboratory on all of the official oleoresins show that this property varies greatly depending on the particular individual under consideration. Furthermore, that no general statement can be made as to its value in fixing the standards of these preparations, but that it is of importance when considered in connection with individual cases as will be brought out later.

For the manner in which this constant was determined in the laboratory, see the *United States Pharmacopœia,* ninth revision, (1916), p. 591.

Saponification Value:

The saponification values of the official oleoresins, as determined in this laboratory and elsewhere,[1]) indicate that this property may be an important factor in fixing standards for these preparations. The results obtained by Parry, Harrison and Self, and others show that in the case of the oleoresin of aspidium, the saponification value varies directly as the filicin content, and is, therefore, useful as a check on the determination of the latter. Considered in connection with such of these preparations as contain easily oxidizable substances, an abnormally high saponification value is very likely caused by an increase in the acid content due to the action of the oxygen of the air, and is thus an indication of an old product[2]) or of improper care in storing. As an example, the oleoresin of lupulin may be cited. In this case, a high saponification value signifies an old preparation or one that has been prepared from deteriorated drug.[3]) These factors, together with the influence of the solvent employed and the method of preparation on this property, will

[1] Saponification values have only been determined in the past in the case of the oleoresin of aspidium and in one instance in the case of the oleoresin of cubeb.

[2] See oleoresin of aspidium.

[3] See oleoresin of lupulin.

be considered in greater detail under the treatment of the individual members.

The manner in which this constant was determined in the laboratory is described on p. 590 of the *United States Pharmacopœia,* ninth revision.

Iodine value:

The determination of the iodine value as an aid to the standardization of the oleoresins appears to have been first employed by the firm of Dieterich in Helfenberg in 1904, however, only in the case of the oleoresin of aspidium. It has since received further practical application, in connection with the same preparation, by the English firm of Evans Sons, Lescher and Webb, while a number of similar determinations have been made by the author. The results[1]) obtained with respect to this preparation show that the iodine value varies directly as the filicin content, and, therefore, serves as another check on the determination of the latter constituent.

With respect to the other official oleoresins, it may be stated that, as a general rule, the iodine value is high in the case of those preparations which contain a large amount of unsaturated constituents of ether fatty or volatile oil.[2]) Further than this, it may be influenced largely by the nature of the other constituents of these preparations and will be considered in detail in connection with the treatment of each individual.

For the method employed in the laboratory in the determination of this constant, see the *United States Pharmacopœia,* ninth revision, p. 590.

Special Tests

While the different official oleoresins can, as a rule, be identified without difficulty, the use of various adulterants in their preparation, through ignorance in some cases, or with willful intent on the part of unscrupulous manufacturers, has made it necessary to guard against this practice by making use of certain qualitative and quantitative tests. As will be brought out later, such tests have been applied principally to the preparations official in foreign countries, namely: the oleoresins

[1] See under oleoresin of aspidium.
[2] See under oleoresin of cubeb.

of aspidium and cubeb. No tests of this, or, as a matter of fact, of any kind have been included in the *United States Pharmacopœia.* It is thought, however, that if interest in these preparations could be awakened in this country, the need of similar precautions with respect to all of the official oleoresins would become apparent.

Qualitative Tests:

Inasmuch as the common physical properties, such as odor, taste and appearance, are very characteristic of the oleoresins, it is hardly necessary to resort to other means for their identification. It appears, however, that the use of the so-called false cubebs in the preparation of the oleoresin of cubeb has made necessary a more certain method of identification. Such a method, based on the red color produced when concentrated sulphuric acid is added to the oleoresin prepared from the genuine fruit,[1]) has, therefore, been given in most of the late European pharmacopœias. Likewise, the use of other species of fern in the preparation of the oleoresin of aspidium caused a qualitative test for this preparation to be included in the late editions of the Austrian, Hungarian and Netherlands pharmacopoeias. For the details of these methods, see qualitative tests under the respective oleoresins.

Quantitative Tests:

On the whole, very little has been done in the past toward developing quantitative methods for the evaluation of the oleoresins. This condition is perhaps due, for the main part, to an imperfect knowledge of the chemistry of most of these preparations, as well as to the lack of exact information concerning the constituents of therapeutic value. In the case of the oleoresin of aspidium, however, the therapeutic value of the preparation has been shown to depend upon a number of acid constituents, the quantity present varying through natural and artificial causes. As a result, various methods[2]) for the determination of the total acid content have been devised and are in use at the present time, a modification of the original method of Fromme being officially recognized in the late edition of the British and Swiss

[1] Dekker states that the so-called false cubebs give a yellow color with concentrated sulphuric acid. Pharm. Ztg. (1912), 84, p. 845.

[2] See under oleoresin of aspidium.

pharmacopoeias. The only other work of this nature appears to have been done quite recently (1914) by the H. K. Mulford Co. in the standardization of the oleoresins of capsicum and ginger. This firm has devised a physiological method for this purpose based on the extreme pungency of these preparations, the highest dilutions in which these preparations (on the average) are still perceptable to the taste being taken as standards.

Experiments conducted in the laboratory in preparation for this monograph have shown, not only that there is an opportunity for improving on some of the above mentioned methods, but that there is need for the development of quantitative methods which may be applied to the other individuals of this class as well. With respect to the forepart of thiss tatement, it is thought that a gravimetric method for the estimation of the pungent principles (gingerol) in ginger would be an improvement over the physiological method of the Mulford Co. as personal idiosyncrasy would thus be eliminated. Trials with the method of Garnett and Grier[1]) (for the estimation of gingerol in ginger) adapted to the oleoresin appear to indicate the correctness of this opinion. In the case of the oleoresin of capsicum, however, the physiological method apparently offers the only practical course at the present time, in view of the fact that the active constituent, capsaicin, is present in such minute quantities that an accurate gravimetric determination would be a difficult matter.

In considering the application of new methods, the work done in this laboratory on the oleoresin of pepper may be cited. Since the therapeutical value of this preparation is apparently due to its piperine content, a method for the quantitative determination of this constituent appeared to be desirable. With this object in view, the nitrogen present was determined by the Kjeldahl method and the piperine content computed therefrom. Some very interesting results were obtained.[2]) As to further possibilities along this line the determination of the apiol content of the oleoresin of parsley, or the estimation of the quantity of total acid resins present in the oleoresin of cubeb may be mentioned.

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[1] See under oleoresin of ginger.
[2] See under oleoresin of pepper.

Adulterations

The examination of commercial samples of the oleoresins has shown that they are all adulterated at times. With respect to most of these preparations, adulteration is thought to be accidental, *e. g.* the presence of copper in nearly all samples due to the use of copper utensils in the manufacture of the same, or the use of ripe instead of unripe fruits in the preparation of the oleoresin of cubeb. In some cases, however, adulteration has been practiced with willful intention to defraud, as for example, the addition of fatty oils to the oleoresins of aspidium and cubeb. Other instances of this kind will be given consideration under the treatment of the individual oleoresins.

PART II—INDIVIDUAL OLEORESINS

OLEORESIN OF ASPIDIUM

Synonyms

Aceite de Helecho Macho, Sp. P. 1905.
Aetheres pafran-kivonat, Hung. P. 1880.
Aetherhaltiges Farrenkrautextract, Aust. P. 1844.
Aetherisches Farrnkrautextract, Pruss. P. 1830.
Aetherisches Farrnkrautwurzel Extract, Bad. P. 1841.
Alvejuuriekstrakti, Finn. P. 1914.
Balsamo de Helecho, Dorvault, *L'Officine,* Sp. Trans. 1879.
Balsamum Filicis, Pareira, Mat. Med. 1854.
Baumé de Fougère, Dorvault, L'Officine, 1898.
Braegne-Extract, Dan. Mil. P. 844.
Bregnerod Extract, Nor. P. 1870.
Bregnerodekstrakt, Nor. P. 1895.
Bregnerotekstrakt, Nor. P. 1913.
Estratto di Felce Maschio, Swiss. P. 1907.
Estrato di Felce Maschio Etereo, Ital. P. 1902.
Ethereal Extract of Male Fern, Journals.
Extract of Male Fern, Jap. P. 1907.
Extract van Mannetjes-Varen, Nethl. P. 1871.
Extracto de Feto Macho, Port. P. 1876.
Extracto de Feto Macho Ethereo, Port. P. 1876.
Extracto Etereo Helecho, Sp. P. 1884.
Extracto Ethereo de Helecho Macho, Sp. P. 1905.
Extracto oleo-resinoso de Helecho, Dorvault, *L'Officine,* Sp. Trans. 1879.
Extractu de Filice Mascule, Roum. P. 1874.
Extractu di Felce Machio, Swiss. P. 1865.
Extractum Aethereum Filicis, Sp. P. 1884.
Extractum Aethericum Filicis, Fr. P. 1866.
Extractum Aethericum Filicis Maris, Fr. P. 1866.
Extractum Aspidii, Nor. P. 1854.
Extractum di Felce Machio Etereo, Port. P. 1876.
Extractum Filicis, G. P. 1900.
Extractum Filicis aethereum, Pruss. P. 1861.
Extractum Filicis liquidum, B. P. 1914.
Extractum Filicis Maris aethereum, Ital. P. 1902.
Extractum Filicis oleoso-resinosum, Jourdan, Univ. P. 1832.
Extractum Radicis Filicis Maris athereum, Bad. P. 1841.

Extractum Stipitum Aspidii, Nor. P. 1854.
Extrait de Fougère, Belg. P. 1906.
Extrait de Fougère Mâle, F. P. 1908.
Extrait Ethéré de Fougère, Belg. P. 1854.
Extrait Ethéré de Fougère Mâle, Fr. P. 1866.
Extrait oléo-résineux de Fougère, Bern. P. 1852.
Extrait oléo-résineux de Fougère Mâle, Fr. P. 1908.
Farnextrakt, Ger. P. 1900.
Farrenkrautextrakt, Bern. P. 1852.
Farrnwurzel Extract, Swiss. P. 1865.
Filicis Extractum, Belg. P. 1906.
Filixextrakt, Journals.
Huile de Fougère Mâle, Belg. P. 1854.
Huile de Fougère de Peschier, Bern. P. 1852.
Liquid Extract of Fern Root, Br. P. 1864.
Liquid Extract of Male Fern, Br. P. 1885.
Oil of Filix mas, Parrish, Treat. on Pharm. 1867.
Oil of Male Fern, Journals.
Oleoresin of Fern, U. S. P. 1870.
Oleoresin of Male Fern, U. S. P. 1910.
Oleoresina Aspidii, U. S. P. 1910.
Oleo-resina de Helecho, Dorvault, L'Officine, Sp. Trans. 1879.
Oleoresina Filicis, U. S. P. 1860.
Oleo-Resina Filicis, Peschier, Ver. P. der Lond., Edinb., and Dub. Med. Coll. 1827.
Oléo-résine de Fougère, Dorvault, L'Officine, 1898.
Oleum Filicis, Hung. P. 1861.
Oleum Filicis Maris, Sp. P. 1905.
Oleum Filicis Maris aethereum, Swiss. P. 1865.
Oleum Filicis Peschieri, Pareira, Mat. Med. 1854.
Oleum Filicis pingue resinosum, Geiger's P. 1835.
Oleum Radicis filicis, Strump. Allg. P. 1861.
Orbunksrot Extrakt, Swed. P. 1901.
Pafran-Kivonat, Hung. P. 1871.
Varenextract, Neth. P. 1905.
Wurmfarnextrakt, Swiss. P. 1893.
Wurmfarnoel, U. S. Disp. 1907.

History

The oleoresin of aspidium, or *Huile de Fougère Mâle* as it was originally known, was first prepared by Peschier in 1825.[1] The advantages of Peschier's preparation over the forms in which male fern was being administered at the time were quickly noted and it received almost immediate recognition throughout Europe. The rapidity with which it was taken up by the medical profession is evidenced in the fact that it was mentioned in the *Vereinigte Pharmacopœen der Londoner, Edingurgher und Dubliner Medicinæ Collegien,* a German translation of the pharmacopœias of London, Edinburgh and Dublin, which appeared in 1827, and, that two years later (1829), it became official in the Prussian Pharmacopœia. Its introduction into other European pharmacopœias followed, as a general rule, in the chronological order of their appearance or revision, whereas, it was the last of this class of preparations to be admitted to the *United States Pharmacopœia* previous to the ninth revision, having been recognized for the first time in the edition of 1870. At the present time, it is the only preparation of this kind which is official in all of the national pharmacopœias. However, it is only in the United States where it is officially recognized under the title oleoresin, it being classed as an extract in all of the foreign pharmacopœias. For a better appreciation of this fact, see the preceding table of synonyms.

A better idea of the popularity of this preparation and the rate at which it came into prominence will be obtained from the following table in which are chronologically enumerated the names of the pharmacopœias of the countries, states and municipalities where it first received official recognition, also, the dates of appearance of the succeeding editions in which it occurs.

Prussian Pharmacopœia — 1829, 1846, 1862.
Pharmacopœia of Baden — 1841.
Austrian Pharmacopœia — 1844, 1869, 1889, 1906.
Pharmacopœia of Schleswig-Holstein — 1844.

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[1] Gebhardt in 1821, and Morin in 1824, in their analyses of male fern, extracted the rhizomes with ether and obtained what they termed a thick, green, fatty oil. This was, of course, the *Huile de Fougère* of Peschier. Neither of these investigators, however, pointed out its value as a galenical preparation, although, the latter stated that he considered it to be the therapeutically active principle of the rhizomes.

Swedish Pharmacopœia — 1846, 1869, 1879, 1888, 1901, 1908.
Pharmacopœia of Berne — 1852.
Belgian Pharmacopœia — 1854, 1885, 1906.
Norwegian Pharmacopœia — 1854, 1870, 1879, 1895, 1913.
Pharmacopœia of Hannover — 1861.
Pharmacopœia of Hessia — 1862.
British Pharmacopœia — 1864, 1867, 1885, 1898, 1814.
Swiss Pharmacopœia — 1865, 1 872, 1893, 1907.
French Pharmacopœia — 1866, 1884, 1908.
Austrian Pharmacopœia — 1869, 1889, 1906.
Hungarian Pharmacopœia — 1871, 1888, 1909.
Netherlands Pharmacopœia — 1871, 1889, 1905.
German Pharmacopœia — 1873, 1882, 1890, 1900, 1910.
United States Pharmacopœia — 1870, 1880, 1890, 1900, 1910.
Roumanian Pharmacopœia — 1874.
Portuguese Pharmacopœia — 1876.
Spanish Pharmacopœia — 1884.
Italian Pharmacopœia — 1892, 1902, 1909.
Danish Pharmacopœia — 1893, 1907.
Japanese Pharmacopœia — 1907.
Russian Pharmacopœia — 1910.
Finnish Pharmacopœia — 1914.

Drug Used, Its Collection, Preservation, Etc.

The rhizomes directed by all of the present day pharmacopoeias to be used in the preparation of the oleoresin of aspidium are those of the male fern[1] now referred by botanists to the genus *Dryopteris* as *Dryopteris Filix-mas* (Linné) Schott. As male fern, especially in the older works on pharmacy, has been referred to genera other than *Dryopteris*, the following table of botanical synonyms is given:

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[1] The rhizomes of ferns other than those which have been officially recognized are said to yield oleoresins which are active in the expulsion of the tapeworm.

Kuersten states that the rhizomes of *Aspidium athamanticum* Kunze yield a preparation which is as active as that obtained from male fern. Arch. d. Pharm. (1891), 229, p. 258.

Lauren reports the use of an extract in Finland prepared from *Aspidium spinulosum* Sw. which he states is very active as a teniafuge. Finska Laegaresaellck. Handl. (1897), p. 9; Pharm. Centralh, (1897), 39, p. 775.

Rosendahl suggests that the rhizomes of *Dropteris dilata* replace those of *Dryopteris Filix-mas* in the preparation of the official oleoresin as he has found them to be four times as active as the latter in the expulsion of *Bothryocephalus latus*. Hygienic Lab. Bull. No. 87, p. 250.

Aspidium Filix-mas Swartz.
Aspidium mildeanum Goeppert.
Lastrea Filix-mas Presl.
Nephrodium Filix-mas Michaux.
Polypodium Filix-mas Linné.
Polystichum Filix-mas Roth.
Tectarea Filix-mas Cavan.
Polypodium-nemorale Salisbury.
Polystichum durum et induratum Schur.
Polystichum abbreviatum De Candolle.

In addition to the rhizomes of *Dryopteris Filix-mas* (Linné) Schott, the *United States Pharmacopœia* also permits the use of the rhizomes of *Dryopteris marginalis,* Linné formerly referred to the genius *Aspidium* as *Aspidium marginale* Schwartz. It should be noted in this connection that the official recognition of *Dryopteris marginalis* Linné appears to have been based on the somewhat doubtful statements of but three persons made back in the seventies. These men, Patterson,[1] Cressler,[2] and Kennedy,[3] respectively, reported that they had prepared oleoresins from the rhizomes of this fern. Two of them, Cressler and Kennedy, also stated that their preparations were found to be active in the expulsion of tape worm, while Patterson merely reported that his preparation resembled the German oleoresin of male fern in appearance and taste. There does not appear to be any evidence in the literature to show that an oleoresin authentically prepared from this rhizome was ever given a trial by a reputable physician. Furthermore, there is no evidence to the effect that the rhizome is ever used in preparing the oleoresin at the present time, a statement which has also been made by Rusby.[4]

The definition of Aspidium as given in the ninth revision of the *United States Pharmacopœia* is as follows: "The rhizome and stipes of *Dryopteris Filix-mas* (Linné) Schott, or of *Dryopteris marginalis* (Linné) Asa Gray (Fam. *Polypodiaceae),* collected in the autumn, freed from the roots and dead portions of rhizomes and stipes and dried at a temperature not exceeding 70° C. Preserve aspidium in tightly closed containers and protect from light."

[1] Am. Journ. Pharm. (1875), 47, p. 292.
[2] Cressler states that he prepared an oleoresin from what he thought to be male fern, but which later proved to be *Aspidium marginale.* Ibid., (1878), 5, p. 290.
[3] Ibid. (1879), 51, p. 382.
[4] Drugg. Circ. (1910), 54, p. 616.

With further reference to the species of drug specified by the Pharmacopœia, it should be stated that the male fern of commerce, obtained from Europe, is frequently contaminated with the rhizomes of other species of fern, principally those of *Dryopteris spinulosa* Kunze. Pendorff (1903), who examined 20 samples of the commercial drug, reported that 12 of them contained over 50 per cent. of rhizomes of this species.

The pharmacopœial directions concerning the collection of the rhizomes in autumn are in keeping with specifications given in most of the foreign pharmacopoeias[1] and are based on the results of extensive investigations carried out in continental Europe and England. Analyses of the drug harvested at different periods of the year have shown autumn to be the season in which the therapeutically active constituents are present in greatest amount. Thus, the firm of Caesar and Loretz, in their *Berichte* for 1898, state that the amount of active constituents present does not begin to approach the maximum until the month of August and that it again begins to diminish in October. They, therefore, conclude that the rhizomes should be harvested only in the months of August, September and October. Similar conclusions were drawn by Ed. Schmidt[2] from a series of observations made in France in 1903. The following table compiled by the latter shows the variation in crude filicin content of the ethereal extracts (oleoresins) prepared from the rhizomes harvested during six consecutive months of the year.

TABLE 8.—*Variation of crude filicin content due to season.*

Time of harvesting	Crude filicin content of oleoresins prepared from rhizomes gathered in the—			
	Forest near Paris	Jura Mts.	Vosges Mts.	Vosges Mts. Peeled Rhiz.
	Per cent	Per cent	Per cent	Per cent
May	9.70	12.78	13.76	———
June	10.80	13.86	15.65	12.75
July	10.86	14.60	17.70	14.85
August	11.64	17.80	19.70	15.60
September	13.78	19.60	20.76	17.76
October	11.80	18.68	19.80	16.70

[1] The Spanish Pharmacopoeia (1905) directs thae the rhizomes be collected at the end of spring or in the autumn.

[2] *Thèse pour l'obtention du Diplôme de Docteur de l'Université de Paris* (1903), p. 116.

The table not only shows a variation in the crude filicin content due to season, but also points out the fact that there is a very considerable variation due to the locality[1] in which the rhizomes are grown. This factor, while evidently overlooked by the United States Pharmacopœial Revision Committee, appears to be of considerable importance in influencing the quality of the oleoresin. Further proof of this is to be found in the reports of Van Aubel,[2] Madsen,[3] Mátzdorff,[3] and Caesar and Loretz.[4]

Further inspection of the pharmacopoeial definition shows that the official drug is intended to be represented by the whole rhizome and stipe deprived only of the roots and dead portions, which is also in conformity with the description generally found in foreign pharmacopoeias. This is a wise provision in that the rhizomes not only contain less of the active constituents when peeled[5] but deteriorate much more rapidly. On the other hand, compliance with this specification would appear to be a difficult problem for the pharmacist as practically all of the drug on the American market is peeled. The latter statement is based on the examination of a number of samples in the laboratory[6] and on the reports of pharmaceutical manufacturers[7] and others[8].

In the drying of the rhizomes, the *United States Pharmacopœia* specifies that the temperature shall not exceed 70°C. This temperature is thought to be too high, as filmaron, the most active constituent therapeutically, melts at 60°C and is very prone to undergo decomposition.[9] The directions as given

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[1] A variation due principally to soil and climate.

[2] Van Aubel (1896) states that the rhizomes growing in Wolmar on the shores of the Aa and those growing in the Jura and Vosges mountains yield an oleoresin which is more active therapeutically than that prepared from the rhizomes growing in Italy.

[3] Madsen (1897) and Matzdorff (1901) report the oleoresin prepared from Russian rhizomes to be the most active.

[4] Caesar and Loretz attribute the uniform activity of the oleoresin prepared by them to the fact that they obtain their supply of rhizomes from the same locality each year.

[5] See preceding table by Schmidt.

[6] Of the sixteen samples of male fern rhizomes purchased from various sources in the United States and examined in the laboratory all but three were in the peeled condition.

[7] Letters received from a number of pharmaceutical manufacturers in this country indicate that the drug as usually received from Europe is peeled.

[8] Plaut (1914) states that though the *U. S. Pharmacœpia* requires the use of unpeeled aspidium, none such is to be found on the market.

[9] Kraft (1902).

in the Belgian Pharmacopœia (1906), "dry at a temperature below 40°C," or the Norwegian Pharmacopœia (1914), "dry at a temperature not exceeding 60°C," appear to be more rational.

In connection with the pharmacopœial provision concerning the preservation of the drug, attention is called to the fact that the late edition of the German Pharmacopœia (1910) requires that the dried rhizomes be kept over freshly calcined lime. Such a procedure was shown by Hager, as early as 1871, to render the oleoresin prepared therefrom less liable to form a deposit.

The fact that the *United States Pharmacopœia* does not specify a time limit for the consumption of the drug is unfortunate in view of the rapidity with which it is known to deteriorate.[1] So important is this factor, that the French Pharmacopoeia (1908) directs that only the recently collected and freshly dried rhizomes be employed and the other European pharmacopœias commonly specify that they be renewed annually. That there is need of similar restrictions in this country will become evident from the following table showing the results obtained in the examination of fourteen samples of commercial rhizomes. Six of these samples were purchased from importers and drug millers in the United States during the winter and spring of 1909 and 1910, respectively. The other specimens were received in January of 1913 and represent samples obtained from abroad as well as in this country. In each case, the rhizomes were sorted, those showing a green fracture having been separated from those showing an internal brown color.

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[1] Peschier as early as 1825 noted that the therapeutic activity of the rhizomes diminished on ageing and recommended that they should be consumed within a period of less than two years after harvesting.

Caesar and Loretz state that they prepare the year's supply of oleoresin immediately after harvesting and drying the rhizomes to insure the maximum activity of the preparation.

TABLE 9.—*Percentage of green rhizomes in samples of male fern purchased from drug millers and jobbers.*

Sample No.	Date of purchase	Source	Content of green rhizomes
			Per cent.
1	December, 1909	United States	6.5
2	" "	" "	18.0
3	" "	" "	0.0[1]
4	April, 1910	" "	8.0
5	" "	" "	0.0
6	" "	" "	53.7
7	January, 1913	" "	0.0
8	" "	" "	9.2
9	" "	England	0.0
10	" "	"	0.0
11	" "	Germany	4.2
12	" "	"	8.5
13	" "	France	0.0
14	" "	"	0.0

[1] Composed entirely of Osmunda rhizomes.

It will be noticed that even the rhizomes purchased in Germany were not in good condition. As these rhizomes were obtained in January, they should have shown an internal green coloration had they consisted of the fresh stock harvested in the preceding autumn. From this, it appears that the German supply for exportation, at least, is not renewed yearly as it should be, but is allowed to accumulate and deteriorate.

U. S. P. Text and Comments Thereon.

Oleoresin of aspidium was admitted to the *United States Pharmacopœia* in 1870 and has been official in all subsequent editions.

1870

Oleoresina Filicis

Oleoresin of Fern

Take of Male Fern,[1] in fine powder,[3] twelve troyounces; Ether[4] a sufficient quantity.

Put the male fern into a cylindrical glass percolator, provided with a stop-cock, and arranged with cover and receptacle suitable for volatile liquids,[5] press it firmly, and gradually pour ether upon it, until twenty-four fluidounces of liquid have slowly passed.[6] Recover[7] the greater part of the ether by distillation on a water-bath, and expose the residue, in a capsule, until the remaining ether has evaporated.[8] Lastly, keep the oleoresin in a well-stopped bottle.[9]

1880

Oleoresina Aspidii

Oleoresin of Aspidium

[Oleoresina Filicis, Pharm., 1870]

Aspidium,[1] in No. 60 powder,[3] *one hundred parts* .....100.
Stronger Ether,[4] *a sufficient quantity.*

Put the aspidium into a cylindrical glass percolator, provided with a cover and receptacle suitable for volatile liquids,[5] press it firmly, and gradually pour stronger ether upon it, until one hundred and fifty (150) parts of liquid have slowly passed.[6] Recover[7] the greater part of the ether by distillation on a water-bath, and expose the residue, in a capsule, until the remaining ether has evaporated.[8]

Keep the oleoresin in a well stopped bottle[9].

Note. Oleoresin of aspidium usually deposits, on standing, a granular crystalline substance.[10] This should be thoroughly mixed with the liquid portion, before use.[11]

1890

Oleoresina Aspidii

Oleoresin of Aspidium

Aspidium,[1] recently[2] reduced to No. 60 powder,[3] *five hundred grams* ..... 500 Gm.
Ether[4] *a sufficient quantity.*

Put the aspidium into a cylindrical glass percolator, provided with a stopcock, and arranged with cover and receptacle suitable for volatile liquids.[5] Press the drug firmly, and percolate slowly with ether, added in successive portions, until the drug is exhausted.[6] Recover the greater part of the ether from the percolate by distillation on a water-bath, and, having transferred the residue to a capsule, allow the remaining ether to evaporate spontaneously.[8]

Keep the oleoresin in a well-stoppered bottle.[9]

NOTE. Oleoresin of Aspidium usually deposits, on standing, a granular-crystalline substance.[10] This should be thoroughly mixed with the liquid portion before use.[11]

1900

Oleoresina Aspidii

Oleoresin of Aspidium

Aspidium,[1] recently[2] reduced to No. 40 powder,[3] *five hundred grammes* ..... 500 Gm.

Acetone,[4] *a sufficient quantity.*

Introduce the Aspidium into a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and a receptacle suitable for volatile liquids.[5] Pack the powder firmly and percolate slowly with acetone, added in successive portions, until the Aspidium is exhausted.[6] Recover[7] the greater part of the acetone from the percolate by distillation on a water-bath, and, having transferred the residue to a dish, allow the remaining acetone to evaporate spontaneously in a warm place.[8] Keep the oleoresin in a well-stoppered bottle.[9]

NOTE. Oleoresin of aspidium usually deposits, on standing, a granular crystalline substance.[10] This should be thoroughly mixed with the liquid portion before use.[11]

Average dose .....2 Gm. (30 grains).

1910

Oleoresina Aspidii

Oleoresin of Aspidium

Oleores. Aspid.—Oleoresin of Male Fern

Aspidium,[1] recently[2] reduced to No. 40 powder,[3] *five hundred grammes* ..... 500 Gm.

Ether,[4] *a sufficient quantity.*

Place the aspidium in a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and a receptacle suitable for volatile liquids.[5] Pack the powder firmly, and percolate slowly with ether, added in successive portions, until the drug is exhausted.[6] Recover[7] the greater part of the ether from the percolate by distilling on a water bath, and, having transferred the residue to a dish, allow the remaining ether to evaporate spontaneously in a warm place.[8] Keep the oleoresin in a well-stoppered bottle.[9]

NOTE.—Oleoresin of Aspidium, on standing, usually deposits a granular crystalline substance.[10] This should be thoroughly mixed with the liquid portion before use.[11]

Average Dose—Caution! Single dose, once a day, Metric, 2 Gm.—Apothecaries, 30 grains.

1.) The Pharmacopoeia of 1870 recognized but one species of fern *(Aspidium Filix-mas)* as the source of the official drug, hence, the directions: "Take of Male Fern, etc." In the subsequent editions, *Aspidium marginale* was also recognized as a cource of supply. In these editions, the drug is, therefore, referred to by the generic name, *Aspidium.* The species from which the official drug is obtained are now referred by botanists to the genus *Dryopteris.* See page 969 under "Drug used, its collection, preservation, etc."

2.) Owing to the fact that the drug deteriorates rapidly when in the powdered condition, the last three editions of the Pharmacopœia have specified that the rhizomes be preserved whole and that they may be reduced to a powder shortly before using. For factors causing the deterioration of the drug, see under "Drug used, its collection, preservation, etc."

3.) In the last two editions of the Pharmacopoeia, it is directed that the drug be employed in the form of a moderately coarse powder (No. 40). In the previous editions, a fine powder (No. 60) was specified. The coarser powder posesses distinct advantage in that it is better adapted to percolation and can be produced with a greater degree of uniformity.

4.) It will be observed that the pharmacopœias of 1870, 1880 and 1890 directed that the drug be extracted with ether; that acetone was the menstruum specified in the Pharmacopœia of 1900; and that ether is again directed to be used for this purpose by the present Pharmacopœia.

These changes appear to have been made for economic reasons as is evidenced in the following statement by Beringer (1916): "In the Eighth Revision, acetone was directed in place of ether, because at that time the former was cheaper. As it is now permissable to use denatured alcohol in the manufacture of ether, that solvent is made so cheaply that it is again advantageous to use it in place of acetone." If the comparative cost of the two solvents was the factor which induced the Revision Committee to make the last change, it is indeed fortunate that ether was the cheaper inasmuch as it has proven to be the more desirable from a scientific standpoint as well.

Acetone, although the official menstruum for the preparation of this oleoresin for more than a decade, does not appear to have been employed for this purpose to any considerable ex-

tent by the manufacturer. This statement is based upon the examination of a number of commercial samples purchased at various times during the past ten years. While the reason for the above condition does not become apparent from the literature, it is thought that it is to be attributed to the fact that acetone yields a product of inferior quality, rather than to the relatively low cost of ether. In support of this supposition, attention is called to the statement of Dunn (1909), who reports that it is necessary to purify the oleoresin made with acetone by dissolving the same in ether, also, to the observations made in the laboratory.

Experiments conducted in the laboratory have shown that the oleoresin, when prepared with acetone, is brown in color and always contains considerable deposited matter. While the greater bulk of the deposited material has the appearance of extractive matter and is very likely of no consequence from a therapeutical standpoint, portions of it answer to the descriptions of filixnigrin and filix acid, decomposition products of the therapeutically active constituents. The latter observation is in keeping with that of Kraft (1902), who found that filmaron, the most important of the therapeutically active constituents, decomposes in acetone solution yielding the above mentioned decomposition products. It was also noted that the amount of deposited material increases much more rapidly in the preparations made with acetone than in those in which ether was used as the menstruum for extracting the drug.

As previously stated, ether has proven to be the more satisfactory solvent for scientific as well as economic reasons. In fact it has been found to be superior to any of the solvents which have been experimented with in this connection, namely: benzin, benzene, chloroform and carbon disulphide. See Part I, page 921, under "Solvents." At the present time, it is the solvent universally employed in the manufacture of the oleoresin, which is in itself a good reason for its adoption by the Pharmacopoeia. Furthermore, the product obtained with ether is perfectly homogenous and forms a deposit only after long standing, the constituents of therapeutic value evidently undergoing no decomposition in ethereal solution. However, the quality of the preparation, even when ether is employed in extracting the drug, is influenced to a certain extent by the purity of the solvent.

Alcohol and water appear to be the impurities which tend to exert a deleterious influence upon the finished product. Thus, Daccomo and Scoccianti (1896) observed that ether containing a considerable amount of alcohol did not completely extract the therapeutically active constituents from the drug and that the oleoresin obtained was more prone to form a deposit than when ether of a greater degree of purity was used. See also page 984 under "Yield of oleoresin." Similar effects were observed by the firm of Caesar and Loretz (1899.) The presence of water is so great a factor in promoting decomposition (hydrolysis?) that the German Pharmacopœia (1910) directs that the rhizomes be preserved over freshly burned lime, a procedure which was recommended by Hager as early as 1871. Further evidence of the undesirability of the presence of water is to be found in the Norwegian (1913) and Finnish (1914) pharmacopœias, which direct that the ethereal tincture be dried with anhydrous sodium sulphate or fused calcium chloride previous to the removal of the solvent by distillation.

5.) For a description of the various forms of percolators designed for extraction with volatile solvents, see Part I under "Apparatus used."

6.) All editions of the Pharmacopœia, including the present, direct that the drug be extracted by the process of simple percolation even though the advantages of a continuous extraction apparatus in the handling of a volatile solvent like ether have been repeatedly pointed out. See Part I under "Solvents" and under "Apparatus used."

Of special interest in this connection is the work of Matzdorff (1901), the results of which show that the therapeutically active constituents are not completely extracted by simple percolation as ordinarily carried out, but that complete extraction is effected in a comparatively short time with the use of a Soxhlet's apparatus.

7.) In connection with the recovery of the solvent by distillation, attention is again directed to the deleterious effect of the presence of moisture and to the manner in which the same is directed to be removed by the Norwegian and Finnish pharmacopoeias. See above.

Attention is also invited to the pharmacopoeial directions regarding distillation, namely that it be conducted on a water

bath. Inasmuch as Kraft (1902) states that filmaron melts at 60°C and undergoes decomposition at higher temperatures, it is thought that the pharmacopoeial directions should contain a warning against exceeding this temperature during distillation.

8.) The removal of a part of the solvent by spontaneous evaporation as directed by the Pharmacopœia tends to operate against obtaining a uniform product as the time required to accomplish the same varies with the temperature. If evaporation is allowed to proceed at a low temperature (winter temperature), the preparation will be exposed to the action of the air for a very considerable length of time and partial oxidation of some of the constituents will very likely result.

The complete removal of the solvent can be accomplished much more rapidly by heating the preparation on a water bath, and without injury, if the temperature is kept below 60°C. By such a procedure, the above conditions are eliminated and a more uniform product will be obtained.

9.) The oleoresin should be kept in well-stoppered bottles as it becomes rancid on prolonged exposure to the air due to the hydrolysis and partial oxidation of the glycerides composing the fatty oil.

10.) For a discussion of the nature of the deposit which forms in the oleoresin on standing, see pages 992 and 1004 under "Constituents of therapeutic importance," and under "Other properties."

11.) As to the propriety of the pharmacopœial directions concerning the mixing of the deposit with the liquid portion before dispensing, there is some doubt. The question, however, is one which should be decided by the pharmacologist rather than the pharmacist and will, therefore, not be considered here.

The use of an alkali, ammonia as suggested by Beringer (1892), for the purpose of facilitating the admixture of the precipitate with the liquid portion should be condemned as a dangerous practice. The danger lies in the fact that the slightly soluble toxic constituents are converted into soluble compounds by union with the alkali and are thereby rendered readily absorbable.

Of further interest in this connection is the procedure recommended by Seifert (1881) and Kraemer (1884) for avoiding

the formation of a deposit, namely: that the ethereal tincture be kept on hand and that the oleoresin be prepared therefrom just previous to dispensing.

Yield

The yield of oleoresin, when ether is the solvent employed in extracting the drug, is commonly stated to be 10 to 15 per cent. in the various dispensatories and American text-books on pharmacy. As a matter of fact, the amount of oleoresin actually obtained is about 7 to 10 per cent. (See the tables which follow.) When petroleum ether or benzene is used, the yield is slightly lower, as a rule, whereas, it is much higher (about 18 per cent.) when acetone is employed. These statements refer to the yield as found for the air dried drug. When the latter is dried at a temperature of 100 to 110°C, the percentage of oleoresin obtained will naturally be somewhat higher as is shown in the table immediately following.

TABLE 10.—*Yield of oleoresin as reported in the literature.*

Date	Observer	Yield of oleoresin to				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per ct.	
1826	von Esenbeck	.....	.....	5.63	.....	Rhizomes harvested in August.
1827	Van Dyk	37.5	.....	7.30	.....	
"	Zeller	32.0	.....	.....	.....	Rhizomes harvested in September.
1828	Meylink	.....	.....	6.04	.....	
"	Winkler	15.6	.....	.....	.....	Rhizomes harvested in February.
1829	Haendess	.....	.....	8.85	.....	
1844	Hornung	.....	.....	8.33	.....	
1851	Bock	.....	.....	12.87	.....	Peeled rhizomes dried at 100° C.
1852	von der Marck	.....	.....	7.80	.....	Portion of rhizomes having borne fronds the previous year.
		.....	.....	8.20	.....	Portion of rhizome bearing fronds.
		.....	.....	8.50	.....	Portion of rhizome to develop fronds the next year.
1876	Kruse	.....	.....	10.30	Petrol. Ether 9.3	Rhizomes harvested in April. Dried at 110° C.
		.....	.....	12.40	8.4	Rhizomes harvested in July Dried at 110° C.
		.....	.....	11.50	9.1	Rhizomes harvested in October. Dried at 110° C.
1887	Kremel	29.0	.....	14.00	.....	
1888	Trimble	.....	.....	6.51	Benzin 5.9	
1891	Nagelwoort (1)	.....	.....	5.60	.....	Rhizomes harvested in July, 1889.
		.....	.....	6.20	.....	Rhizomes harvested in September, 1889.
		.....	.....	5.70	.....	Rhizomes harvested in October, 1889
		.....	.....	6.00	.....	Rhizomes harvested in December. " " "
		.....	.....	8.50	.....	
		.....	.....	8.00	.....	Rhizomes harvested in February, 1890. " "
		.....	.....	11.00	.....	" " "
		.....	.....	13.00	.....	
		.....	.....	6.00	.....	Rhizomes harvested in April. 1890.
		.....	.....	6.50	.....	" " "
		.....	.....	5.70	.....	" " "
1892	Beringer	.....	18.0	.....	Benzin 16.18	
"	Sherrard	.....	.....	9.27	.....	Whole Rhizomes.
		.....	.....	9 87	.....	" "
		.....	.....	7.26	.....	Peeled "
		.....	.....	8.90	.....	" "
1898	Bellingrodt	.....	.....	5.90	.....	Rhizomes from "Rheinische Tiefebene (Calcar)."
		.....	.....	6.12	.....	Rhizomes from "Rheinische Tiefebene (Dinslaken)."
		.....	.....	8.92	.....	Rhizomes from "Voreifel (Aachen.)"
		.....	.....	9.96	.....	Rhizomes from "Hocheifel (Gerolstein.)"
		.....	.....	9.50	.....	Rhizomes from "Taunus (Braubach.)"
		.....	.....	9.88	.....	Rhizomes from "Westerwald auf Thonschiefer (Daaden.)"

[1] Ed. Schmidt, Thèse pour l'Obtention du Diplôme du Docteur l'Université de Paris, 1903, p. 78.

TABLE 10.—Continued.

Date	Observer	Yield of oleoresin to				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per ct.	
1898	Bellingrodt—Con.	.....	.....	9.95	.....	Rhizomes from "Westerwald auf Basalt boden (Daaden.)"
		.....	.....	8.90	.....	Rhizomes from "Hansruck (Simmern)'
1899	Hausmann	.....	.....	8.50	.....	Rhizomes from "St. Gallen, Switzerland."
		.....	.....	10.00	.....	Rhizomes from "Bludenz (Vorarlberg)."
		.....	.....	8.00	.....	Rhizomes from "Appenzell, Switzerland."
		.....	.....	9.30	.....	Rhizomes from "Bierberwier, Tyrol."
1902	Buttin.....	.....	.....	8.00	.....	Rhizomes harvested in spring.
1903	Schmidt, E.[1]	.....	.....	6.60	.....	Whole rhizomes from near Paris harvested in September.
		.....	.....	9.60	.....	Whole rhizomes from the Vosges Mts. harvested in September.
		.....	.....	9.10	.....	Whole rhizomes from the Jura Mts. harvested in September.
		.....	.....	6.40	.....	Peeled rhizomes from the Vosges Mts. harvestsd in September.
		.....	.....	6.90	.....	Whole rhizomes from near Paris harvested in October.
		.....	.....	9.80	.....	Whole rhizomes from the Vosges Mts. harvested in October.
		.....	.....	9.30	.....	Whole rhizomes from the Jura Mts. harvested in October.
		.....	.....	7.00	.....	Peeled rhizomes from the Vosges Mts. harvested in October.
1905	Dietrich.....	.....	.....	9.94 to 10.60	.....	From air dried rhizomes.
		.....	.....	Up to 11.20	.....	From rhizomes dried at 100° C.
1906	Röder	.....	.....	9.22 to 10.1	.....	Yield obtained when the product was heated at 95° C for 2 hours, cooled in a desiccator & weighed.
1906	Wollenweber..	.....	.....	10.30	Benzene 9.81	Air dried rhizomes extracted in a Soxhlet's apparatus.
		.....	.....	10.00	10.10	Exiccated rhizomes extracted in a Soxhlet's apparatus.
		.....	.....	.....	Petrol. Ether 9.8	Air dried rhizomes extracted in a Soxhlet's apparatus.
		.....	.....	.....	9.5	Exiccated rhizomes extracted in a Soxhlet's apparatus,

[1] l. c., p. 110.

TABLE 10.—Continued.

Date	Observer	Yield of oleoresin to				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per ct.	
1908	Vanderkleed(1)	.....	.....	.....	Solvent? 6.68	Reported as yield of oleoresin.
		.....	.....	.....	10.003	
		.....	.....	.....	17.90	
1909	Vanderkleed ..	.....	.....	.....	10.33	
1911	Rosendahl.....	.....	.....	10.00	.....	Rhizomes harvested in May.
		.....	.....	12.50	.....	Rhizomes harvested in August.
		.....	.....	11.50	.....	Rhizomes harvested in October.
1913	Harrison & Self	.....	.....	9.50	.....	Rhizomes from "Harz."
		.....	.....	11.60	.....	" " "
		.....	.....	8.80	.....	" " "
		.....	.....	7.90	.....	" " "
		.....	.....	8.30	.....	" " "
		.....	.....	7.70	.....	" " "Bayern"
		.....	.....	9.70	.....	" " "
		.....	.....	8.60	.....	" " "
		.....	.....	7.50	.....	" " "Schwarzwald, Wuertemberg."
		.....	.....	7.00	.....	" " "Mosel, Rhein-Preussen."
		.....	.....	10.90	.....	"
914	Riedel	.....	.....	9.40 to 9.70	.....	"
	Vanderkleed ..	.....	.....	.....	Solvent? 6.85 to 10.12	Average yield of oleoresin is reported as 8.23 per cent.

[1] The high yield (1.79 per cent.) obtained in this instance is suggestive of the use of acetone as the menstruum for exhausting the drug. It may, however, have been due to the extensive adulteration of the latter with the rhizomes of *Dryopteris spinulosa*. Rosendahl (1911) obtained 17.0 per cent. of oleoresin from the rhizomes of this species by extraction with ether.

TABLE 11.—*Yield of oleoresin obtained in the laboratory.*

Date	Observer	Yield of oleoresin to				Remarks
		Alcohol	Acetone	Ether	Benzin	
		Per ct.	Per ct.	Per ct.	Per ct.	
1909	DuMez & Baker.....	.....	18.27	9.3	.....	Represents the yield using a Soxhlet's extraction apparatus.
"	DuMez & Beedle	.....	.....	9.7	.....	Represents the yield using a Soxhlet's extraction apparatus.
1910	DuMez & Netzel.....	43.33 (1)	16.10	8.70	7.5	Represents the yield using a Soxhlet's extraction apparatus.

(1) The alcoholic extract was obtained by simple percolation.

An examination of the first of the foregoing tables reveals the fact that the yield is influenced to a very considerable extent by the condition of the drug from which the oleoresin is prepared. Thus, for instance, the amount obtained is less when the powdered whole rhizomes are used than when peeled rhizomes are employed. This is to be expected in view of the fact that the outer layers contain little that is soluble in the solvent (ether) usually made use of. It will also be noticed that natural causes, such as, locality in which the rhizomes are grown, and time of harvesting are important factors in this connection. These influences will be brought out more clearly on an inspection of the following table which shows the results of this nature obtained by Ed. Schmidt.

TABLE 12.— *Effect of locality in which the rhizomes are grown and the time of harvesting on the yield of oleoresin.*

Time of harvesting	Peeled rhizomes from—			Whole rhizomes from—
	Forest near Paris	Vosges Mts.	Jura Mts.	Vosges Mts.
	Per cent.	Per cent.	Per cent.	Per cent.
May	4.00	7.00	6.40	.....
June	4.80	7.60	7.00	4.90
July	5.60	8.70	8.00	5.70
August	6.20	9.00	8.40	6.00
September	6.60	9.60	9.10	6.40
October	6.90	9.80	9.30	7.00

In addition to the comments already made with regard to the influence of the solvent on the yield, the observations of Daccomo and Sccocianti (1896) are of importance in this connection. These investigators found that the amount of oleoresin obtained, when ether was employed for extracting the drug, depended to some extent on the purity of the former. Thus, ether, specific gravity 0.720 gave 10 per cent. of oleoresin, whereas, ether, specific gravity 0.756 yielded 17 per cent. It was further pointed out, however, that the greater yield was not desirable as in this case the preparation did not contain all of the therapeutically active constituents and in addition was more prone to form a deposit on standing.

Chemistry of the Drug and Oleoresin.

Tabulation of Constituents.

A survey of the voluminous literature[1] pertaining to the chemistry of the male fern rhizome shows the constituents of pharmaceutical interest to be as follows: volatile oil, fatty oil, filix acid, albaspidin, flavaspidic acid, aspidinol, flavaspidinin (phloraspin), filmaron, filixnigrin, chlorophyll, filix tannic acid, wax, sugar, starch and inorganic constituents. Of these substances, the following have been identified in the oleoresin obtained by extracting the drug with ether:

Volatile oil[2]	0.40	to 0.45	per cent
Fatty oil[3]	70.00	to 75.00	" "
Filix acid[4]	5.75	to 12.48	" "
Albaspidin[5]	Av.	0.05	" "
Flavaspidic acid[5]	"	2.50	" "
Aspidinol[5]	"	0.10	" "
Flavaspidinin[5]	"	0.10	" "
Filmaron[5]	"	5.00	" "

[1] The following have reported more or less complete analyses of the male fern rhizome or of the ethereal extract: Gebhardt, cited by Geiger, Mag. f. Pharm. (1824), 7, p. 38; Morin, Journ. de Pharm. et de Chim. (1824), 10, p. 223; Buchner, Rep. f. d. Pharm. (1827), 27, p. 337; Batso, Trommsdorff's n. Journ. d. Pharm. (1827), 14, p. 294; Peschier, Ibid. (1828), 17, p. 9; Luck, Jahrb. f. prakt. Pharm. (1851), 14, p. 129; Bock, Arch. d. Pharm. (1851), 115, p. 257; Kruse, Ibid (1876), 209, p. 24; Daccomo, Annali di Chim et Farmak. (1887), 87, p. 69; Boehm, Arch. f. Exp. Path. u. Pharmak. (1896), 38, p. 35; Kraft, Schweiz. Wochenschr. f. Chem. u. Pharm. (1902), 40, p. 322.

[2] The percentage of volatile oil as given above has been computed on the basis of an average yield of 10 pr cent. of oleoresin.

[3] The quantity of fatty oil present in the oleoresin has been shown to vary with the strength of the ether employed in extracting the drug and with the degree to which the latter has been exhausted. These factors, however, are not sufficient to explain the large variation in oil content as found by various investigators. The variation is more probably due to the different methods employed in its estimation. Thus, Bock reports the presence of 42 per cent of fatty oil, Arch. d. Pharm. (1851), 115, p. 266; Kremel estimates it at 40 to 45 per cent, Pharm. Post d. Pharm. (1887), 20, p. 525; Wollenweber at 70 to 75 per cent, Arch. d. Pharm. (1906), 244. p. 467.

[4] There is a very considerable difference in the filix acid content of the oleoresin as reported in the literature. This is due, principally, to the natural variation in the filix acid content of the drug and to the different methods employed in its estimation. The limits as given above are those obtained by the method of Fromme and represent the percentage occuring in the oleoresin prepared from the better rhizomes. Under these conditions, Madsen found 5.8 to 12.1 per cent, Arch. f. Pharm. og. Chem. (1897), 54, p. 269; Gehe & Co., 5.78 to 11.32 per cent, *Handels-Ber.* (1897), p. 60; Bellingrodt, 5.75 to 10.75 per cent, Apoth. Ztg. (1898), 13, p. 869; Caesar and Loretz, 8.65 to 12.48 per cent, *Geschaefts-Ber.* (1901), p. 68.

Filixnigrin[5]	" " 6.00 " "
Chlorophyll[6]	—— " "
Wax[7]	—— " "
Ash	" 3.50 to 5.00 " "

Occurrence and Description of Individual Constituents.

Volatile oil.[8] The volatile oil as described by Ehrenberg is a clear yellow liquid having a specific gravity of 0.85 to 0.86 at 15°C, and is stated by him to be composed principally of fatty acid esters of hexyl and octyl alcohol, the acids ranging from propionic to caproic.

The quantity of essential oil present in the rhizomes is stated to vary with the seasons of the year, 0.04 to 0.045 per cent. being contained therein at the time of the year when the drug is usually collected.[9]

Fatty oil.[10] The fatty oil as obtained from the male fern rhizomes by extraction with ether and subsequent purification is stated by Katz[11] to be composed of the glyceryl esters of oleic, palmitic, cerotic and butyric acids.[12]

Filix acid[13] *(Filicin)*[14] Filix acid ($C_{35}H_{38}O_{12}$) crystalizes

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[5] Kraft, Schweiz. Wochenschr. f. Chem. u. Pharm. (1902), 40. p. 323.

[6] Bock, Arch. d. Pharm. (1851), 115, p. 266.

[7] Kraft, l. c.

[8] The volatile oil as described above is that obtained from the rhizomes by steam distillation and in all probabilities differs somewhat from the same as it exists in the galenical oleoresin.

[9] Ehrenberg reports the presence of volatile oil as follows: rhizomes gathered in April, 0.008 per cent; in June .025 per cent; in September, October and November, 0.04 and 0.045 per cent. Arch. d, Pharm. (1893), 231, p. 345.

[10] The fatty oil of male fern was probably first isolated by Luck. In 1851, he reported that the oily portion (*filixoline*) of the ethereal extract was a glyceride yielding *filomysilsaeure* and *filixolinsaeure* upon saponification. Jahrb. f. prakt. Pharm. (1851), 22. p. 130.

From Luck's description it is considered that these acids were in all probability butyric and oleic, respectively.

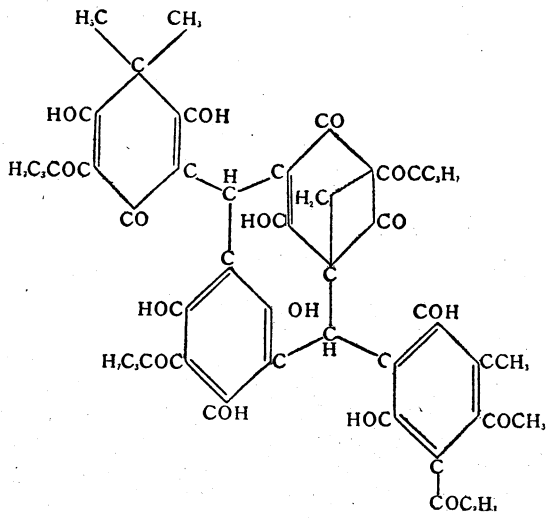
[11] Arch. d. Pharm. (1898), 236, p. 655.

[12] Butyric and oleic acids have also been identified by Farup in the fatty oil obtained from *Aspidium Spinulosum.* In addition a phytosterol, linolinic, and probably isolinolinic acid are stated to have been detected. Arch. d. Pharm. (1904), 242, p. 17.

[13] The term *filixsaeure* was first used by Luck to designate this constituent. Filix acid is the translation given above rather than the usual English form, *filicic acid,* to avoid confusion with the *filicinsaeure* of Boehm, a reduction product of the former, Ann .d. Chem. (1899), 307, p. 249, or the *Acidum filiceum* of Batso, a supposedly volatile acid which the latter isolated from the ethereal extract. Tromsdorff's n. Journ. d. Pharm. (1827), 14, p. 249.

[14] *Filicin* is the term introduced by Poulsson to designate the crystalline form of filix acid as he was of the opinion that it also existed in the amor-

in small yellow plates melting at 184 to 185° C. It is difficulty soluble in water, alcohol, and ether, quite readily soluble in ethyl acetate. According to Boehm,[15] its constitution[16] is probably represented by the following structural formula:



Filix acid has been found to be present in the male fern rhizome[17] in quantities varying from 0.268 to 2.159 per cent, the variation in content depending principally upon the location in which the rhizomes are grown and on the time of harvesting.[18]

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phous state. Arch. f. Exp. Path. u. Pharm. (1895), p. 357. The term is now usually employed to designate the mixture of acid substances obtained in the quantitative evaluation of the oleoresin. It should not be confused with the ***Filicina*** of Batso, supposedly an alkaloid isolated from the ethereal extract. l. c.

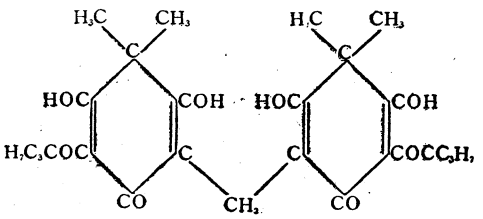
[15] Ann. d. Chem. (1901), 318, p. 256.

[16] The following investigators have contributed work on the constitution of filix acid: Luck, Ann. d. Chem. (1845), 54, p 119; Jahrb. f. prakt. Pharm. (1851), 22, p. 129; Grabowski, Ann. d. Chem. (1867), 143, p. 279; Daccomo, Ber. d. deutsch. Chem. Gesell. (1888), 21, p. 2962; Gaz. Chim. Ital. (1895), 24, 1, p. 511; Ibid. (1896), 26, 2, p. 441; Paterno, Ber. d. deutsch. Chem. Gesell. (1889), 22, p. 463; Schiff, Ann. d. Chem. (1889), 253, p. 236; Poulsson, Arch. f. Exp. Path. u. Pharm. (1895), 35, p. 97; Boehm, Ibid. (1897), 38. p. 35; Ann. d. Chem. (1898, 302, p. 171.

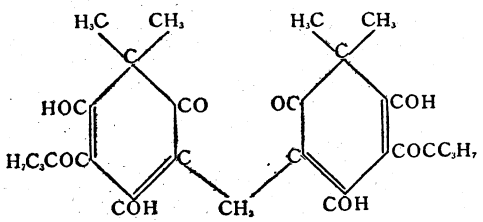
[17] Filix acid has also been isolated by Hausmann from *Athyrium Filix femina* Roth. Arch. d. Pharm. (1899), 237. p. 556, and has been identified by Bowman in *Aspidium rigidum* Swartz. Am. J. Pharm. (1881), 53, p. 389.

[18] Matzdorff, Apoth. Ztg. (1901), 16, p. 274.

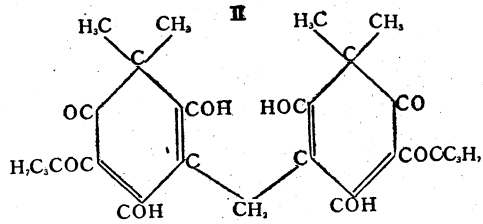
Albaspidin.[19] Albaspidin crystallizes in fine colorless needles melting at 147 to 148°C. It is readily soluble in ether, chloroform and benzol, difficultly soluble in alcohol, acetone and glacial acetic acid. Its constitution is stated to be represented by one of the three following formulae:[20]



I



II



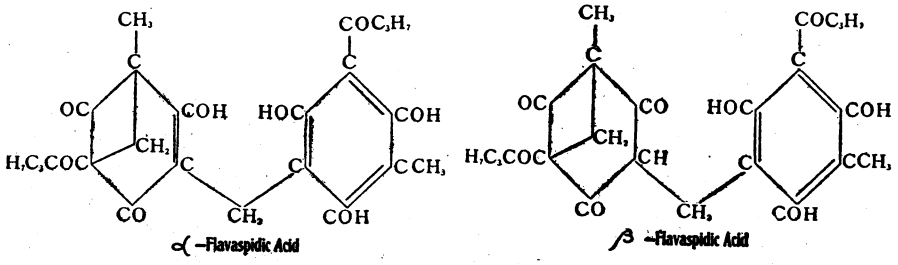
III

Flavaspidic acid. Flavaspidic acid ($C_{24}H_{28}O_8$) was first isolated from the ethereal extract by Boehm. It is stated to exist in two forms (α and β) which differ in their melting points, the α-flavaspidic acid melting at 92°C and the β-modification at 156°C. The α-acid on heating is converted into the β-acid

[19] Albaspidin should not be confused with aspidin. Hausmann has shown the latter to be a constituent of *Dryopteris spinulosa* O. Kuntze, but that it is not present in *Dryopteris filix mas* Schott. Arch. d. Pharm. (1899), 237, p. 544.

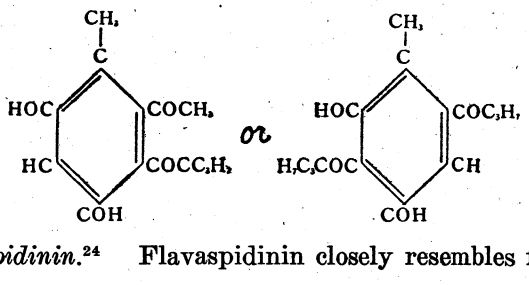
[20] Boehm, Arch. f. Exp. Path. u. Pharm. (1897), 38, p. 35; Ann. d. Chem. (1901), 318, p. 268.

which may be crystallized from hot benzol or glacial acetic acid. The β-form is converted into the α-modification on crystallizing the former from alcohol. The α-acid is thought to be the enol-, the β-acid the keto-form. The structure is shown in the following formulae:[21]



Flavaspidic acid has been isolated from the male fern rhizome in quantities varying from 0.10 to 0.15 per cent.[22]

Aspidinol. Aspidinol ($C_{12}H_{16}O_4$) crystallizes in small yellowish-white needles melting at 156 to 161°C. It is difficultly soluble in petroleum ether and benzol, readily soluble in ether, alcohol, chloroform, carbon disulphide and acetone. The following two formulae have been suggested by Boehm as representing the structure of this compound:[23]



Flavaspidinin.[24] Flavaspidinin closely resembles flavaspidic

[21] Boehm, Ann. d. Chem. (1901), 318, p. 253; Ibid. (1903, 329, p. 310.

[22] In addition to establishing the presence of flavaspidic acid in the male fern rhizome, Hausmann has also isolated this compound from *Athyrium Filix femina* Roth. and *Aspidium spinulosum* Swartz. Arch. d. Pharm. (1899), 237, p. 556.

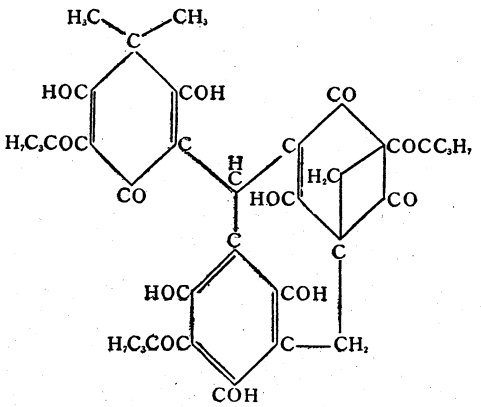
[23] Arch. f. Exp. Path. u. Pharm. (1893), 33, p. 35; Ann. d. Chem. (1901), 318, p. 245; *Ibid.* (1903), 329, p. 286.

[24] Kraft. Schweiz. Wochenschr. f. Chem. u. Pharm. (1902), 40, p. 323.

The "phloraspin" ($C_{23}H_{28}O_8$) of Boehm is probably identical with flavaspidinin. The pale yellow crystals obtained from the alcoholic solution melt at 211°C, and are stated to be almost insoluble in ether, petroleum ether, benzene and carbon disulphide, but more readily soluble in acetone, chloroform, hot absolute alcohol, ethyl acetate, glacial acetic acid and boiling xylene. Ann. d. Chem. (1903), 329, p. 338.

acid. It crystallizes from ethyl acetate in nearly colorless prisms melting at 199°C. It is soluble in methyl alcohol, difficultly soluble in ether, carbon disulphide and alcohol, readily soluble in warm benzene, chloroform, ethyl acetate, acetone and amyl alcohol.

Filmaron.[25] Filmaron ($C_{47}H_{52}O_{16}$) is a light yellow, amorphous powder melting at about 60°C. It is insoluble in water, difficultly soluble in alcohol, methyl alcohol and petroleum ether, readily soluble in acetone, chloroform, ether, ethyl, acetate, benzene, carbon disulphide, carbon tetrachloride, amyl alcohol and glacial acetic acid. In acetone solution, at ordinary temperatures, or upon warming with alcohol, it gradually decomposes into filix acid and filixnigrin. The following structural formula has been suggested by Kraft:



Filixnigrin.[26] Filixnigrin is the term used by Kraft to designate the mixture of brown to black amorphous decomposition products of the foregoing constituents. These decomposition products differ from the mother substances in that they are insoluble in petroleum ether. They have been isolated from the etheral extract. To what extent they occur in the plant, if at all, has not been determined.

Chlorophyll. The green coloring matter of the male fern rhizome and of the oleoresin prepared therefrom is generally conceded by the various investigators to be chlorophyll, al-

[25] Kraft, l. c.
[26] Kraft, *l. c.*

though, no attempt appears to have been made to determine its composition. Work upon the pigments present in a closely related species of fern, *Aspidium Filix femina* Roth. has resulted in the isolation of carrotin ($C_{16}H_{32}O$) and three aspidiophylls, $C_{208}H_{347}O_{32}N$, $C_{240}H_{320}O_{31}N_2$ and $C_{210}H_{346}O_{48}N_{20}$.[27]

The amount of chlorophyll present in the rhizome varies with its age and with the season of the year.[28]

Wax. The wax occurring in the male fern rhizome has not been studied from a chemical standpoint, although its presence in the ethereal extract was observed at a very early date.[29]

Filix Tannic Acid.[30] Filix tannic acid ($C_{41}H_{48}NO_{24}$) is a glucoside breaking down upon hydrolysis into hexose and a mixture of reddish-brown compounds.[31] It is readily soluble in water and dilute alcohol.

Filix tannic acid usually constitutes about 7 per cent. of the rhizome, as much as 7.8 per cent, having been isolated therefrom.[32]

Ash. Analyses[33] of the male fern rhizome have shown the ash to contain the basic elements, K, Na, Ca, Mg, Al and Fe combined with the acid radicles Cl′, SO_4'', PO_4''', SiO_3'' and

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[27] Ebard, Ann. Inst. Pasteur (1899), 13, p. 456. The more recent work of Willstaetter and his pupils on the chlorophylls isolated from more than 200 different plants belonging to numerous families indicates that magnesium is a constant consituent of the molecule, which is considered by them to be a methyl phytyl ester of the tricarboxylic acid, chlorophyllin, $C_{31}H_{29}N_4Mg(COOH)_3$. Viewed in this light, the above formulae for the aspidiophylls are erroneous in that they contain no magnesium and express molecular weights which are much too high. Ann. d. Chem. (1908), 358, p. 267; *Ibid.* (1910), 378, p. 1.

[28] Kruse has observed that the rhizomes collected in April and October have a more intense green color than those gathered in July. Arch. d. Pharm. (1876), 209, p. 24.

[29] Batso, Trommsdorff's n. Journ. d. Pharm. (1827), 14, p. 294; Peschier *Ibid.* (1828), 17, p. 5 and Bock, Arch. d. Pharm. (1851), 115, p. 266, report the presence of a stearin-like substance in the ethereal extract.

Caesar and Loretz have observed that rhizomes rich in wax yield an ethereal extract which is not fluid at the ordinary temperature. *Gechaefts Ber.* (1897), p. 62.

[30] In the light of our present knowledge concerning the chemistry of male fern, filix tannic acid is not considered to be a constituent of the oleoresin when prepared with ether. As its presence in the latter has been reported by early investigators, the above description has been included here. See analysis by Bock, Arch. d. Pharm. 1851, 115, p. 266.

[31] Malin, Ann. d. Chem. (1867), 115, p. 276; Wollenweber, Arch. d. Pharm. (1906), 244, p. 480.

[32] Wollenweber, l. c.

[33] Bock, Arch. d. Pharm. (1851), 115, p. 257; Spies, Jahresb. d. Pharm. (1860), 20, p. 15.

CO_3". Hell and Company[34] report the presence of 0.0144 per cent. of copper. Spies, however, was unable to detect the presence of either copper or manganese.

The ash content of the dried rhizomes varies, about 2.0 to 3.0 per cent being the usual amount obtained.[35]

Constituents of Therapeutic Importance

The value of the oleoresin of aspidium as a teniafuge has at various times been attributed to either its filix acid[1] or volatile oil[2] content. Comparatively recent pharmacological investigation,[3] however, has shown that the property of expelling the tape worm is not due to a single constituent, but is shared by a number of the acid-like components, namely: filix acid, flavaspidic acid, albaspidin, aspidinol, flavaspidinin and filmaron. Of these substances, filmaron is the most active and is stated by Jacquet[4] and others to be the constituent of most importance therapeutically.

The diminution in the therapeutic activity of the oleoresin on ageing has been found to be due to the breaking down of some of these constituents into compounds which are inert or less active as teniafuges. Of the decomposition products tested by Straub, phloroglucin, filicin acid and butyric acid were found to be non-toxic when administered to frogs.[5] Filix acid on the other hand was found to be toxic. Its value as a teniafuge is, however, doubtful.[6]

Physical Properties

Color: The color of the oleoresin varies to a considerable extent depending principally on the condition of the drug from which it is prepared. It is described by various writers as being yellowish-green, green, dark green or greenish-brown.

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[34] Pharm. Post (1894), 27, p. 168; Journ. de Pharm. et de Chim., 139, p. 493.

[35] Bock gives the ash content of the air dried rhizomes as 2.13 per cent., Kruse as 1.90 to 2.2 and Spies as 2.74. For the exsicated rhizomes, the latter obtained 3.19 per cent.

[1] Poulsson, Arch. f. Exp. Path. u. Pharmak. (1891), 29, p. 9.

[2] Kobert, Therap. Monatsch. (1893), p. 136.

[3] Straub, Arch. f. Exp. Path. u. Pharmak. (1902), 48, pp. 1–47.

[4] Therap. Monatsh. (1904), 18, p. 391.

[5] *l. c.*

[6] Boehm, Arch. f. Exp. Path. u. Pharmak. (1897), 38, p. 35.

When prepared from the freshly dried and powdered rhizomes gathered in the autumn,[1] it usually has an olive-green color when spread out in a thin layer on a white porcelain surface. A brownish-green color is an indication of the use of old deteriorated drug[2] in its preparation, whereas, a deep green color suggests adulteration with salts of copper or chlorophyll.[3]

The nature of the solvent employed in extracting the drug is also stated to have an influence on the color of the preparation, the use of ether (specific gravity 0.720) yielding an oleoresin of a green color, whereas, the color is brownish-green when ether (specific gravity 0.728) is employed.[4]

Odor: The odor of the oleoresin is peculiar, like that of male fern.

Taste: The preparation has a bitter, nauseous, subacrid taste.

Consistence: The oleoresin when freshly prepared is homogeneous and is of about the same degree of fluidity as castor oil. It is variously stated as being of the consistence of syrup, fresh honey or an oily extract.

Solubility: The oleoresin when prepared with ether forms clear or slightly cloudy solutions with acetone, ether, chloroform and carbon disulphide.[5] It is partially soluble in carbon tetrachloride, benzene, methyl alcohol, ethyl alcohol (95 per cent.), glacial acetic acid and petroleum ether. The degree to which it is soluble in the last three solvents mentioned has been made the basis of tests for the detection of adulteration with castor oil.

According to Hill (1913), not less than 8 volumes of the oleoresin should be soluble in 10 volumes of petroleum ether, a lesser degree of solubility indicating adulteration. Jehn and

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[1] The oleoresin prepared from rhizomes gathered in October is stated by Kruse (1876) to have a more intense green color than that prepared from rhizomes gathered in July.

Caesar and Loretz in their *Berichte* for 1913 state the condition of the season in which the rhizomes are harvested has an influence on their color which becomes evident in the oleoresin, *e. g.* the oleoresin, when prepared from the rhizomes gathered in a dry season, is often very dark green in color.

[2] Buchner (1826) found that when the drug was kept in an open container for more than a year a brown instead of a green colored oleoresin was obtained.

[3] Wepen and Lueders (1892), Beckurts and Peters (1893) and others.

[4] Bellingrodt. (1898).

[5] This statement holds good only for the freshly prepared oleoresin and does not apply when the same contains deposited material.

Crato[1] state that the presence of castor oil is indicated when more than 50 per cent. of the oleoresin is soluble in 95 per cent. alcohol. Solubility tests made in the laboratory with glacial acetic acid have shown that not over 10 per cent. by volume of the oleoresin is soluble in the latter, a greater degree of solubility indicating adulteration with castor oil.

Specific gravity: Observations made in the laboratory show that the specific gravity should be above 1.000 when determined at 25°C. This is in keeping with the findings of Parry (1911) and Hill (1913), respectively, even though their determinations were made at 15°C. It is also the standard given in the late edition of the British Pharmacopoeia. A specific gravity of less than 1.000 usually indicates adulteration with castor oil or a preparation naturally low in filicin content. It may, however, be due to the addition of chlorophyll as pointed out by Hill, or to the presence of unevaporated solvent. These details, together with the effect produced by the use of different solvents in the extraction of the drug are brought out in the following tables:

TABLE 13.—*Specific gravities of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Specific gravity
				At 25° C
1	1910	DuMez & Netzel	Alcohol	1.166
2	"	" "	Acetone	1.052
3	"	" "	Ether	1.012
4	"	" "	Petrol. ether	0.995
1	"	DuMez	Acetone	1.048
2	"	"	Ether	1.000
1	1916	"	Acetone	1.009 [2]
2	"	"	Ether	0.997 [2]

[1] *Kommentar zum Arzneibuch fuer das deutsche Reich* (1901), p. 258.

[2] Same as 2 and 3 after having stood in the laboratory for 6 years. Both contained a heavy deposit which was not mixed with the liquid portion when the specific gravity was redetermined.

TABLE 14.—*Specific gravities of commercial samples.*

Sample No.	Date	Observer	Source	Specific gravity
				At 15° C
1	1911	Parry	Not given	0.973 (1)
2	"	"	"	0.973 (1)
3	"	"	"	0.974 (1)
4	"	"	"	0.975 (1)
5	"	"	"	0.975 (1)
6	"	"	"	0.988 (1)
1	1912	Southall Bros. & Barclay	"	0.9745 (1)
2	"	" "	"	0.0800 (1)
3	"	" "	"	1.0148
4	"	" "	"	1.0200
5	"	" "	"	1.0205
6	"	" "	"	1.0231
				Temp. (?)
1	1913	Bohrisch	German firms	0.9836 (3)
2	"	"	"	0.9842 (3)
3	"	"	"	0.9888 (4)
4	"	"	"	1.0109
				At 25° C
1	1913	DuMez	Manila, P. I.	0.977 (3)
2	"	"	England	0.985 (1)
3	"	"	"	0.9889 (1)
4	"	"	United States	1.001
5	"	"	Germany	1.003
6	"	"	England	1.003 (1)
7	"	"	United States	1.008 (2)
8	"	"	Germany	1.008
				At 15° C
1	1913	Harrison & Self	Germany	0.987 (4)
2	"	"	"	0.997
3	"	"	"	1.015
4	"	"	"	1.020
5	"	"	"	1.029
6	"	"	"	1.029
1	"	Hill	Europe	0.9829 (3)
2	"	"	"	0.9850
3	"	"	"	0.9921
4	"	"	"	0.9944
5	"	"	"	0.9980 (2)
6	"	"	"	0.9980 (1)
7	"	"	England	0.9985 (1)
8	"	"	"	1.000
9	"	"	"	1.000
10	"	"	"	2.0006 (1)
11	"	"	"	1.0036
12	"	"	"	1.0045
13	"	"	"	1.0065
14	"	"	"	1.0075
15	"	"	England	1.0090
16	"	"	Europe	1.0109
17	"	"	"	1.0179
18	"	"	"	1.0190
19	"	"	"	1.0227
20	"	"	England	1.0233
21	"	"	Europe	1.0235
22	"	"	"	1.0240
23	"	"	"	1.0249
1	"	Southall Bros. & Barclay	Not given	1.025
2	"	" "	"	1.025
1	1915	" "	"	0.9985
2	"	" "	"	1.0110
3	"	" "	"	1.021
4	"	" "	"	1.023
5	"	" "	"	1.030
				At 25° C
1	1916	DuMez	Squibb & Sons	0.9808 (3)
2	"	"	Lilly & Co.	0.9947 (3)
3	"	"	Parke, Davis & Co.	1.0103
4	"	"	Stearns & Co.	1.0379 (3)

[1] Adulterated with castor oil.
[2] Contained added chorophyll.
[3] Low in crude filicin content.
[4] Referred to as suspicious.
[5] Contained ether.

Refractive index: A refractive index of not less than 1.490 at 40°C is required for this oleoresin by the late edition of the British Pharmacopoeia. This is in accordance with the observations of Hill (1913). The statement by Parry (1911), that the refractive index should not be below 1.500 when determined at 20°C is confirmed by the results which were obtained by Harrison and Self (1913), and is more in conformity with the observations made in this laboratory at 25°C. When the oleoresin is properly prepared, ether being the menstruum used, the refractive index appears to vary directly as the crude filicin content. A low refractive index, therefore, indicates a preparation naturally low in filicin content. With respect to the commercial oleoresins, however, a low refractive index may also result from adulteration with castor oil or chlorophyll, or may be due to the presence of unevaporated solvent as is shown in the tables which follow:

TABLE 15.—*Refractive indices of laboratory preparations.*

Sample No.	Date	Observer	Solvent	Refractive index
				At 25° C
1.....	1913	DuMez.....	Ether.....	1.500
				At 20° C
1.....	"	Harrison & Self.....	"	1.4995
2.....	"	" "	"	1.5018
3.....	"	" "	"	1.5036
4.....	"	" "	"	1.5088
5.....	"	" "	"	1.5088
6.....	"	" "	"	1.5102
7.....	"	" "	"	1.5120
8.....	"	" "	"	1.5122
9.....	"	" "	"	1.5126
10.....	"	" "	"	1.5145
11.....	"	" "	"	1.5157
1.....	1916	DuMez.....	Acetone.....	1.500[1]
2.....	"	"	Ether.....	1.498[1]

[1] These figures represent the refractive indices of oleoresins which had stood in the laboratory for six years.

TABLE 16—*Refractive indices of commercial oleoresins.*

Sample No.	Date	Observer	Source	Refractive index
				At 15° C
1	1911	Evans Sons, Lescher & Webb	Not stated	1.484 [2]
2	"	" "	"	1.485 [2]
3	"	" "	"	1.501
4				1.501
				At 20° C
1	"	Parry	"	1.484 [1]
2	"	"	"	1.484 [1]
3	"	"	"	1.487 [1]
4	"	"	"	1.488 [3]
5	"	"	"	1.4885 [1]
6	"	"	"	1 493 [1]
				At 15° C
1-16	1912	Evans Sons, Lescher & Webb	"	1.507 to 1.509
				At 20° C
1	"	Southall Bros. & Barclay	"	1.4830 [1]
2	"	" "	"	1.4840 [1]
3	"	" "	"	1.5040
4	"	" "	"	1.5055
5	"	" "	"	1.5065
6	"	" "	"	1.5210(?)
				At 25° C
1	1913	DuMez	England	1.484 [1]
2	"	"	"	1.485 [1]
3	"	"	Manila, P. I.	1.489
4	"	"	United States	1.490
5	"	"	"	1.490
6	"	"	Germany	1.492
7	"	"	England	1.493
8		"	Germany	1.494
				At 20° C
1	"	Harrison & Self	Germany	1.4910 [3]
2	"	"	"	1.4944
3	"	"	"	1.4984
4	"	"	"	1.5055
5	"	"	"	1.5080
6	"	"	"	1.5084
				At 40° C
1	"	Hill	Europe	1.4823 [1]
2	"	"	"	1.4869 [1]
3	"	"	"	1.4874 [1]
4	"	"	"	1.4880
5	"	"	"	1.4909
6	"	"	"	1.4915 [2]
7	"	"	"	1.4920
8	"	"	England	1.4922
9	"	"	Europe	1.4925
10	"	"	"	1.4935
11	"	"	"	1.4940
12	"	"	"	1.4945
13	"	"	England	1.4960
14	"	"	"	1.4965
15	"	"	Europe	1.4980
16	"	"	"	1.4985
17	"	"	"	1.4988
18	"	"	"	1.4990
19	"	"	"	1.5006
20	"	"	"	1.5025
21	"	"	"	1.5036
				At 15° C
1-7	"	Evans Sons, Lescher & Webb	Not stated	1.500 to 1.510
8	"	" "	"	1.495 [3]
9	"	" "	"	1.497 [3]
10	"	" "	"	1.499 [3]
				At 25° C
1	"	Southall Bros. & Barclay	"	1.4975
2	"	" "	"	1.5115
1	1915	Southall Bros. & Barclay	"	1.4976
2	"	" "	"	1.4983
3	"	" "	"	1.5000
4	"	" "	"	1.5001
5	"	" "	"	1.5020
				At 25° C
1	1916	DuMez	Stearns & Co.	1.4953 [4]
2	"	"	Lilly & Co.	1.4988 [4]
3	"	"	Squibb & Sons	1.4993 [5]
4			Parke, Davis & Co.	1.4998

[1] Samples adulterated with castor oil.
[2] Samples contained added chlorophyll.
[3] Samples are referred to as being suspicious.
[4] Low in crude filicin content.
[5] Contained unevaporated solvent.

Chemical Properties.

Loss in weight on heating: Hill (1913) stated that the oleoresin when heated at 100°C should not lose more than 6 per cent. of its weight, a greater loss indicating the presence of unevaporated solvent. The statement is confirmed by other data of this nature reported in the literature as well as by the results obtained in the laboratory as is shown in the tables which follow:

TABLE 17—*Laboratory preparations—Loss in weight on heating.*

Sample No.	Date	Observer	Solvent	Per cent of loss on heating
				At 100° C
1	1887	Kremel	Alcohol	17.40
2	"	"	Ether	0.70
1	1904	Dieterich	"	4.51
				At 110° C
1	1916	DuMez	Acetone	2.51
2	"	"	Ether	2.37

TABLE 18.—*Commercial oleoresins—Loss in weight on heating.*

Sample No.	Date	Observer	Source	Per cent. of loss on heating
				At 100° C
1	1891	Dieterich	Germany	2.70
1	1893	"	"	1.15
2	"	"	"	1.60
3	"	"	"	1.75
1	1894	"	"	1.90
2	"	"	"	2.32
3	"	"	"	3.65
1	1895	"	"	1.75
1	1896	"	"	1.62
1	1897	"	"	4.52
2	"	"	"	4.72
1	1901	"	"	5.23
1	1903	"	"	5.52
2	"	"	"	7.38
1	1904	"	"	2.96
2	"	"	"	3.09
1	1905	"	"	5.06
2	"	"	"	7.51
1	1913	Hill	Europe	2.43
2	"	"	"	2.44
3	"	"	England	2.57
4	"	"	Europe	2.69
5	"	"	"	3.55
6	"	"	"	3.63
7	"	"	"	3.65
8	"	"	"	4.23
9	"	"	"	4.40
10	"	"	"	4.57
11	"	"	"	4.64
12	"	"	"	4.84
13	"	"	England	5.03
14	"	"	Europe	5.22
15	"	"	"	6.50 (1)
16	"	"	"	6.52 (1)
17	"	"	"	6.60 (1)
18	"	"	"	6.68 (1)
				At 100 to 105° C
1	1914	Linke	Brückner, Lampe & Co.	3.20
2	"	"	Caesar & Loretz	3.25
3	"	"	Merck & Co.	6.85
				At 110° C
1	1916	DuMez	Parke, Davis & Co.	1.75
2	"	"	Stearns & Co.	2.03
3	"	"	Lilly & Co.	6.01
4	"	"	Squibb & Sons	7.18 (1)

(1) Unevaporated solvent (ether) was present.

Ash Content: The results of this nature reported in the literature, as well as those obtained in the laboratory, indicate that the ash content of the oleoresin, when prepared with ether, seldom exceeds 0.50 per cent, which is the standard given in the Belgian and Spanish pharmacopœias. With respect to the commercial samples examined in the laboratory, the high ash content obtained was due to the presence of copper, evidently a result of the use of copper utensils in the manufacture of these preparations. The results of the determinations made in the

laboratory and those reported in the literature are given in the tables which follow:

TABLE 19.—*Ash contents of laboratory preparations.*

Sample No.	Date	Observer	Solvent	Per cent of ash.
1.....	1904	Dieterich	Ether.....	0.36
1.....	1916	DuMez	"	0.26
2.....	"	"	Acetone	0.31

TABLE 20—*Ash contents of commercial oleoresins.*

Sample No.	Date	Observer	Source	Per cent of ash	Foreign constituents
1.....	1891	Dieterich	Germany	0.40	
1.....	1893	"	"	0.45	
2.....	"	"	"	0.50	
3.....	"	"	"	0.50	
1.....	1894	"	"	0.42	
2.....	"	"	"	0.50	
3.....	"	"	"	0.55	
1.....	1895	"	"	0.50	
1.....	1896	"	"	0.45	
1.....	1897	"	"	0.43	
2.....	"	"	"	0.52	
1.....	1901	"	"	0.32	
1.....	1903	"	"	0.27	
2.....	"	"	"	0.30	
3.....	"	"	"	0.39	
1.....	1904	"	"	0.36	
2.....	"	"	"	0.83	
1.....	1905	"	"	0.26	
2.....	"	"	"	0.46	
1.....	1914	Linke	Brueckner, Lampe & Co...	0.34	Copper
2.....	"	"	Caesar & Loretz.....	0.41	"
3.....	"	"	Riedel	0.52	"
4.....	"	"	Merck & Co.....	0.52	
1.....	1916	DuMez.....	Lilly & Co.....	0.58	Copper
2.....	"	"	Squibb & Sons.....	0.54(1)	"
3.....	"	"	Parke, Davis & Co.....	0.80	"
4.....	"	"	Stearns & Co.....	0.82	

(1) Contained unevaporated solvent—ether.

Acid number: The acid numbers 82.2 and 82.7 were obtained for the oleoresins prepared in the laboratory. Inasmuch, however, as these preparations were made six years previous to the time when the determinations were made, it is thought that the value of this constant would be somewhat lower for the oleoresin when freshly prepared. This statement is based on the assumption that the acidity of the preparation will increase on standing due to the partial hydrolysis of the glycerides of the fatty acids and to the breaking down of the complex substances constituting the so-called crude filicin.

In the case of the commercial samples, the acid numbers were found to vary as a rule in the same direction as the filicin content. It would appear, therefore, that the value obtained for this constant might serve as a check on the latter determination. The results obtained in the determination of the acid numbers of the preparations examined in the laboratory and those reported by Kremel follow:

TABLE 21.—*Acid numbers of laboratory preparations.*

Sample No.	Date	Observer	Solvent	Acid number
1	1887	Kremel	Alcohol	23
"	"	"	Ether	50 to 70
1	1916	DuMez	"	82.7(1)
2	"	"	Acetone	82.2(1)

(1) These preparations were 6 years old when the acid number was determined.

TABLE 22.—*Acid numbers of commercial oleoresins.*

Sample No.	Date	Observer	Source	Acid Number
1	1916	DuMez	Stearns & Co.	50.2
2	"	"	Squibb & Sons	65.9(1)
3	"	"	Lilly & Co.	72.9
4	"	"	Parke, Davis & Co.	87.8

(1) Contained ether.

Saponification value: Determinations made by Parry in 1911 lead him to state that the saponification value of this preparation should not be lower than 230, corresponding to a crude filicin content of not less than 22 per cent. The values obtained for this constant in the laboratory and those reported by Harrison and Self agree, as a rule with this statement, when the minimum filicin content is taken as 20 per cent. A value of less than 230 in the case of commercial samples has been shown to be due in general to adulteration with castor oil. In a few instances, however, it is to be attributed to the presence of unevaporated solvent, or to a low filicin content due to the use of a poor quality of drug in the manufacture of the oleoresin.

The relatively high values obtained in the laboratory for the old preparations low in filicin content (16.0 and 16.27 per cent, respectively) is very likely due to the effect caused by the hydrolysis of the constituents of high molecular weight with the formation of acids of comparatively low molecular weight.[1] The saponification values found for the preparations examined in the laboratory as well as those reported in the literature are given in the tables which follow:

TABLE 23 —*Saponification values of laboratory preparations.*

Sample No.	Date	Observer	Solvent	Saponification value
	1887	Kremel	Ether	116 to 165
1-20	1911	Parry	"	230 to 250
1	1913	DuMez	Acetone	208.8
2	"	"	Ether	229.3
1	"	Harrison & Self	"	225.0
2	"	"	"	227.0
3	"	"	"	236.5
4	"	"	"	248.0
5	"	"	"	248.9
6	"	"	"	251.5
7	"	"	"	252.0
8	"	"	"	254.5
9	"	"	"	255.0
10	"	"	"	259.0
11	"	"	"	259.0
1	1916	DuMez	Acetone	245.2 (1)
2	"	"	Ether	246.4 (1)

(1) Old preparations low in filicin content.

[1] See under "Chemistry of the drug and oleoresin."

TABLE 24—*Saponification values of commercial oleoresins.*

Sample No.	Date.	Observer.	Source.	Saponification value
1	1904	Dieterich	Germany	204.4
2	"	"	"	234.2
1	1911	Evans Sons, Lescher & Webb	Not given	195.2 (1)
2	"	"	"	220 4
3	"	"	"	248.8
1	"	Parry	"	197.0 (1)
2	"	"	"	200.0 (1)
3	"	"	"	207.0 (1)
4	"	"	"	208.0 (1)
5	"	"	"	210.0 (1)
6	"	"	"	221.0 (1)
1	1912	Southall Bros. & Barclay.	"	195.1 (1)
2	"	" "	"	204.6 (1)
3	"	" "	"	235.4
4	"	" "	"	241.0
5	"	" "	"	256.3
6	"	" "	"	258.2
1	1913	DuMez	England	195.7 (1)
2	"	"	Manila, P. I.	200.3 (2)
3	"	"	England	202.4 (1)
4	"	"	United States	206.7 (2)
5	"	"	England	208.7 (1)
6	"	"	Germany	214.6 (2)
7	"	"	"	225.5
8	"	"	United States	240.5
1	"	Harrison & Self.	Germany	205.0 (3)
2	"	"	"	213.0
3	"	"	"	218.0
4	"	"	"	223.0
5	"	"	"	225.0
6	"	"	"	237.0
1	"	Southall Bros. & Barclay.	Not given	225.1
2	"	" "	"	263.1
1	1915	" "	"	206.5
3	"	" "	"	236.0
3	"	" "	"	250.0
4	"	" "	"	253.1
5	"	" "	"	254.6
1	1916	DuMez	Stearns & Co.	190.0 (2)
2	"	"	Lilly & Co.	211.4 (2)
3	"	"	Squibb & Sons	233.2 (4)
4	"	"	Parke, Davis & Co	249.1

[1] Adulterated with castor oil.
[2] Low in crude filicin content.
[3] Referred to as suspicious.
[4] Contained ether.

Iodine value: Observations made in the laboratory indicate that the oleoresin should have an iodine value of not less than 99, corresponding to at least 20 per cent. of crude filicin. Preparations giving a lower value than this were found to be low in crude filicin content due to adulteration with castor oil or to the presence of unevaporated solvent. On the other hand, it was observed that a high iodine value does not always signify a high filicin content, *e. g.* iodine values of 106.3 and 108.1 were obtained for preparations containing only 16.0 and 16.27 per cent. of crude filicin, respectively. As the latter were

old and contained deposited material equal to nearly one-half of their bulk, the high iodine values obtained for the supernatent liquid portions were very likely due to the concentration of the compounds of a lesser degree of saturation (glycerides of the unsaturated fatty acids) as a result of the decomposition and deposition of the more highly saturated compounds (crude filicin). The results obtained in the determination of this constant are shown in the following tables:

TABLE 25.—*Iodine values of laboratory preparations.*

Sample No.	Date	Observer	Solvent	Iodine value
1.....	1911	Evans Sons, Lescher & Webb.....	Ether.....	101.8
1.....	1913	DuMez	Acetone	95.3
2.....	1913	"	Ether.....	99.8
1.....	1916	"	Acetone	106.3 [1]
2.....	1916	"	Ether.....	108.1 [1]

[1] These preparations were six years old when examined.

TABLE 26.—*Iodine values of commercial oleoresins.*

Sample No.	Date	Observer	Source	Iodine value
1.....	1804	Dieterich	Germany.....	100.6
2.....	"	"	"	84.2
1.....	1911	Evans Sons, Lescher & Webb	Not given.....	89.2 [1]
2.....	"	" "	" "	92.3 [1]
3.....	"	" "	" "	95.9
4.....	"	" "	" "	99.1
1.....	1913	DuMez	England	85.8 [1]
2.....	"	"	Manila, P. I.....	87.2 [2]
3.....	"	"	England.....	89.4 [1]
4.....	"	"	United States.....	94.4
5.....	"	"	Germany.....	97.1
6.....	"	"	England.....	98.3 [1]
7.....	"	"	Germany.....	100.2
8.....	"	"	United States.....	101.5
1.....	1916	"	Squibb & Sons.....	95.3 [3]
2.....	"	"	Stearns & Co.....	97.7 [2]
3.....	"	"	Lilly & Co.....	98.2 [2]
4.....	"	"	Parke, Davis & Co.....	103.2

[1] Adulterated with castor oil.
[2] Low in crude filicin content.
[3] Contained ether.

Other Properties

The oleoresin, when freshly prepared, is homogeneous, but upon standing, a deposit is formed therein as a result of the breaking down of some of its constituents. The precipitated

material has been identified by Boehm[1] as crystalline filix acid and a wax-like substance. Kraft,[2] in a later investigation, confirmed the findings of Boehm insofar as they concerned the presence of filix acid. The wax-like material, however, he found to be composed of a number of substances, decomposition products of the therapeutically active constitutents, which he designated as filixnigrin. As the deposit has been found to be active[3] in the expulsion of tapeworm, although in a much lesser degree than the oleoresin proper, the *United States Pharmacopœia* directs that it be mixed with the liquid portion before dispensing.

Special Qualitative Tests

A number of the European pharmacopœias prescribe tests for the determination of the quality of this preparation. These tests are of two kinds, namely, those which have for their object the establishment of the presence of the constituents of therapeutic value, *i. e.* the substances of an acid character known collectively as crude filicin, and those which serve to identify starch when present. The former are based on the fact that the above mentioned constituents of an acid character may be precipitated directly by means of certain solvents, or from alkaline solutions by means of acids. The following are the official tests of this nature:

Tests for Filicin.

Austrian Pharmacopoeia (1906): Upon adding an excess of petroleum ether to the oleoresin dissolved in a small quantity of ethyl ether, a white precipitate should be produced.

Netherlands Pharmacopœia (1905): If 0.025 gram of the oleoresin dissolved in 2 cubic centimeters of ether be shaken with 5 cubic centimeters of a saturated barium hydroxide solution and 5 cubic centimeters of water, the aqueous portion, when separated and filtered, should give a flocculent precipitate on being acidified with hydrochloric acid.

Hungarian Pharmacopœia (1909): If 0.25 gram of the extract be dissolved in 2 cubic centimeters of ether and shaken with 10 cubic centimeters of lime water, the aqueous portion filtered and acidified with hydrochloric acid, a copious white precipitate should be formed.

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[1] Arch. f. exp. Path. u. Pharmak. (1897), 38, p. 35.

[2] Kraft (1902).

[3] Reuter, Pharm. Ztg. (1891), 36, p. 245; Straub, Arch. f. exp. Path. u. Pharmak. (1902), 48, p. 1.

The application of these tests in the laboratory has shown that they are of practically no value as an indication of the quality of the oleoresin, as preparations very low in crude filicin content give comparatively heavy precipitates when treated as described above. Furthermore, they do not serve as a means of identification as oleoresins prepared from the rhizomes of certain other species of fern[1] behave in a similar manner when subjected to these conditions.

Tests for Starch

A test for the presence of starch has been included in those pharmacopœias in which the oleoresin is directed to be prepared by the process of maceration, namely, the German and Japanese. In these instances, it serves as a means of distinguishing between preparations which have been filtered as officially directed and those which have been merely strained through cloth as is often the case. A similar test is also found in the pharmacopœias of those countries (Hungary, Spain and Switzerland) in which this preparation is frequently made by maceration, although the official process is that of percolation. The test as officially recognized in the different countries is identical with that described in the German Pharmacopœia. It is as follows:

The oleoresin, when diluted by shaking with glycerin, should not show the presence of starch grains under the microscope.

Experience in the application of this test to the preparations examined in the laboratory has shown that it is unsatisfactory when carried out as described above. The fault lies in the fact that the glycerin cannot be thoroughly mixed with the oleoresin by shaking. If mixing is effected by trituration in a mortar, the results are better, although there is considerable danger in rupturing the starch grains by this procedure.

In addition to the foregoing, special tests have been proposed for the detection of adulterants when present. They are as follows:

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[1] See under "Drug used, its collection, preservation, etc."

Tests for the Presence of the Oleoresin of Dryopteris Spinulosa.

Hausmann found that the male fern of commerce frequently contained large quantities of the rhizomes of *Dryopteris spinulosa* Kunze. He therefore devised a test for the detection of the use of the latter in the preparation of the oleoresin. It is based on the fact that the rhizomes of *Dryopteris spinulosa* Kunze contain aspidin, whereas those of the official species, *Dryopteris Filixmas* Schott do not.

Hausmann's Method (1899): Dissolve a small amount of crude filicin[1] in as small a quantity of absolute ether as possible and set the solution aside in a desiccator. If aspidin is present, the thick solution will form a crystalline brine in a few hours, when the needle-like crystals of the former can easily be identified under the microscope. If aspidin is not present, the solution undergoes no change, even on long standing except to deposit a granular substance.

Tests for the Presence of Castor Oil

The tests for the presence of castor oil are based on the solubility of the oleoresin in various solvents and are discussed under the heading, "Solubility."

Tests for the Presence of Salts of Copper

The tests for the presence of salts of copper involve an examination of the ash of the oleoresin and are discussed under the general treatment of the subject, "Ash content."

Special Quantitative Tests.

A great deal of work has been done with reference to the evaluation of this preparation, and as a result, a number of methods for the quantitative estimation of the constituents of therapeutic importance has been devised. The chemical methods may be conveniently divided into two groups, the one including those methods which have for their object the quantitative determination of the filix acid; and the other comprising the methods in which the quantity of the total constituents of an acid character is determined.

[1] See under "Special quantitative methods".

Methods for the Determination of Filix Acid.

As the oleoresin was originally thought to owe its teniafuge properties to its filix acid content, the determination of this constituent naturally received consideration first. The nature of the methods devised for its estimation and their subsequent development is illustrated in the descriptions which follow:

Method of Kremel (1887): Place a weighed quantity (about 10 grams) of the oleoresin in a flask and macerate it successively with several portions of petroleum ether when the greater part will be dissolved leaving the filix acid as an insoluble residue. Collect the latter on a filter and wash with more petroleum ether. Then dissolve it while on the filter in hot alcohol, remove the latter by evaporation and again wash with petroleum ether to remove the last traces of fat. Finally dry and weigh.

Method of Bocchi (1896):[1] Dissolve 1 to 2 grams of the oleoresin in a small quantity of ether, place the solution in a separatory funnel and shake it with successive portions of lime water until the shakings become colorless and remain clear on the addition of acetic or hydrochloric acids. Filter the united lime water solutions into a separatory funnel and acidify with hydrochloric acid when a dirty yellow precipitate will form. Dissolve the latter by shaking with carbon disulphide added in successive portions, unite the shakings, filter and remove the solvent by evaporation on a water bath. Dry and weigh the residue which is pure filix acid.

Method of Kraft (1896): Add a solution composed of 2 grams of potassium carbonate, 40 grams of water and 60 grams of alcohol (95 per cent.) to 5 grams of the oleoresin in a suitable flask and shake for 15 minutes. Filter 83 grams of this liquid into a separatory funnel, add 9 grams of dilute hydrochloric acid, 50 grams of ether and 35 grams of water and shake vigorously. After the mixture has separated draw off the lower hydro-alcoholic liquid and repeat the shaking, using 35 grams more of water. Separate the latter and run the remaining ethereal solution into a tared Erlenmeyer flask of 100 cubic centimeters capacity. Distill off the greater part of the ether and evaporate the remainder down to about 2 grams. Dissolve the dried mass in 1.5 grams of amyl alcohol and precipitate the filix acid by the addition of 30 cubic centimeters of methyl alcohol (5 cubic centimeters added at once and the remainder drop by drop.) Allow the precipitate and supernatant liquid to stand over night in a cool place, then collect the former on a tared filter and wash it with 15 cubic centimeters of methyl alcohol (use 3 portions of 5 cubic centimeters.) Finally, dry the precipitate at a temperature between 60° and 70°C and weigh. The weight obtained will represent the filix acid contained in 4 grams of the oleoresin.

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[1] The procedure as outlined above really gives the amount of total acid substances (crude filicin) present, but is described here as it was proposed by its originator as a method for the determination of the filix acid content.

Original Method of Fromme (1896): Dissolve 1.5 to 2 grams of the oleoresin in 2 grams of ether, and thoroughly mix the solution in a porcelain dish (diameter 8 to 10 centimeters) with 3 grams of calcined magnesia (or 8 grams of burned lime.) Allow the ether to evaporate completely and triturate the remaining dry pulverent mass with water, added gradually until a thin brine is formed. Set the mixture aside until the magnesia has settled, then decant the supernatant aqueous portion on a dry filter. Continue to repeat this operation, using fresh portions of water, until the filtrate no longer gives a precipitate when acidified with hydrochloric acid. Place the combined filtrates (usual weight 200 to 250 grams) in a separatory funnel, acidify with hydrochloric acid and shake out the precipitate with carbon disulphide added in successive portions (20, 10 and 10 cubic centimeters.) Filter the united carbon disulphide shakings into a round-bottom flask of 100 cubic centimeters capacity and evaporate to dryness on a water bath. Dissolve the crude filix acid obtained in this manner in 10 drops of amyl alcohol, using a gentle heat if necessary, then add 10 cubic centimeters of methyl alcohol (added drop by drop at the beginning and later rapidly.) Set the liquid containing the crystals aside in a cool place for 12 hours, then collect the latter on a tared filter, and, after washing with several 5 cubic centimener portions of methyl alcohol, dry at a temperature between 60° and 70°C and weigh.

Improved Method of Fromme (1897): Place 5 grams of the oleoresin, 30 grams of ether and 100 grams of a solution of barium hydroxide (1 per cent.) in a 200 cubic centimeter flask and shake for 5 minutes. Then run the mixture into a separatory funnel, and, after allowing it to stand for 10 to 15 minutes, run off into another separatory funnel 86 grams (corresponding to 4 grams of the oleoresin) of the lower aqueous layer. Acidify by the addition of hydrochloric acid (25 to 30 drops) and shake out with ether (in 25, 15, 10 and 10 cubic centimeter portions.) Filter the combined ether washings into a 100 cubic centimeter flask and evaporate to dryness on a water bath. Dissolve the residue in 1 cubic centimeter of amyl alcohol by heating over a free flame and precipitate the pure filix acid with 30 cubic centimeters of methyl alcohol (added drop by drop until a permanent precipitate is produced, and the remainder at once.) After the liquid has stood quietly in a cool place for 10 to 12 hours, collect the precipitate on a tared filter, wash with methyl alcohol (two 5 cubic centimeter portions,) press the filter between porous plates, dry at an initial temperature of 40°C and finally at 80°C, and weigh.

Stoder's Method (1901): Dissolve 5 grams of the oleoresin in 20 cubic centimeters of ether, add 100 cubic centimeters of a freshly prepared solution of barium hydroxide (2 per cent.) and shake the mixture frequently during 1 hour. After allowing the mixture to stand quietly for a short time, separate the lower aqueous layer by filtration. Collect 86 cubic centimeters of this portion (corresponding to 4 grams of the oleoresin) in a separatory funnel and acidify with 10 cubic centimeters of dilute hydrochloric acid. Shake out the resulting precipitate with three portions of ether (40, 30 and 20 cubic centimeters) added successively, unite the shakings and remove the solvent by distillation. Dissolve the

residue in 1 cubic centimeter of amyl alcohol, and, after the solution has stood in a cool place for 48 hours, add 15 cubic centimeters of methyl alcohol. After standing for 24 hours more, collect the precipitated filix acid on a filter, wash with 5 cubic centimeters of methyl alcohol, dry on a water bath and weigh.

It will be noticed that the preceding methods, with the exception of the one devised by Kremel, are very similar in general outline, practically the only difference being found in the procedure by which the crude filix acid is directed to be purified. This difference is of special importance, however, as the weight of the product finally obtained will naturally vary with the degree to which purification has been effected, and this in turn will cause the computed percentage to vary, as is shown in the following table:

TABLE 27.—*Variation in filix acid content due to the difference in the methods employed in its determination.*

Date	Observer	Per cent. of filix acid by the method of			
		Bocchi	Kraft	Fromme (Original)	Fromme (Improved)
1897	Gehe & Co.	13.24 to 30.35	.....	3.28 to 11.32	.....
1897	Madsen	.....	.....	13.07	12.10
1897	"	.....	.....	6.58	5.85
1898	Plzak	.....	6.48	6.00	5.20

The above table shows further that the filix acid is obtained in the state of greatest purity when the improved method of Fromme is employed. And this method was usually given preference in the valuation of the oleoresin until it was discovered that the teniafuge properties were not due to the filix acid, alone, but were to be attributed in part to the presence of a number of other substances as well, compounds resembling acids to a certain extent in their chemical behavior.

Methods for the determination of the Crude Filicin.

With the above mentioned advance in our knowledge concerning the therapeutic constituents of this preparation, the methods for the determination of the filix acid lost their value and have since been superceded by those which have for their

object the determination of the quantity of total active constituents (crude filicin) present. The methods which have been proposed for this purpose are as follows:

Method of Bulle (1867):[1] Add a liberal amount of water to a weighed portion of the oleoresin contained in a suitable flask and heat on a water bath at 40° to 50°C. Add sufficient ammonia water to produce a strong odor of the same after vigorously shaking. Allow the mixture to stand in cold water for 3 or 4 hours and add 1/5 to ¼ of its volume of a saturated solution of salt, then filter. Wash the flask and filter with the salt solution, diluted with 6 parts water, until the filtrate no longer gives a precipitate with hydrochloric acid. Add dilute hydrochloric acid to the filtrate until precipitation is complete, collect the precipitate on a filter, wash and dry over sulphuric acid until of constant weight.

Method of Daccomo and Sccocianti (1896):[2] Dissolve 1 to 3 grams of the oleoresin in a small quantity of ether and shake the solution for ½ hour with an equal volume of an aqueous copper acetate solution. Allow the mixture to stand and separate, decant the ethereal liquid and collect the precipitate on a tared filter. Wash it successively with water, alcohol and ether, then heat at 100°C until of constant weight. When dry 111.55 parts of the precipitate represent 100 parts of filix acid.

Method of Schmidt (1903):[3] Place 5 grams of the oleoresin in a mortar and convert it to a coarse powder by triturating it with a sufficient quantity of calcined magnesia. Then add 250 cubic centimeters of water and thoroughly mix. After the magnesia has settled, decant the aqueous portion on a filter. Repeat this operation twice using 150 cubic centimeters of water each time. Transfer the combined filtrate to a separatory funnel and add hydrochloric acid in sufficient quantity to produce complete precipitation. Shake out the precipitate with ether, specific gravity 0.720 to 0.722, added in successive portions (100, 50 and 30 cubic centimeters.) After filtering the ethereal shakings, remove the solvent by distillation and dry the residue at 100°C.

Method of Fromme (1905):[4] Dissolve 5 grams of the extract in 30 grams of ether, add 100 grams of a saturated solution (3 per cent.) of barium hydroxide, and shake the mixture vigorously during several minutes. Transfer to a separator, and run 86 grams (4 grams of the extract) of the lower equeous layer into a flask of 200 cubic centimeters capacity. Add 2 grams of hydrochloric acid (25 per cent.) and shake out with 3 portions of ether, 25, 15, and 10 cubic centimeters. Separate the ether, and filter each portion successively through the same plain double filter into an

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[1] Cited by Doesterbehn (1898).

[2] This procedure was proposed as a method for the estimation of the filix acid. As its nature and the results obtained in its application show that it is in reality a method for determining the total constituents of an acid character, it has been included here.

[3] The method proposed by Goris and Voisin (1913) is almost identical with the above, the only difference being that 2 to 3 grams of the oleoresin are taken instead of 5 grams as directed by Schmidt.

[4] This is the method (but slightly modified) which is official in the British, Finnish and Swiss pharmacopœias.

Erlenmeyer flask of 200 cubic centimeters capacity which has been previously weighed. Wash the filter with 10 cubic centimeters more of ether, and finally distill off the ether and dry the residue at 100°C. Weigh after allowing it to stand in a desiccator for half an hour. The weight multiplied by 25 will give the percentage of crude filicin in the sample.

The striking similarity in the above methods is quite apparent and needs no special mention. Attention, however, is invited to the principal point of difference, namely, the reagent employed for the purpose of rendering the constituents to be determined soluble in water. In the methods under consideration, ammonia water, magnesium oxide and barium hydroxide have been made use of. As the amount of crude filicin obtained has been shown to depend to a considerable extent upon which one of these reagents is employed, the difference in the results reported in the literature in this connection is readily accounted for. The importance of this factor is clearly brought out in the following data obtained by Hill:

TABLE 28—*Influence of different alkalies on the percentage of crude filicin obtained.*

Alkali	K_2CO_3	1 per cent KOH	6 per cent. KOH	$Mg(OH)_2$	$Ca(OH)_2$	$Ba(OH)_2$
Per cent. of crude filicin	37.6	37.9	38.8	13.6	20.0	21.6

These results would appear to indicate that potassium hydroxide is the most efficient reagent for effecting a soluble combination of the constituents comprising the so-called crude filicin. The data, however, are misleading in that the strong alkali combines with other material therapeutically inert, and thereby causes the results to be high. While there is no information of a physiological nature at hand to substantiate the statement that barium hydroxide is the best reagent for this purpose, it is nevertheless, thought to be the most satisfactory from a chemical stand point at least. The method of Fromme, in which the latter is directed to be used, was, therefore, employed in the evaluation of the oleoresins examined in the laboratory. The results obtained in these analyses, together with those reported by other workers are given in the table which follows:

TABLE 29.—*Crude filicin content of laboratory samples of the oleoresin determined by Fromme's method.*

Sample No.	Date	Observer	Solvent	Crude filicin
				Per cent
1	1898	Bellingrodt	Ether	18.20
2	"	"	"	18.96
3	"	"	"	19.82
4	"	"	"	20.38
5	"	"	"	20.87
6	"	"	"	21.76
7	"	"	"	21.85
8	"	"	"	24.32
1	1899	Caesar & Loretz	"	31.44[1]
2	"	"	"	27.48[2]
1	1913	Bohrisch	"	18.22
1	"	DuMez	Acetone	13.79
2	"	"	Ether	20.37
1	"	Harrison & Self	"	19.30
2	"	"	"	19.70
3	"	"	"	21.50
4	"	"	"	21.90
5	"	"	"	24.10
6	"	"	"	24.20
7	"	"	"	24.50
8	"	"	"	24.70
9	"	"	"	26.50
10	"	"	"	27.70
11	"	"	"	28.0
1	1914	Linke	"	19.30
2	1916	DuMez	Acetone	16.00[3]
2	"	"	Ether	16.27[3]

[1] Ether, specific gravity 0.720.

[2] Ether, specific gravity 0.728.

[3] Oleoresins which were prepared in 1910 and had deteriorated. Examined shortly after being prepared, the ethereal oleoresin showed a crude filicin content of 26.35 per cent.

From the foregoing, it is apparent that the crued filicin content is influenced[1] by the age of the oleoresin as well as by the solvent which has been employed in its preparation. In the case of acetone, the low results obtained are not due to the incomplete extraction of the constituents to be determined, as might be inferred, but rather to the relatively large amount of total extractive matter obtained. It will be noticed that when the oleoresin is fresh and ether is the solvent which has been used in its preparation, the crude filicin content is usually above 20 per cent. This is in accordance with the requirements of the British Pharmacopœia and is thought to be a more reasonable standard than that adpoted by the Swiss, or the Finnish pharmacopœias. The former requires a filicin content of 26 to 28 per cent, while the latter specifies a minimum content of 26 per cent. This statement is further supported by the results obtained in the examination of commercial samples as is shown in the following compilation of such data:

[1] For the effect of the condition of the rhizomes used on the crude filicin content, see under "Drug used, its collection, preservation, etc."

TABLE 30.—*Crude filicin content of commercial samples of the oleoresin determined by Fromme's method.*

Sample No.	Date	Observer	Source	Crude filicin
				Per cent.
1	1901	Caesar & Loretz	Prepared by the firm	21.40
2	"	" "	" "	26.15
3	"	" "	" "	27.37
4	"	" "	" "	28.17
5	"	" "	" "	30.00
6	"	" "	" "	30.12
7	"	" "	" "	30.80
8	"	" "	" "	30.92
1	1903	" "	" "	27.08
2	"	" "	" "	28.22
3	"	" "	" "	28.78
4	"	" "	" "	29.39
5	"	" "	" "	30.05
6	"	" "	" "	36.60
1	1911	Evans Sons, Lescher & Webb.	England	26.30
2	"	"	"	28.00
1	"	Parry	"	8.40 (1)
2	"	"	"	8.60 (1)
3	"	"	"	8.80 (1)
4	"	"	"	9.00 (1)
5	"	"	"	9.20 (1)
6	"	"	"	10.80 (1)
1 to 16	1912	Evans Sons, Lescher & Webb.		22.90 to 26.30
1	"	Southall Bros. & Barclay	"	6.09 (1)
2	"	" " "	"	7.16 (1)
3	"	" " "	"	26.04
4	"	" " "	"	28.76
1	1913	Bohrisch	Germany	14.85
2	"	"	"	15.42
3	"	"	"	16.00
4	"	"	"	24.00
1	"	DuMez	England	8.79
2	"	"	United States	14.36
3	"	"	Germany	16.55
4	"	"	England	17.51 (1)
5	"	"	Germany	20.32
6	"	"	United States	20.77
1 to 7	1913	Evans Sons, Lescher & Webb.	Not given	21.3 to 25.30
8	"	"	" "	15.60 (1)
9	"	"	" "	19.60 (1)
10	"	"	" "	19.70 (1)
	"	Goris & Voisin	Germany	13.61 to 19.00
	"	" "	Switzerland	7.13 to 24.00
	"	" "	France	20.60 to 22.13
1	"	Harrison & Self	Germany	13.70
2	"	" "	"	19.10
3	"	" "	"	21.20
4	"	" "	"	24.80
5	"	" "	"	25.80
6	"	" "	"	28.10 (1)
1	"	Hill	England	11.60 (1)
2	"	"	"	13.20 (1)
3	"	"	"	14.10
4	"	"	Not given	18.10
5	"	"	" "	18.92
6	"	"	" "	19.30
7	"	"	" "	20.22
8	"	"	" "	20.67
9	"	"	" "	21.57
10	"	"	" "	21.60
11	"	"	" "	22.00
12	"	"	" "	22.65
13	"	"	" "	23.10

TABLE 30.—Continued.

Sample No.	Date	Observer	Source	Crude filicin
				Per cent.
14	1913	Hill	Not given	23.72
15	"	"	" "	23.75
16	"	"	" "	24.50
17	"	"	" "	24.55
18	"	"	" "	25.15
19	"	"	" "	25.27
20	"	"	" "	27.10
21	"	"	" "	27.82
22	"	"	" "	28.10
23	"	"	" "	29.75
1	1914	Linke	Merck & Co.	20.40
2	"	"	Brueckner, Lampe & Co.	21.67
3	"	"	Caeser & Loretz	27.22
1	1915	Southall Bros. & Barclay	Not given	20.40
2	"	" " "	" "	21.60
3	"	" " "	" "	24.20
4	"	" " "	" "	24.60
5	"	" " "	" "	27.70
1	1916	DuMez	Stearns & Co.	7.79 [2]
2	"	"	Lilly & Co.	17.57
3	"	"	Squibb & Sons	19.04
4	"	"	Parke, Davis & Co.	22.66

[1] These samples were adulterated with castor oil.

[2] Apparently an oleoresin from some species of fern other than *Dryopteris filix-mas*.

In addition to the information given in table No. 29, table No. 30 reveals the fact that a low filicin content in the commercial oleoresins is frequently due to adulteration with castor oil.

Physiological Tests.

In view of the difference in toxicity of the various constituents of the oleoresin with respect to the tapeworm, a physiological method for the evaluation of this preparation would appear to be desirable. The method proposed for this purpose by Yagi indicates the possibilities along this line. However, as there is no available information regarding its application, aside from that given by the originator, no statement can be made concerning its practical value. A description of the method for conducting the test follows:

Method of Yagi (1914): After thoroughly drying in a desiccator, accurately weigh 1 gram of the oleoresin and dissolve it in 25 cubic centimeters of ether. Bring the therapeutically active constituents into aqueous solution by shaking the ethereal liquid with a saturated solution of magnesium hydroxide, using 50 cubic centimeters of the latter for every

cubic centimeter of the former. Filter and divide the filtrate into several parts. Prepare solutions of different dilution from these parts by adding a measured amount of water to each. Then immerse 5 earthworms in each of these solutions and note the maximum dilution in which all 5 are killed. For computing the relative value of the preparation compare these results with those obtained when using a standard solution prepared by dissolving a weighed amount of filix acid, filmaron or albaspidin in water in the same manner as described above for the oleoresin. In the case of these standard solutions the limit of toxicity is given as follows: filmaron, 3 parts in 1,000,000; filix acid 4 parts in 1,000,000; albaspidin 1 part in 100,000.

Adulterations

The efforts which have been made in recent years to standardize this preparation have resulted in the discovery that the commercial article is very frequently adulterated, the latter being accomplished in a variety of ways.

The method usually resorted to by unscrupulous manufacturers in order to increase their profits consists of diluting the finished product with some comparatively cheap material. Castor oil[1] has generally been used for this purpose. In some cases, the oleoresin is prepared from deteriorated brown rhizomes and made to assume the green color of the official preparation by the addition of chlorophyll or salts of copper.[2]

Adulteration, however, is not limited to the addition of foreign materials to the finished product, but may take place in the drug from which the oleoresin is prepared. The forms in which the drug may be contaminated are conveniently classed under three heads, *viz.:* (a) the substitution of old deteriorated rhizomes for the fresh material, (b) the admixture of chaff and dead stipe bases with the rhizomes, and (c) the admixture of rhizomes of unofficial species of fern with those of the official species. For a discussion of these conditions, see under "Drug used, its collection, preservation, etc."

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[1] Parry (1911); Evans Sons, Lescher and Webb (1911); and others.

[2] Weppen and Lueders (1892); Beckurts and Peters (1893); Pendorff (1913); and others.

A trace of copper is usually present in the commercial product as a result of the use of copper utensils in the manufacture of the preparation. (See under "Ash").

OLEORESIN OF CAPSICUM

Synonyms

Aetherische Spanishpfefferextrakt, Nat. Disp. 1884.
Capsicum,[1] Chem. & Drugg. (1913), 82, p. 470.
Capsicol, Vierteljahrschr. f. prakt. Pharm. (1873), 22, p. 507.
Ethereal Extract of Capsicum, Am. Journ. Pharm. (1849), 21, p. 114.
Extractum Capsici aethereum, Hirch, Univ. P. 1902, **No. 1905.**
Oleoresin of Red Pepper, Stevens, Pharm. and Disp. (1909), p. 255.
Oleoresina Capsici, U. S. P. 1910.
Oléorésine de Capsique, U. S. Disp. 1907.
Spanishpfefferextrakt, Nat. Disp. 1884.
Spanishpfeffer-Oelharz, Nat. Disp. 1884.

History

The oleoresin of capsicum appears to have been first prepared by Procter in 1849, and it was through his efforts that it was introduced into the *United States Pharmacopœia* of 1860. Up to the present time, no such preparation appears in any of the foreign pharmacopœias. A similar preparation known as capsicin has, however, been in use in Europe since 1873.[2]

Drug Used, Its Collection, Preservation, Etc.

The drug directed to be used by the present edition of the *United States Pharmacopœia* is "the dried ripe fruits of *Capsicum fructescens* Linné[3] (Fam. *Solanaceae),* without the presence or admixture of more than 2 per cent. of stems, calyxes or other foreign matter." The preceding editions of the Pharmacopœia since 1880 have specified the use of the species known as *Capsicum fastigiatum* Blume. The change is evidently due to the fact that the leading commercial varieties of Cayenne pepper are at the present time being received from Africa and Japan and

[1] For other uses of the term capsicin, see under "Chemistry of capsicum and its oleoresin."

[2] Buchheim states that capsicin (the ethereal extract of capsicum) was being prepared and sold by Merck of Darmstadt in 1873. Vierteljahrschr. f. prakt. Pharm. (1873), 22, p. 507.
Capsicin, as found on the market in England, is stated to be indefinite in that it may be an alcoholic, a chloroformic, an ethereal or an acetone preparation. Chem. and Drugg. (1913), 82, p. 470.

[3] This is also the species recognized by the French Pharmacopœia. In the other European pharmacopœias, in which this drug occurs, it is usually the the larger fruited variety, *Capsicum annum,* which is designated.

belong to the first mentioned species[4] which has also been known as *Capsicum baccatum* Vell.

The fruit is plucked when ripe, exposed to the sun until dried, and then usually packed in suitable shape for market. It should be preserved in the whole condition in a cool place,[5] and preferably in a closed container as it is prone to become rancid owing to the large amount of fatty oil which it contains.

U. S. P. Texts and Comments Thereon.

The oleoresin has been official in the *United States Pharmacopœia* for the past half century having been recognized for the first time in the edition of 1860.

1860

Oleoresina Capsici

Oleoresin of Capsicum

Take of Capsicum,[1] in fine powder,[2] twelve troy-ounces;
Ether[3] a sufficient quantity.

Put the capsicum into a cylindrical percolator,[4] press it firmly, and gradually pour ether upon it until twenty-four fluid ounces of filtered liquid have passed.[5] Recover from this, by distillation on a water-bath, eighteen fluid-ounces of ether,[6] and expose the residue, in a capsule, until the remaining ether has evaporated.[7] Lastly, remove, by straining, the fatty matter which separates on standing,[8] and keep the Oleoresin in a well-stoppered bottle.[10]

1870

Oleoresina Capsici

Oleoresin of Capsicum

Take of Capsicum,[1] in fine powder,[2] twelve troyounces;
Ether[3] a sufficient quantity.

Put the capsicum into a cylindrical percolator, provided with a stop-cock, and arranged with cover and receptacle suitable for volatile liquids,[4] press it firmly, and gradually pour ether upon it, until twenty-four fluid ounces of liquid have slowly passed.[5] Recover the greater part of the ether by distillation on a water-bath,[6] and expose the residue in a capsule, until the remaining ether has evaporated.[7] Lastly, remove, by straining, the fatty matter which separates on standing,[8] and keep the Oleoresin in a well-stoppered bottle.[10].

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[4] Tolman and Mitchell, Bull. 163, Bur. of Chem. (1913), p. 9.
[5] Brown, Bull. 150, Kentucky Agric. Exp. Sta. (1910), p. 131.

1880

Oleoresina Capsici

Oleoresin of Capsicum

Capsicum,[1] in No. 60 powder,[2] *one Hundred parts* 100
Stronger Ether,[3] *a sufficient quantity.*

Put the capsicum into a cylindrical percolator, provided with a cover and receptacle suitable for volatile liquids,[4] press it firmly, and gradually pour stronger ether upon it, until one hundred and fifty (150) parts of liquid have slowly passed.[5] Recover the greater part of the ether by distillation on a water-bath,[6] and expose the residue, in a capsule, until the remaining ether has evaporated.[7] Lastly, pour off the liquid portion,[8] transfer the remainder to a strainer, and, when the separated fatty matter (which is to be rejected) has been completely drained, mix all the liquid portions together.[9]

Keep the oleoresin in a well stopped bottle.[10]

Preparation. Emplastrum Capsici.

1890

Oleoresina Capsici

Oleoresin of Capsicum

Capsicum,[1] in No. 60 powder,[2] *five hundred grammes* .....500 Gm.
Ether,[3] *a sufficient quantity.*

Put the capsicum into a cylindrical glass percolator, provided with a stopcock, and arranged with cover and receptacle suitable for volatile liquids,[4] Press the drug firmly, and percolate slowly with ether, added in successive portions, until the drug is exhausted.[5] Recover the greater part of the ether from the percolate by distillation on a water-bath,[6] and, having transferred the residue to a capsule, allow the remaining ether to evaporate spontaneously.[7] Then pour off the liquid portion, transfer the remainder to a strainer, and, when the separated fatty matter (which is to be rejected) has been completely drained, mix the liquid portions together.[8]

Keep the oleoresin in a well-stoppered bottle.[10].

Preparation: Emplastrum Capsici.

1900

Oleoresina Capsici

Oleoresin of Capsicum

Capsicum,[1] in No. 40 powder,[2] *five hundred grammes* .....500 Gm.
Acetone,[3] *a sufficient quantity.*

Introduce the capsicum into a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and a receptacle suitable for volatile liquids.[4] Pack the powder firmly, and percolate slowly with acetone, added in successive portions, until eight hundred cubic centimeters of percolate have been obtained.[5] Recover the greater part of the acetone from the percolate by distillation on a water-bath,[6] and, having transferred the residue to a dish, allow the remaining acetone to evaporate spontaneously in a warm place.[7] Then pour off the liquid portion,[8] transfer the remainder to a glass funnel provided with a pledget of cotton, and when the separated fatty matter (which is to be rejected) has been completely drained, mix the liquid portions together.[9] Keep the oleoresin in a well-stoppered bottle.[10]

Average dose.—0.030 Gm. = 30 milligrammes (½ grain).

1910

Oleoresina Capsici

Oleoresin of Capsicum

Oleores. Capsic.

Capsicum,[1] in No. 40 powder[2] *five hundred grammes* .....500 Gm.
Ether,[3] *a sufficient quantity.*

Place the capsicum in a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and a receptacle suitable for volatile liquids.[4] Pack the powder firmly and percolate slowly with ether, added in successive portions, until the percolate measures *eight hundred mils.*[5] Recover the greater part of the ether from the percolate by distillation on a water-bath,[6] and, having transferred the residue to a dish, allow the remaining ether to evaporate spontaneously in a warm place.[7] Then pour off the liquid portion,[8] transfer the remainder to a glass funnel provided with a pledget of cotton, and, when the separated fatty matter (which is to be rejected) has been completely drained, mix the liquid portions together.[9] Keep the oleoresin in a well-stoppered bottle.[10]

Preparation — Eplastrum Capsici.

Average Dose.—Metric, 0.03 Gm.—Apothecaries, ½ grain.

1) For a description of the drug, see pag 1017 under "Drug used, its collection, preservation, etc."

2) The editions of the Pharmacopœia previous to that of 1900 directed that the drug be reduced to a fine powder (No. 60) for percolation. As a No. 40 powder has been found to be equally satisfactory for this purpose, the last two editions of the Pharmacopœia have specified the use of the coarser powder.

3) Ether is the solvent which is directed to be used in the extraction of the drug at the present time. Previous editions of the Pharmacopœia, with the exception of that 1900, also, specified the use of ether for this purpose. The use of acetone as directed by the Pharmacopœia of 1900 was unsatisfactory as the large amount of extractive matter obtained caused the residue which remained upon the evaporation of the solvent to assume a semi-solid gelatinous form, and thus increased the difficulty of separating the liquid portion.

Among the other solvents which have received consideration in this connection, benzin is worthy of mention. The reports of Maisch, Trimble and Beringer, respectively, (see part I, pages 923 and 924) indicate that it is a good solvent for the oleoresinous constituents of capsicum and that the product obtained is equal in quality to that yielded by ether. Experiments conducted in the laboratory confirm these observations. The solvent used in the laboratory, however, was petroleum ether, boiling temp. 45 to 50° C., as the composition of ordinary commercial benzin varies to a considerable extent.

4) The Pharmacopœia of 1860 directed that the extraction of the drug be carried out in an ordinary glass percolator. As a considerable amount of solvent was lost under these conditions, the subsequent editions of the Pharmacopœia have specified that a form of percolator adapted to the use of volatile liquids be employed for this purpose. For a description of such forms, see Part I, under "Apparatus used."

5.) Of interest in connection with the preparation of this oleoresin is the fact that the pharmacopœial directions concerning the amount of percolate to be collected have been changed no less than three times. The first change appeared in the Pharmacopœia of 1880, and was apparently instituted for economic reasons as the amount of percolate directed to

be collected was reduced from approximately 2 cubic centimeters for each gram of drug used (24 fluid ounces for 12 troy ounces of drug) to 1.5 cubic centimeters. In the succeeding edition of the Pharmacopœia (edition of 1890), the second change was made, the directions being to continue percolation until the drug is exhausted. The third change occurs in the Pharmacopœia of 1900, which directs that 1.6 cubic centimeters of percolate be collected for each gram of drug taken.

The reason for making the second change does not become apparent from the information at hand. The third change, however, appears to have been instituted primarily for the purpose of reducing the amount of solid fats (mainly palmitin and stearin) extracted in order that the separation of the liquid portion constituting the oleoresin might be accomplished more easily.

In commenting further upon these changes, it is stated that, in the preparation of the oleoresin in the laboratory, no greater difficulty was experienced in the separation of the liquid portion when the amount of sold fats present was large than when the quantity present was relatively small. From this standpoint, therefore, the last change does not appear to have been warranted. For economic reasons, however, the change was desirable since at least twice as much ether was required for the complete exhaustion of the drug as is ordinarily used when proceeding according to the directions given in the last edition of the Pharmacopœia.

It is thought that the present pharmacopœial method could be still further improved through the use of some form of continuous extraction apparatus for exhausting the drug. Not only would this procedure result in the saving of a large amount of solvent, but the time required to complete the preparation of the oleoresin would be considerably shortened.

6) The Pharmacopœia of 1860 directed that only ¾ of the menstruum contained in the percolate be recovered by distillation on a water bath. In all of the subsequent editions the directions are to recover the greater part of the solvent, no specific amount being mentioned. In this connection, it may be stated that the preparation will not be injured even if all of the solvent is recovered under the above conditions. In case this is done, however, it is necessary to use ether in re-

moving the thick liquid from the flask so that no particular advantage is gained by such a procedure.

7) In all editions of the Pharmacopœia in which this preparation is official, it is directed that the last traces of solvent be allowed to evaporate spontaneously at room temperature. Since the complete removal of the solvent can be accomplished much more rapidly by heating the ethereal liquid on a water bath, and without injury to the finished product, it is thought that such a procedure would be a desirable improvement over the present pharmacopœial method.

8–9) The liquid portion constituting the oleoresin is directed to be separated from the solid fats, which precipitate upon the removal of the solvent, by decantation, and straining through a pledget of cotton. Experience has shown that this may be accomplished much more rapidly and satisfactorily by the aid of a force filter. By this procedure a more complete separation can be effected without washing the residue on the filter with a portion of the solvent as has been suggested by some and thus, the necessity of further exposure of the preparation to the air is done away with.

With further reference to the removal of the solid fats, attention is called to the fact that the degree to which this is accomplished depends upon the temperature at which the operation is carried out. The preparation when made during the summer may be perfectly homogeneous at the time, but deposit fat during the winter. In order to secure a more uniform product, it is therefore, thought that the Pharmacopœia should direct that the mixture be chilled to a definite temperature previous to the separation of the liquid portion.

10) The oleoresin should be kept in well-stoppered bottles for the same reasons as are given in the comments on the oleoresin of aspidium. See page 979.

Yield

The average yield of oleoresin is usually about 15 to 18 per cent. when ether is the solvent employed in exhausting the drug. It is about the same when alcohol, acetone, petroleum ether, carbon disulphide or chloroform are used. In this connection, attention is called to the fact that the total amount of

extract obtained and the oleoresin are not identical, the latter consisting only of the oily, liquid portion of the former. Thus, it will be observed, upon examining the tables which follow, that the total amount of extract obtained with acetone may amount to 25 per cent. of the drug operated upon, whereas, the yield of oleoresin is only about 18 per cent. The factor which appears to influence the yield to the greatest extent is the temperature at which the preparation is completed. This is due to the fact that the oleoresin is saturated with solid fats (principally palmitin) and, that these will be precipitated to a greater or lesser degree depending on the temperature at which the preparation is finally strained. The finished product will, therefore, contain a relatively small amount of these fats, and the yield will be correspondingly low when made during the cold winter months, whereas, the opposite will be the case when the oleoresin is prepared in the hot months of summer. The following tables show the yield of oleoresin, as reported in the literature, likewise, that obtained in the laboratory:

TABLE 31.—*Yield of oleoresin as reported in the literature.*

Date	Observer	Yield of oleoresin to				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per cent.	
1853	Bakes	25.00				
888	Trimble			19.50	Benzin 18.15	
1892	Beringer		18.00	17.32	21.00	Yield of oleoresin when prepared by the U. S. P. method.
		28.00	25 00		25.00	Total yield of extract on complete exhaustion of the drug.
1892	Sherrard			15.50		
				17.40		
				18.30		
				18.40		
1896	Alpers			19.00		Oleoresin from which deposited fat had been removed.
1898	Winton, Ogden & Mitchell			15.81		Total ether extract from "Chilli colorado."
				16.85		Total ether extract from Natal capsicum.
				21.31		Total ether extract from Nepaul capsicum.
				16.19		Total ether extract from Zanzibar capsicum.
1903	Southall Bros. & Barclay			15.67		*Capsicum minimum* total yield to ether, sp. gr. 0.717.
				15.34		*Capsicum annum* total yield to ether, sp. gr. 0.717.
1905	Vanderkleed				Solvent(?) 9.40 to 23.90	Reported as yield of oleoresin. The average yield of 8 samples is given as 18.13 per cent.
1905	Gerrard	Alcohol (90%) 26.40		18.20	Benzin 18.60; Petrol Ether 16.40; Carbon disulphide 16.70; Chloroform 17.50	Represents yield of total extractive matter.
1907	Patch	16.20 to 26.50				Total alcoholic extract. Results obtained in the examination of 10 samples of capsicum.
1908	"	15.0 to 25.20				Total alcoholic extract.
1908	Vanderkleed				Solvent(?) 11.59 to 18.35	Reported as yield of oleoresin: Represents the yield from 3 samples of capsicum.
1909	"				14.34 to 17.95	Reported as yield of oleoresin. Results obtained in the extraction of 5 samples of capsicum.
1910	Southall Bros. & Barclay				Benzene 14.00 to 15.40	Total benzene extract.
1910	Eldred			11.00 to 26.00		Reported as yield of ether soluble oleoresin. The avererage yield obtained from 48 samples of capsicum is given as 18.00 per cent.
1910	Vanderkleed				Solvent(?) 15.10 to 22.27	Reported as yield of oleoresin. Results obtained in extracting 7 samples of capsicum.

TABLE 31.—*Yield of oleoresin as reported in the literature*—Continued.

Date	Observer	Yield of oleoresin to				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per cent.	
1911	Vanderkleed—Continued.	.....	.....	.....	Solvent(?) 14.70	Reported as yield of oleoresin.
		.....	.....	.....	17.93	
1912	Johnson and Johnson.....	.....	.....	16.00 to 19.00	.....	Reported as yield of ether extract.
1912	Vanderkleed.....	.....	.....	.....	Solvent(?) 14.41 to 16.70	Reported as yield of oleoresin.
1913	Patch.....	19.00 to 24.00	.....	.....	.....	Total alcoholic extract. Results obtained in extracting 4 samples of capsicum.
1913	Vanderkleed.....	.....	.....	.....	Solvent(?) 13.10 to 18.10	Reported as yield of oleoresin. Seven samples of capsicum were extracted.
1913	Englehardt.....	.....	.....	.....	Solvent(?) 11.00	Reported as yield of oleoresin.
		.....	.....	.....	11.30	
		.....	.....	.....	13.10	
		.....	.....	.....	14.80	
		.....	.....	.....	15.26	
		.....	.....	.....	15.80	
1914	Rippetoe.....	17.02 to 24.46	.....	16.49 to 17.88	.....	Total yield of extract.
1914	Riedel.....	31.90 to 35.30	.....	.....	.....	Total yield of extract.
1914	Vanderkleed.....	.....	.....	.....	Solvent(?) 13.00 to 18.00	Reported as yield of oleoresin. The average yield from 15 samples is given as 10.00 per cent.
1915	Vanderkleed.....	.....	.....	.....	Solvent(?) 13.85 to 20.84	Reported as yield of oleoresin. The average yield of 6 samples is given as 16.65 per cent.

TABLE 32—*Yield of oleoresin obtained in the laboratory.*

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per cent.	
1910..	DuMez & Netzel.	25.12	20.25	18.33	Benzine 16.50.....	Represents total extract.
1916..	Du Mez	29.90	22.48	19.98	Petrol. Ether. 18.82.....	" " "
		16.40	17.50	16.14	16.18.....	Represents oleoresin separated from deposited fat and other extractive matter.

Chemistry of the Drug and Oleoresin.

Tabulation of Constituents.

The reported analyses[1] of the various varieties of red peppers show the constituents of pharmaceutical interest to be as follows: fixed oil, volatile oil, fatty acids, capsaicin, capsicine, resin, mucilage, starch, coloring matter and inorganic substances. Most of these substances have been identified in the oleoresin prepared by extracting the fruits with ether. They are the following:

Fatty Oil	Capsaicin	Coloring Matter
Fatty Acids	Capsicine	Ash
Volatile Oil	Resin	

Occurrence and Description of Individual Constituents.

Fatty Oil. Work on the oil of capsicum has practically been limited to that obtained from the variety official in most of the continental pharmacopœias, namely: *Capsicum annum* L. The properties of the oil of *Capsicum fastigiatum* Bl. as observed by Goetz appears to indicate that it is very likely identical with the former.[2] The oil as obtained from the seeds of *Capsicum annum* Bl.[3] is a yellowish brown, mobile liquid, specific gravity 15.5°C 0.91095; iodine value (Huebls) 119.5; saponification value (Koettsdorffer) 187.2. It is composed of the glyceryl esters of oleic, palmitic and stearic acids.

The oil of capsicum is located in the seeds and is variously stated to comprise from 20[4] to 24.06[5] per cent. of these organs in *Capsicum annum.* The yield as computed by Goetz for the entire fruit of *Capsicum fastigiatum* is 8.4 per cent.. The yield in the case of *Capsicum fructescens* does not appear to have been determined.

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[1] Taylor, Am. Journ. Pharm. (1857), 29, p. 303; Buchheim, Vierteljahrschr. f. prakt. Pharm. (1872), 4, p. 507; Proc. A. Ph. A. (1873), 22, p. 106; Strohmer, Chem. Centralb. (1884), 55, p. 557; Pabst, Arch. d. Pharm. (1892), 230, p. 108; Tolman and Mitchell, Bull. No. 163, Bur. of Chem., Dep. of Agr. (1913), p. 9.

[2] Goetz obtained 15.7 per cent of a yellowish-brown fixed oil from the seeds of *Capsicum fastigiatum* Bl., specific gravity at 25°, 0.919. Goetz, unpublished results.

[3] Buchheim, *l. c.;* Pabst, *l. c.;* von Bitto, Landwirt. Versuchs-Stat. (1896), 46, p. 310; Meyer-Essen, Chemiker Ztg. (1903), 27, p. 958.

[4] Meyer-Essen, *l. c.*

[5] von Bitto, *l. c.*

Fatty Acids.[6] The free fatty acids present have been identified as oleic, palmitic and stearic, palmitic acid predominating in the fruits of *Capsicum annum.* The proportions of these acids as they occur in the fruit of *Capsicum fastigiatum* or *C. fructescens* have apparently not been determined to date.

Volatile Oil. The presence of a volatile oil was first noted in the fruits of *Capsicum annum* by Taylor.[7] Pabst isolated a small amount of a volatile liquid having the odor of parsley from the same. Inasmuch as the oleoresin, when prepared from *Capsicum fructescens* has a distinct odor, it is quite probable that a similar volatile oil is also present in the fruit of this variety.

Capsaicin[9] Capsaicin is the sharp tasting constituent of the fruits of the various varieties of red pepper. It crystallizes from petroleum ether in colorless plates melting at 60.5°C (Morbitz), 63 to 63.5°C (Micko), 64.5°C (Nelson).[10] The substance is stated to be soluble in water (1:30,000), petroleum ether (1:3,633), ether, alcohol, carbon disulphide and chloroform. According to Morbitz, its composition is represented by the formula $C_{35}H_{54}N_3O_4$. Micko[11] does not agree with the latter and has proposed the formula $,CH_3O.C_{17}H_{24}NO.OH$, as also representing the structure in part.

Capsaicin is stated by Morbitz to be present in the fruit of *Capsicum fastigiatum* to the extent of 0.05 to 0.07 per cent.

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[6] Buchheim, Pabst, von Bitto, *l. c.*

[7] *l. c.*

[9] The term *capsicin* was first used to designate the sharp tasting principle principle in red peppers. Bucholz, Taschenb. f. Scheidkuenst. u. Apoth. (1816), 37, p. 1; Landerer, Vierteljahresschr. f. prakt. Pharm. (1854), 3, p. 34. The name was also applied to the ethereal extract of capsicum as marketed by Merck and Co. See note by Buchheim, Vierteljahrschr. f. prakt. Pharm. (1873), 22, p. 507. Later it was used to indicate a coniine-like alkaloid isolated from the fruit of *Capsicum fastigiatum* by Thresh. Pharm. Journ. (1876), 35, p. 941.

In 1873, Buchheim gave the name *Capsicol* to a dark red oily liquid (our present oleoresin) which he considered to be the pungent principle.

Capsaicin is the term which was introduced by Thresh to denote the sharp tasting substance isolated by him from the fruits of *Capsicum fastigiatum.* Pharm. Journ. (1876), 36, p. 21. It is the name now generally employed to indicate this substance, although, Morbitz (*l. c.*) subsequently proposed the name *Capsicutin.*

A more recent investigator, Gabriel de la Puerta, has given the name "capsic acid" to the irritant principle isolated from pimenta. Ann. de la Soc. Espanola de fis. y. quim. (1905), No. 23; Am. Drugg. & Pharm. Rec. (1906), 48, p. 40.

[10] Chem. News (1911, 103, p. 111.

[11] Chem. Centralbl. (1899), 70, p. 293.

The amount present in *Capsicum fructescens* has not been reported.

Capsicine. According to Felletar[12] and Thresh,[13] capsicine is present in the fruits of *Capsicum annum* and *C. fastigiatum.* The latter describes it as an alkaloid possessing an odor similar to that of coniine. The hydrochloride is stated to have been isolated in the crystalline form and to be precipitated from aqueous solution by the usual alkaloidal reagents. Pabst[14] states that the base is not a normal constituent of the fruits of *Capsicum annum,* but that it is formed when the latter are stored or by the action of various reagents.

Resin. Resin is mentioned by several investigators[15] as a constituent of the fruits of the red peppers. Apparently nothing has been done toward determining its composition or properties.

Coloring Matter. The red color of the capsicum fruit as well as that of the ethereal extract appears to have attracted the attention of all investigators, although, Pabst, is the only observer who attempted to identify the substance. He concluded, from saponification experiments, that it was a cholesterin ester of a fatty acid.[16]

Ash. According to von Bitto,[17] the ash of capsicum is composed of the basic elements, K, Na, Mg, Ca, Fe, Al and Mn combined with the acid radicles Cl', SiO_3'', SO_4'', PO_4''', NO_3' and CO_3''.

The ash content of red pepper varies with the variety of the fruit.[18] That of the commercial drug is also influenced by the presence of sand. The ash of *Capsicum fructescens* (sand free) amounts to about 4.90 per cent of the dried fruit.[19]

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[12] Vierteljahrschr. f. prakt. Pharm. (1868), 17, p. 360; Buchner's Repert. f. d. Pharm. (1828), 27, p. 35; Proc. A. Ph. A., (1871), 19, p. 289.

[13] Pharm. Journ. (1876), 35, p. 941.

[14] *l. c.*

[15] Strohmer, Pabst, Tolman and Mitchell, *l. c.*

[16] Pabst, *l. c.*

[17] Landw. Versuchsstat. (1893), 42, p. 369.

[18] Tolman and Mitchell give the ash content of sand free *Capsicum annum* as 6.69 to 7.54 per cent. Bull. 163, Bur. of Chem., Dept of Agr., Washington, 1913.

[19] McKeown gives the ash content of *Capsicum fastigiatum* as 4.50 to 4.95 per cent. Am. Drugg. (1886), 14, p. 128.

Tolman and Mitchell report the sand free ash content of *Capsicum fructescens* (African) as 4.49 to 5.44 per cent, that of the fruits of the same variety coming from Japan as 4.60 to 5.35 per cent, *l. c.*

Constituents of Therapeutic Importance

The early investigators assigned the intensely irritating properties of the oleoresin of capsicum to various substances supposed to be contained therein. Bracconot[1] and Buchheim[2] thought it due to the oily constituents, Felletar[3] attributed the action to a liquid organic base, and Pabst[4] to a resin intimately mixed with the red pigment. The irritating principle is now known to be the crystalline constituent, capsaicin.[5] The latter has not been isolated in sufficient quantities to permit of an extensive investigation of its physiological properties. It is, however, known to act as a rubefacient when applied externally, and to be extremely pungent to the taste, its sharpness being perceptible in aqueous solution, 1 part to 11 million parts of water.[6]

Physical Properties

Color: The color of the oleoresin, when the latter is spread out in a thin layer on a white porcelain surface, is a characteristic light brownish-red. The descriptions of the color given in pharmaceutical literature vary to a considerable extent (light reddish-brown to dark brown) owing very likely to a difference in the conditions under which the observations were made.

Odor: The odor of the preparation is rather faint, but characteristic, resembling that of the red peppers.

Taste: It is extremely pungent and should be tasted with caution. The taste is usually described as being hot and fiery, or burning.

Consistence: The consistence of the oleoresin varies with the amount of solid fats (palmitin and stearin) present,[7] and with

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[1] Ann. Chim. Phys. (1817), 6, p. 122.
[2] Vierteljahresschr. f. prakt. Pharm. (1873), 22, p. 507.
[3] *Ibid.* (1868), 17, p. 360.
[4] Arch. d. Pharm. (1892), 230, p. 108.
[5] Micko, Zeitschr. f. Unters. Nahr.-u. Genussm. (1898), 12, p. 215.
[6] Morbitz, Pharm. Zeitschr. f. Russland, (1897), p. 372.
[7] See under "Methods of preparation".

the temperature. At ordinary temperatures the degree of fluidity is usually such that it can be readily poured. It should be homogeneous and not contain a deposit of fat.

Solubility: The oleoresin, when prepared with ether, is soluble in acetone, ether, chloroform, carbon tetrachloride, carbon disulphide, petroleum ether, oil of turpentine[1] and solutions of the caustic alkalies. It should not be soluble to any great extent in 90 per cent. alcohol, solubility therein indicating that alcohol was the menstruum used in the preparation of the oleoresin.

Specific gravity: The specific gravity of the oleoresin determined at 25°C was found to be 0.925 to 0.932 when ether was the solvent employed in extracting the drug. When alcohol or acetone were employed for this purpose, the results were almost the same, whereas petroleum ether yielded a product of low specific gravity. The low specific gravity observed in the one case, where acetone was used in the preparation of the oleoresin, was not due to the nature of the solvent, but to the more complete removal of the solid fats. The variation in the amounts of the latter retained in the finished product is thought to be the chief factor influencing the specific gravity of this preparation. In the case of the commercial samples, however, the presence of unevaporated solvent must also be taken into consideration as is shown in the tables which follow:

TABLE 33—*Specific gravities of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Specific gravity
				At 25° C
1.....	1910	DuMez & Netzel.....	Alcohol.....	0.932
2.....	"	" "	Acetone.....	0.933
3.....	"	" "	Ether.....	0.932
4.....	"	" "	Benzine.....	0.925
1.....	1916	DuMez.....	Alcohol.....	0.926
2.....	"	"	Acetone.....	0.919
3.....	"	"	Ether.....	0.925
4.....	"	"	Petrol. ether.....	0.914

[1] King's *American Dispensatory* (1900), p. 1331.

TABLE 34—*Specific gravities of commercial oleoresins.*

Sample No.	Date	Observer	Source	Specific gravity
				At 25° C
1.....	1916	DuMez	Squibb & Sons.....	0.910[1]
2.....	"	"	Lilly & Co.	0.919
3.....	"	"	Sharp & Dohme	0.928

[1] o ntained ether

Refractive index: Determinations made in the laboratory show that the oleoresin should have a refractive index of about 1.47 when observed at 25°C. A refractive index lower than this was found to be due to the presence of unevaporated solvent. The solvent employed in extracting the drug or the variation in solid fat content appears to have very little influence, if any, on this constant. The results obtained in the laboratory in the examination of the oleoresin follow:

TABLE 35 – *Refractive indices of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Refractive index
				At 25° C
1.....	1910	DuMez & Netzel	Alcohol	1.463
2.....	"	"	Acetone.....	1.477
3.....	"	"	Ether	1.474
4.....	"	"	Petrol, ether	1.475
1.....	1916	DuMez.....	Alcohol	1.473
2.....	"	"	Acetone.....	1.473
3.....	"	"	Ether	1.474
4.....	"	"	Petrol, ether	1.472

TABLE 36.—*Refractive indices of commercial oleoresins.*

Sample No.	Date	Observer	Source	Refractive index
				At 25° C
1	1916	DuMez	Lilly & Co.....	1.472
2	"	"	Sharp & Dohme	1.473
3	"	"	Squibb & Sons.....	1.467[1]

[1] Contained ether.

Chemical Properties.

Loss in weight on heating: Determinations made in the laboratory show that the oleoresin loses but little in weight on heating at 110°C, a loss of but 0.42 to 2.13 per cent. having been observed for the preparation when free from solvent. The laboratory preparations as a rule showed a smaller loss than the commercial samples, which is very likely due to a difference in the temperature conditions under which the preparations were made. The results obtained in the determinations made in the laboratory are given in the following tables:

TABLE 37.—*Laboratory preparations—loss in weight on heating*

Sample No.	Date	Observer	Solvent	Per cent. of loss on heating
				At 110° C
1	1916	DuMez	Alcohol	0.42
2	"	"	Acetone	0.52
3	"	"	Ether	0.88
4	"	"	Petrol. ether	0.68
5	"	"	Alcohol	5.15[1]
6	"	"	Acetone	0.74
7	"	"	Ether	2.09
8	"	"	Benzine	1.01

[1] Contained alcohol.

TABLE 38.—*Commercial oleoresins—loss in weight on heating.*

Sample No.	Date	Observer	Source	Per cent of loss on heating
				At 110° C
1	1916	DuMez	Sharp & Dohme	1.93
2	"	"	Lilly & Co.	2.13
3	"	"	Squibb & Sons	4.09[1]

[1] Contained ether.

Ash Content: The determinations made in the laboratory show that the ash content of the oleoresin varies with the solvent employed in its preparation. When acetone was the solvent used, the amount of ash obtained did not exceed 0.26 per cent, whereas, the amount was only 0.09 to 0.12 per cent. when the oleoresin was prepared with ether. The variable results obtained in the examination of the commercial samples appear to

indicate the use of different solvents in their preparation. The comparatively high value (0.40 per cent.) obtained in one case, however, may have been due to the copper present. The ash content of the samples examined in the laboratory is given in the tables which follow:

TABLE 39—*Ash contents of oleorsins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Per cent. of ash
1.....	1916	DuMez.....	Alcohol.....	0.39
2.....	"	"	Acetone	0.26
3.....	"	"	Ether.....	0.09
4.....	"	"	Petrol, ether	0.09
5.....	"	"	Alcohol.....	0.39
6.....	"	"	Acetone	0.24
7.....	"	"	Ether	0.12
8.....	"	"	Benzin	0.10

TABE 40.—*Ash contents of commercial oleoresins*

Sample No.	Date	Observer	Source	Per cent. of ash	Foreign constituents
1	1916	DuMez	Squibb & Sons.....	0.09[1]	
2	"	"	Sharp & Dohme.....	0.40	Copper
3	"	"	Lilly & Co.....	0.30	"

[1] Contained ether.

Acid number: The acid numbers, when acetone, ether, or petroleum ether were used in the preparation of the oleoresin, were found to be 106.6, 103.8 and 105 respectively. When alcohol was employed for this purpose, the value obtained for this constant was considerably lower, being 93.5. With respect to the commercial samples examined, the acid number was in all cases found to be much lower. This is thought to be due, in two instances, to a low free acid content (principally palmitic acid) of the drug from which the oleoresins were prepared, or to the more complete removal of these acids in the separation of the deposited material. In the third case, it was caused, in part, at least, by the presence of unevaporated solvent. The acid numbers obtained for the preparations examined in the laboratory are as ofllows:

TABLE 41 – *Acid numbers of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Acid number
1	1916	DuMez	Alcohol	93.5
2	"	"	Acetone	106.6
3	"	"	Ether	103.8
4	"	"	Petrol. Ether	105.0

TABLE 42—*Acid numbers of commercial oleoresins.*

Sample No.	Date	Observer	Source	Acid number
1	1916	DuMez	Squibb & Sons	30.8[1]
2	"	"	Sharp & Dohme	60.3
3	"	"	Lilly & Co.	82.7

[1] Contained ether.

Saponification value: The saponification values obtained for the oleoresins prepared in the laboratory were above 200, as a rule, regardless of the nature of the solvent used in extracting the drug. The comparatively slight variations observed were very likely due to the difference in the degree to which the solid fats (principally palmitin) had been removed. This also accounts for the comparatively low values obtained for the commercial preparations. The exceptionally low value obtained for the sample from Squibb and Sons is to be attributed to the presence of unevaporated solvent. The values obtained for the preparations examined in the laboratory are given in the tables which follow:

TABLE 43—*Saponification values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Saponification value
1	1916	DuMez	Alcohol	203.5
2	"	"	Acetone	209.2
3	"	"	Ether	207.4
4	"	"	Petrol, ether	208.6
5	"	"	Alcohol	196.7
6	"	"	Acetone	202.8
7	"	"	Ether	206.9
8	"	"	Benzin	198.7

TABLE 44—*Saponification values of commercial oleoresins.*

Sample No.	Date	Observer	Source	Saponification value
1.....	1916	DuMez.....	Squibb & Sons.....	193.4 [1]
2.....	"	"	Sharp & Dohme.....	196.9
3.....	"	"	Lilly & Co.	198.3

[1] Contained ether.

Iodine value: An iodine value of 122 to 123.9 was obtained for the oleoresins prepared in the laboratory using ether as the extracting menstruum. Results very near the same were obtained when acetone or petroleum ether were the solvents used. whereas, the preparation when made with alcohol gave a lower value, 109.3 to 105.7. The principal cause[1] for the variation in this constant (aside from the effect which the quality of the drug or the solvent may have thereon) as observed in the case of some of the laboratory preparations, as well as the commercial samples, is thought to be the difference in the degree to which the saturated fats (principally palmitin) have been removed. In the case of one of the commercial samples, however, the low iodine value is to be attributed to the presence of unevaporated solvent. The results obtained in the determinations made in the laboratory together with those reported by Kebler for the total ether extract are given in the tables which follow:

TABLE 45.—*Iodine values of laboratory preparations.*

Sample No.	Date	Observer	Solvent	Iodine value
1.....	1913	Kebler[a].....	Ether.....	107.
2.....	"	"	"	123.4
3.....	"	"	"	125.2
4.....	"	"	"	127.3
5.....	"	"	"	132.0
6.....	"	"	"	137.3
7.....	"	"	"	138 0
8-24.....	"	"	"	110.0 to 14-5.7
1.....	1916	DuMez	Alcohol.....	115.7
2.....	"	"	Acetone	125.2
3.....	"	"	Ether.....	122.0
4.....	"	"	Petrol. Ether	123.7
1.....	"	"	Alcohol	109.3
2.....	"	"	Acetone	118.0
3.....	"	"	Ether.....	102.9
4.....	"	"	Benzin.....	116.9

(a) Kebler's results represent the iodine value of the total ether extract.

[1] Lowenstein and Dunn have shown that heating at 110° C. to remove volatile matter from the total ether extract causes a lowering in the iodine value due to absorption of oxygen by the unsaturated fats. Journ. Indust. and Eng. Chem. (1910). 2, p. 48.

TABLE 46.—*Iodine values of commercial oleoresins.*

Sample No.	Date	Observer	Source.	Iodine value
1.....	1916	DuMez	Squibb & Sons	109.2 [1]
2.....	"	"	Sharp & Dohme	116.2
3.....	"	"	Lilly & Co.....	121.7

[1] Contained ether.

Special Quantitative Tests.

Physiological Test.

As the active constituent is present in the oleoresin in such minute quantities that a gravimetric method for its estimation is not practical at the present time, a physiological method would appear to be the best means to employ in the standardization of this preparation. Such a method is reported to be in use for this purpose by the H. K. Mulford Company. Aside, however, from the fact that the test is based on the ability to detect the pungency of the oleoresin in extremely dilute solutions, and that the firm takes as its standard a preparation which is still pungent to the taste in a maximum dilution of 1 to 150,000, there is no exact information available to show in what manner the same is actually carried out. It is thought, however, that a procedure similar to that developed in this laboratory some years ago (1910) is made use of. The following is a description of this method.

Accurately weigh about 1 drop of the oleoresin contained in a small flask, add 5 cubic centimeters of normal potassium hydroxide solution and heat on a water bath for a short time to saponify the fats. Transfer the saponified material to a 100 cubic centimeter flask, using several portions of water for this purpose, and finally dilute up to the mark with more water. With the aid of a pipette, measure off 5 cubic centimeters of this solution and run it into a graduated cylinder (glass stoppered) of 1,000 cubic centimeters capacity. Dilute this with water added in portions of 100 cubic centimeters, tasting the solution after each addition. Note the highest dilution in which the pungent taste is still distinctly perceptible and compare this with the results obtained using a standard preparation.

As all of the samples prepared in this laboratory were found to be distinctly pungent to the taste in dilutions of 1 to 250,000,

it is thought that the standard employed by the H. K. Mulford Company is rather low. In view of these observations, it would appear that a standard of 1 in 200,000 would be more desirable.

Adulterations

A trace of copper was found in most of the commercial samples examined. See under "Ash content."

OLEORESIN OF CUBEB

Synonyms

Aetherisches Cubebenextrakt, Bern. P. 1852.
Aether-szeszes kubeba kivonat, Hung. P. 1888.
Cubeben Extrakt, Nethl. P. 1902.
Estratto di Cubebe, Swiss P. 1907.
Estratto di Pepe Cubebe, Swiss P. 1865.
Estratto di Pepe Cubebe Etereo, Ital. P. 1902.
Ethereal Extract of Cubeb, Am. Journ. Pharm. (1846), 18, p. 167.
Extract van Staartpeper, Nethl. P. 1871.
Extractu de Cubebe, Roum. P. 1874.
Extractum Cubebae Fluidum, U. S. P. 1850.
Extractum Cubebarum, Aust. P. 1906.
Extractum Cubebarum aethereum, Swiss P. 1865.
Extractum Cubebarum aethereo-spirituosum, Hung. P. 1888.
Extractum Cubebarum oleoso-resinosum, Strump, Allg. P. 1861.
Extractum Kubebae oleo-resinosum, Pruss. P. 1829.
Extrait de Cubebe, Fr. P. 1884.
Extrait éthéré de Cubebe, Bern. P. 1852.
Extrait oléo-sésineux Cubebe, Fr. P. 1884.
Fluid Extract of Cubebs, U. S. P. 1850.
Kubebe Extract, Dan. P. 1869.
Kubebenextrakt, G. P. 1872.
Kubeberextrakt, Dan. 1893.
Kubeba Kivonat, Hung. P. 1875.
Oelig-Harziges Kubebenextrakt, Strump, Allg. P. 1861.
Oleo-Resin of Cubebs, B. P. 1885.
Oleo-Resina Cubebae, B. P. 1885.
Oleoresina Cubebae, U. S. P. 1910.
Oléorésine de Cubebe, U. S. Disp. 1907.
Oleoresinous Extract of Cubeb, Pareira, Mat. Med. 1854.

History

The oleoresin of cubeb, prepared by extracting the drug with ether and then removing the latter by distillation, was first

brought to the attention of the European pharmacist by Hausmann in 1838. Ten years previous (1828), however, Dublanc in France and simultaneously Oberdoerffer in Germany had made known a similar preparation obtained by a rather long and tedious process involving the distillation of the drug with steam and subsequent extraction of the marc with alcohol. The latter became official in the Prussian Pharmacopœia of 1829 and in the Pharmacopœia of Schleswig-Holstein in 1846, while the former first received official recognition in the Baden Pharmacopœia of 1841.

Through the efforts of Procter, a preparation similar to that made by Hausmann was introduced into the *United States Pharmacopœia* of 1850 under the title *Extractum Cubebae Fluidum*. In the edition of 1860, this title was changed to *Oleoresina Cubebae*. The preparation official in the United States at present is the oleoresin obtained by extracting the cubeb with alcohol, whereas, that which is given recognition in the late European pharmacopœias is the product obtained by exhausting the drug with a mixture of alcohol and ether.

The pharmacopœias of the countries, states and municipalities in which this preparation has been officially recognized, together with the dates of appearance of the various editions in which it received such recognition, are enumerated below.

Prussian Pharmacopœia — 1829, 1833, 1868.
Pharmacopœia of Baden — 1841.
Pharmacopœia of Schleswig-Holstein — 1844.
Pharmacopœia of Berne — 1852.
Belgian[1] Pharmacopœia — 1854, 1855.
United States Pharmacopœia — 1850, 1860, 1870, 1880, 1890, 1900, 1910.
Pharmacopœia of Hannover — 1861.
Pharmacopœia of Hessen — 1862.
Swiss Pharmacopœia — 1865, 1872, 1893, 1907.
Austrian Pharmacopœia — 1869, 1889, 1906.
Danish[1] Pharmacopœia — 1869, 1893.
Hungarian Pharmacopœia — 1871, 1888, 1909.
Netherlands Pharmacopœia — 1871, 1902.
German Pharmacopœia — 1873, 1882, 1890, 1900, 1910.
Roumanian Pharmacopœia — 1874.
French Pharmacopœia — 1884, 1908.
British[1] Pharmacopœia — 1885.
Italian Pharmacopœia — 1902, 1909.
Japanese Pharmacopœia — 1907.

[1] Not official in the recent editions.

Drug Used, Its Collection, Preservation, Etc.

The drug recognized by the ninth revised edition of the *United States Pharmacopœia* is "the dried, unripe fruits of *Piper Cubeba* Linné filius (Fam. *Piperaceae),* without the presence or admixture of more than 5 per cent of stems or other foreign matter." Other botanical synonyms for the same frequently met with in the literature are: *Cubeba Cubeba* (Linné filius) Lyons; and *Cubeba officinalis* Mique.

The fruit is supposedly gathered when full grown, but before ripe, and is immediately packed for exportation. That some of the fruit for sale on the American market is not collected until after ripening would appear to be the case from the color of some of the oleoresins prepared by the author, a condition which has also been noted by the others.[1] In addition, it should also be noted that the so-called *false cubebs*[2] are sometimes substituted for the official drug.

As cubeb gradually deteriorates with age,[3] and in the powdered condition becomes rapidly weaker owing to the loss of volatile oil, it should be stored whole, in closed containers, and powdered only as it is used.

U. S. P. Text and Comments Thereon.

The oleoresin has been official in the last seven editions of the Pharmacopœia, having been recognized for the first time in the edition of 1850 under the title *Extractum Cubebae Fluidum.*

1850

Extractum Cubebae Fluidum

Fluid Extract of Cubebs

Take of Cubebs,[1] in powder,[2] a pound; Ether[3] a sufficient quantity.

Put the Cubebs into a percolator,[4] and, having packed it carefully, pour Ether gradually upon it until two pints of filtered liquor are obtained;[5] then distill off, by means of a water-bath, at a gentle heat, a pint and a half of the ether,[6] and expose the residue, in a shallow vessel, until the whole of the ether has evaporated.[7]

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[1] Emanuel (1894) stated that when he reported to the jobber that he had obtained a brown colored oleoresin from the cubeb purchased, the latter replied that, while the *United States Pharmacopœia* specified the unripe fruit, this was rarely found on the market.

[2] The botanical origin of this fruit is not known. Culbreth, *Materia Medica and Pharmacology* (1903), p. 138.

[3] The volatile oil, in part, is converted into the so-called cubeb camphor, especially when stored in a damp place. Schmidt, Ber. d. deutsch chem. Ges. (1877), 10, p. 188.

1860

Oleoresina Cubebae

Oleoresin of Cubeb

Extractum Cubebae Fluidum, *Pharm.*, 1850

Take of Cubeb,[1] in fine powder,[2] twelve troyounces;
Ether [3] a sufficient quantity.

Put the Cubeb into a cylindrical percolator,[4] press it moderately, and gradually pour Ether upon it until twenty-four fluid-ounces of filtered liquid have passed.[5] Recover from this, by distillation on a water-bath, eighteen fluid-ounces of ether,[6] and expose the residue, in a capsule, until the remaining ether has evaporated.[7] Lastly keep the oleoresin in a well-stopped bottle.[8]

1870

Oleoresina Cubebae

Oleoresin of Cubeb

Take of Cubeb,[1] in fine powder,[2] twelve troy-ounces;
Ether [3] a sufficient quantity.

Put the Cubeb into a cylindrical percolator, provided with a stop-cock, and arranged with a cover and receptacle suitable for volatile liquids,[4] press it moderately, and gradually pour ether upon it, until twenty-four fluidounces of liquid have slowly passed.[5] Recover the greater part of the ether by distillation on a water-bath,[6] and expose the residue, in a capsule, until the remaining ether has evaporated.[7] When, after standing in a close vessel, the liquid has deposited a waxy and crystalline matter, decant the oleoresin [8] and keep it in a well-stopped bottle.[9]

1880

Oleoresina Cubebae

Oleoresin of Cubeb

Cubeb,[1] in No. 60 powder,[2] *one hundred parts* 100
Stronger Ether,[3] *a sufficient quantity.*

Put the Cubeb into a cylindrical percolator, provided with a cover and receptacle suitable for volatile liquids,[4] press it firmly, and gradually pour stronger ether upon it, until one hundred and fifty (150) parts of liquid have slowly passed.[5] Recover the greater part of the ether by distillation on a water-bath,[6] and expose the residue, in a capsule, until the remaining ether has evaporated.[7] Transfer the remainder to a close vessel, and let it stand until it ceases to deposit a waxy and crystalline matter. Lastly, pour off the oleoresin.[8]

Keep the oleoresin in a well-stopped bottle.[9]

Preparation: Trochisci Cubebae.

1890

Oleoresina Cubebae

Oleoresin of Cubeb

Cubeb,[1] in No. 30 powder,[2] *five hundred grammes* .....500 Gm.
Ether,[3] *a sufficient quantity.*

Put the Cubeb into a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and receptacle suitable for volatile liquids.[4] Press the drug firmly, and percolate slowly with ether, added in successive portions, until the drug is exhausted.[5] Recover the greater part of the ether from the percolate by distillation on a water-bath,[6] and, having transferred the residue to a capsule, allow the remaining ether to evaporate spontaneously.[7]

Keep the product in a well-stoppered bottle.[8]

NOTE. Oleoresin of Cubeb deposits, after standing for some time, a waxy and crystalline matter, which should be rejected, only the liquid portion being used.[8]

Preparation: Trochisci Cubebae.

1900

Oleoresina Cubebae

Oleoresin of Cubeb

Cubeb,[1] in No. 30 powder,[2] *five hundred grammes* .....500 Gm.
Alcohol,[3] *a sufficient quantity.*

Introduce the cubeb into a cylindrical glass percolator,[4] pack the powder firmly, and percolate slowly with alcohol, added in successive portions, until the cubeb is exhausted.[5] Recover the greater part of the alcohol from the percolate by distillation on a water-bath,[6] and, having transferred the residue to a dish, allow the remaining alcohol to evaporate, with constant stirring, in a warm place.[7] Keep the oleoresin in a well-stoppered bottle.[8]

NOTE. Oleoresin of cubeb deposits, after standing for some time, a waxy and crystalline matter, which should be rejected, the liquid portion only being used.[8]

Average dose. — 0.500 Gm. = 500 milligrammes (7½ grains.)

1910

Oleoresina Cubebae
Oleoresin of Cubeb
Oleores. Cubeb

Cubeb,[1] in No. 30 powder,[2] *five hundred grammes* .....500 Gm.
Alcohol,[3] *a sufficient quantity.*

Place the cubeb in a cylindrical glass percolator,[4] pack the powder firmly, and percolate slowly with alcohol, added in successive portions, until the drug is exhausted.[5] Recover the greater part of the alcohol from the percolate by distillation on a water-bath,[6] and, having transferred the residue to a dish, allow the remaining alcohol to evaporate, in a warm place, stirring frequently.[7] Keep the oleoresin in a well stoppered bottle.[8]

NOTE—Oleoresin of Cubeb, after standing for some time, deposits a waxy and crystalline precipitate, which should be rejected, the liquid, portion only being used.[8]

Preparation—Trochisci Cubebae.

Average Dose—Metric, 0.5 Gm.—Apothecaries, 8 grains.

1) For a description of the drug, see page 1040 under "Drug used, its collection, preservation, etc."

2) The last three editions of the Pharmacopœia have specified that the drug used be reduced to a No. 30 powder for percolation. Previous editions, with the exception of that of 1850, directed that a fine powder (No. 60) be used for this purpose. In the Pharmacopœia of 1850, the degree of fineness was not specified. The coarser powder corresponds more nearly in its composition to that of the whole fruit than does the fine powder, owing to the fact that a relatively large amount of volatile oil is lost in the preparation of the latter.

3) Previous to the edition of 1900, the Pharmacopœia specified the use of ether for extracting the drug, whereas, the last two editions have directed that alcohol be employed for this purpose. The fact, that the latter yields a product differing but slightly in its physical properties from the oleoresin obtained with ether, was pointed out by Procter in 1866, and later confirmed by other investigators. Since the alcoholic preparation appears to be equally as efficient from a therapeutic standpoint, as well, the change from ether to alcohol appears to be justified. The use of a menstruum consisting of equal parts of alcohol and ether, as specified in some of the foreign pharmacopœias, the Austrian, German and Japanese,

does not appear to offer any special advantage either from a pharmaceutic or therapeutic standpoint.

4) In the Pharmacopœias of 1870, 1880 and 1890,t he drug was directed to be extracted in a percolator specially adapted to the use of volatile solvents. See Part I under "Apparatus used." With the change in menstruum (ether to alcohol), a special form of percolator was no longer necessary, and the Pharmacopœia now directs that an ordinary cylindrical, glass percolator be used.

5) In the earlier editions of the Pharmacopœia (1850 to 1880 inclusive), it was directed that percolation be discontinued short of the complete exhaustion of the drug, the object evidently having been to economize in the use of the relatively expensive solvent, ether. With the reduction in the price of the latter, however, the economic factor diminished in importance and as a result the Pharmacopœia of 1890 directed that percolation be allowed to proceed until the drug was exhausted. This is also the procedure given in the more recent editions of the Pharmacopœia, in which alcohol has replaced ether as the extracting menstruum.

In this connection, it is desired to point out that, whereas percolation, when ether is the menstruum used, should be continued to complete exhaustion of the drug in order that the extraction of the total amount of therapeutically active constituents may be assured, this procedure does not appear to be necessary when alcohol is the solvent employed. While this statement is not in conformity with the present pharmacopœial directions governing the extraction of the drug and is not supported by direct experimental evidence, it is thought to be justified in view of the difference in the solubility of the therapeutically active resins in the above mentioned menstrua. The indifferent resin is but slightly soluble in ether. It will, therefore, be extracted but slowly by this solvent and will be present in the percolate even to the last portions. Alcohol, on the other hand, dissolves both, the acid and indifferent resins readily. These substances should therefore be contained *in toto* in the first portions of the percolate. In this case, it would therefore appear that the continuation of the process of extraction to the complete exhaustion of the drug

only serves to load the percolate with undesirable extractive matter such as cubebin.

6–7) The various editions of the Pharmacopœia, since 1870, have directed that the greater part of the solvent be removed from the percolate by distillation on a water bath, and that the remainder be allowed to evaporate spontaneously.

Experience in the laboratory has shown that it is impossible to obtain a uniform product, when operating according to the above directions, unless identical conditions are maintained in each case. This is due to the fact that a comparatively slight variation in the procedure, with respect to the quantity of the solvent removed by distillation or to the temperature at which spontaneous evaporation is allowed to proceed produces a variation in the volatile oil content of the finished product, which in turn affects its physical and chemical properties. It is thought, therefore, that the amount of solvent to be removed by distillation, as well as the temperature at which the last portions are to be removed, should be definitely stated by the Pharmacopœia in order that a more uniform product may be obtained.

8) For a statement concerning the nature of the precipitate which forms in the oleoresin upon standing, see page 1060 under "Other properties."

Since the greater part of the precipitate is composed of material which is of no therapeutic value, it should be removed before dispensing the preparation as directed by the Pharmacopœia.

9) The oleoresin should be kept in well stoppered bottles owing to the fact that it loses volatile oil and undergoes other changes on exposure to the air. See cubeb camphor, page 1050.

Yield

The amount of oleoresin obtained varies to a considerable extent, 10 to 30 per cent, having been obtained when alcohol, acetone or ether were employed as menstrua for the extraction of the drug. When petroleum ether is the solvent made use of, the yield is much lower, 4 to 18 per cent. having been reported in this case. Aside from the effect of the solvent, the principal

factors influencing the yield appear to be the variation in the volatile oil content of the drug from which the oleoresin is prepared and the conditions under which the preparation of the latter has been accomplished. As the volatile oil content of the cubeb fruit is stated to vary from 10 to 18 per cent., a variation of even greater magnitude is to be expected in the amount of oleoresin obtained. While this is true when a vacuum pan is employed in the evaporation of the solvent, the difference is not so great when the pharmacopœial directions are followed as the loss in volatile oil in this case is relatively greater when the fruits contain a large amount of this constituent than when only a small amount is present. The difference is still further decreased when the solvent is evaporated on a water bath under ordinary atmospheric pressures. The following tables show the yield of oleoresin obtained with the use of various solvents:

TABLE 47 —*Yield of oleoresin as reported in the literature.*

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per cent	Per cent	Per cent	Per cent.	
1846	Bell	.....	.....	15.0 to 20.0	.....	
1868	Procter	27.00	.....	21.90	Benzin 16.50	
1867	Pile	.....	.....	.....	Benzin 5.00	Yield to benzin, sp. gr. 86° Baumé.
1868	Heydenreich	.....	.....	23.75	.....	
1877	Griffin	.....	.....	.....	Gasolin 16.50	
1887	Kremel	30.00	.....	22.00	.....	
1888	Trimble	.....	.....	21.26	Benzin 16.65	
1892	Beringer	.....	21.75	.....	.....	The cubebs were completely exhausted.
		.....	24.10	.....	.....	
		.....	25.00	.....	.....	
1892	Sherrard	.....	.....	16.40	.....	
		.....	.....	18.80	.....	
		.....	.....	21.06	.....	
		.....	.....	21.90	.....	
		.....	.....	23.00	.....	
		.....	.....	24.70	.....	
		.....	.....	24.80	.....	
		.....	.....	24.80	.....	
1895	Hyers	14.48	18.48	22.45	Petrol. Ether. 13.47	
1907	Blome	.....	.....	.....	Solvent(?) 18.85 to 26.88	Reported as yield of oleoresin. Results obtained in the extraction of 5 samples of cubeb.
1907	Evans Sons, Lescher & Webb	.....	.....	.....	22.08	
		.....	.....	.....	22.60	
		.....	.....	.....	21.13	
		.....	.....	.....	22.80	
1908	Vanderkleed	.....	.....	.....	Solvent(?) 13.69 to 23.60	Reported as yield of oleoresin. Results obtained in the extraction of 4 samples of cubebs.
1909	.....	.....	.....	.....	Solvent (?) 16.49 to 24.34	Reported as yield of oleoresin.
1910	Southall Bros., & Barclay	.....	.....	.....	Petrol Ether 3.88	On subsequent extraction with alcohol 3.40 to 5.66 per cent. of extractive matter was obtained.
		.....	.....	.....	4.30	
		.....	.....	.....	4.45	
		.....	.....	.....	14.00	
		.....	.....	.....	16.03	
		.....	.....	.....	16.54	
		.....	.....	.....	16.90	
		.....	.....	.....	18.08	
1910	Vanderkleed	.....	.....	.....	Solvent(?) 18.42 to 24.40	Reported as yield of oleoresin. Results obtained in the extraction of 6 samples of cubebs.
1911	Vanderkleed	.....	.....	.....	Solvent(?) 22.14	Reported as yield of oleoresin.
1911	Southall Bros. & Barclay	.....	.....	.....	Petrol Ether 4.66 to 8.78	The average yield of 5 samples of cubebs is given as 6.95.

TABLE 47—*Yield of oleoresin as reported in the literature*—Continued.

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per cent	Per cent	Per cent	Per cent.	
1912	Vanderkleed				Solvent(?) 17.36 to 24.49	Reported as yield of oleoresin. Results obtained in the extraction of 5 samples of cubebs.
1913	Dohme & Engelhardt				Solvent(?) 16.00 to 22.00	Reported as yield of oleoresin.
1913	Vanderkleed				Solvent(?) 21.18	
1914	Riedel				Alcohol and ether 11.10 to 14.70	
1914	Rippetoe	8.87 to 11.04		7.68 to 9.80		Results obtained in the extraction of 6 samples of cubebs. Reported as anhydrous extracts.
1914	Scoville				Solvent(?) 18.10 to 22.00	Reported as yield of oleoresin.
1914	Vanderkleed				Solvent(?) 15.90 to 19.80	Reported as yield of oleoresin. Results obtained in the extraction of 6 samples of cubebs.

TABLE 48—*Yield of oleoresin as obtained in the laboratory.*

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per cent	Per cent	Per cent	Per cent.	
1910	DuMez & Netzel.	27.09	26.07	23.47	Benzin 18.75	Represents the yield using a Soxhlet's extraction app., except in the case of alcohol.
1916	DuMez	16.34	16.76.	15.28	Petrol Ether 13.04	Represents the yield using a Soxhlet's extraction app., except in the case of alcohol.

Chemistry of the Drug and Oleoresin.

Tabulation of Constituents.

We are indebted principally to Bernatzik[1] Schmidt[2] and Schulze[3] for definite information concerning the constituents of the cubeb fruit. According to these investigators, the constituents of importance from a pharmaceutical standpoint are as follows: volatile oil, fatty oil, fat, cubebin, cubebic acid, indifferent resin, coloring matter, starch, gum and inorganic substances. Inasmuch as an attempt to determine the composition of the oleoresin does not appear to have been made since the identification of the above enumerated constitutents, a definite statement concerning its exact composition can not be given.[4] However, a knowledge of the physical properties of the constituents of the fruit warrants the statement that the following are present in the oleoresin when prepared with alcohol or ether:

Volatile oil	Cubebin	Coloring matter
Fatty oil	Cubebic acid (Acid resin)	Ash
Fat	Resin (Indifferent resin)	

Occurrence and Description of Individual Constituents

Volatile Oil.[5] The volatile oil of cubeb is a colorless or pale green, thick fluid possessing a burning, spicy, but not a bitter taste. Its specific gravity varies (0.915 to 0.937 at 15°C) depending on the age of the oil after distillation or the length of time that the fruits have been stored before obtaining the oil. It is strongly refractive and is laevogyrate,—39.45° to

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[1] Buchner's n. Repert. f. d. Pharm. (1865), 14, p. 97.

[2] Arch. d. Pharm. (1870), 191, p. 23.

[3] *Ibid.* (1873, 202, p. 388.

The following are among the early investigators who have reported analyses of the fruit: Trommsdorff, Trommsdorff's n. Journ. der Pharm. (1811), 20, p. 69; Vauquelin, Journ. de Chim. Med. (1820), 21, p. 103; Taschenb. f. Scheidekuenst. (1822), p. 185; Monheim, Buchner's Repert. d. Pharm. (1833), 44, p. 199.

[4] Vieth in an article on the relation between the chemical composition and therapeutic activity of various *balsams* states that *Kubebenextrakt* consists of terpenes (25 per cent.) resin acids (10 per cent.) and resins (25 per cent.) Verh. d. Ges. deutsch. Naturf. u. Aerzte (1905), 2, p. 364.

[5] The above description is for the volatile oil obtained from the fruits by steam distillation and corresponds to the properties as observed by Schmidt, Arch. d. Pharm. (1870), 191, p. 18.

—40.16°. Alcohol, ether, carbon disulphide, petroleum ether, chloroform and fatty oils dissolve it readily.

The investigation of the composition of this oil has been undertaken by a number of workers.[6] Oglialoro[7] noted the presence of a l-terpene (pinene or camphene). Wallach[8] isolated dipentene and cadinene. The presence of the latter has been confirmed by others.[9] Cubeb camphor[10] has also been obtained from certain samples of the oil. It is a sesquiterpene hydrate ($C_{15}H_{24}H_2O$) which forms when the fruits are stored in a damp place or when the oil is exposed to a moist atmosphere. It separates out in the form of rhombic octahedrons when the oil is cooled at a low temperature (−12 to −14°C) for some time.

The yield of the oil is stated by Schimmel & Co.[11] to be from 10 to 18 per cent. A yield as low as 0.4 per cent. has been reported.[12] Schmidt obtained 14.215 per cent. from fresh cubebs and 13.041 per cent. from stored cubebs.[13]

Fatty Oil. Schmidt[14] describes the fatty oil as a thick, dark green liquid congealing at 0°C. It is stated to be slowly but completely soluble in cold alcohol, more soluble in hot alcohol, readily soluble in ether, chloroform, carbon disulphide and fatty oils.

The yield as reported by the above investigator is 1.175 per cent. for fresh cubebs and 1.096 per cent. for fruits which have been stored for some time.

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[6] The earliest work on the constituents of the oil is that of Soubeiran and Capitaine, Ann. d. Chem. (1840), 34, p. 31.

[7] Gaz. Chim. Ital. (1875), 5, p. 497.

[8] Ann. d. Chem. (1887), 238, p. 78.

[9] Schaer and Wyss, Arch. d. Pharm. (1875), 206, p. 216; Umney, Pharm. Journ. (1895), 25, p. 951.

[10] Blanchet and Sell, Ann. d. Chem. (1833), 6, p. 294; Winckler, Buchner's Repert. f. d. Pharm. (1833), 45, p. 397; Bernatzik, Buchner's n. Repert. f. d. Pharm. (1865), 14, p. 97; Schmidt, Ber. d. deutsch. chem. Ges. (1877), 10, p. 188.

[11] Schimmel & Co., Ber. (1897), p. 14.

[12] Busse reports the yield of volatile oil as obtained by various investigators as follows:

Baumé	5.3	per cent.
Schoenwald	7.03	per cent.
Oberdoerffer	12.5	per cent.
Hager	0.4	per cent.
Busse	15.	per cent.

Arch. d. Pharm. (1844), 89, p. 30.

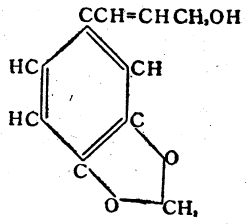
[13] Ibid. (1870), 191, p. 18.

[14] *Ibid.*, p. 34.

Fat. Schmidt[15] obtained 0.511 per cent. of a semi-solid fat from fresh cubebs, 0.408 per cent. from old cubebs. It is stated to be of ointment-like consistence, melting at 30 to 32°C. Hot alcohol, ether, carbon disulphide, chloroform, benzene and petroleum ether dissolve it readily. It is reported to be insoluble in cold alcohol.

Cubebin.[16] Cubebin crystallizes from alcohol in white, odorless needles melting at 125 to 126°C (Schmidt),[17] 132°C (Mameli).[18] The alcoholic solution has a bitter taste. It is only slightly soluble in cold alcohol, quite soluble in hot alcohol, readily soluble in ether, chloroform, carbon disulphide, glacial acetic acid, fatty and volatile oils. The chloroformic solution is laevogyrate. Concentrated sulphuric acid dissolves it with a purple violet color, a reaction which is used as test for the identity of the cubeb fruit and the oleoresin prepared therefrom.

Cubebin was thought by Heldt[19] to be an oxidation product of the sesquiterpene constituent of the volatile oil, $2\ C_{15}H_{24} + 18\ O = C_{30}H_{30}O_9 + 9\ H_2O$. Later work on the determination of its structure, however, has shown this theory to be untenable. The following structural formulas have been brought forward to represent its composition.



(20)

Formula of Pomeranz

[15] *Ibid.*

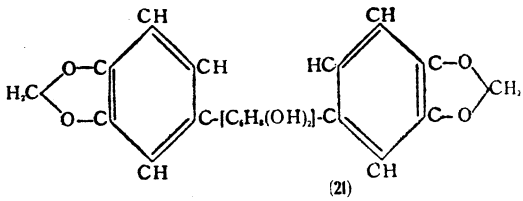
[16] Monheim, Buchner's Repert. f. d. Pharm. (1833), 44, p. 199; Cassola, Journ. d. Chim. Med. (1834), 10, p. 685; Soubeiran and Capitaine, Journ. de Pharm. et de Chim. (1839), 25, p. 355; Ann. d. Chem. (1840), 34, p. 323; Steer, Buchner's Repert. f. d. Pharm. (1838), 11, p. 88; Ibid. (1840), 20, p. 119; Schuck, Buchner's n. Repert. f. d. Pharm. (1852), 1, p. 213; Engelhardt, *Ibid.* (1854), 3, p. 1; Bernatzik, *Ibid.* (1865), 14, p. 97; Schmidt, Arch. d. Pharm. (1870), 191, p. 1; Weidel, Wien. Akad. Ber. (1878), 74, p. 377.

[17] *l. c.*

[18] Chem. Ztg. (1908), 32, p. 46.

[19] Arch. der Pharm. (1870), 191, p. 23.

[20] Monatsch. f. Chem. (1888), 9, p. 323.



(21)

Formula of Mameli

Cubebin occurs in the fruit to the extent of about 2.5 per cent.[22]

Cubebic Acid. (Acid Resin) Cubebic acid, $C_{13}H_{14}O_{71}$ (Schmidt),[23] $C_{28}H_{30}O_7H_2O$ (Schulze),[24] was first described by Bernatzik. It is a white, resinous mass melting at 56°C (Schmidt), 45°C (Schulze) and becoming brown on exposure to the air. It shows only a weak acid reaction. Alcohol, ether, ammonia and the caustic alkalies dissolve it readily.

There is a considerable variation in the cubebic acid content of the fruit as reported in the literature. Schmidt[25] obtained 0.96 per cent. from fresh cubebs and 1.16 per cent. from the fruit which had been stored. Bernatzik reports the presence of 3.458 per cent.[26]

Resin. The so-called indifferent resin, $C_{13}H_{14}O_5$ (Schmidt)[27] is a yellowish-brown, pulverulent mass readily soluble in alcohol and the caustic alkalies, but only slightly soluble in ether, chloroform and carbon disulphide.

The indifferent resin occurs in the fruit to the extent of about 3 per cent. on the average.[28]

Coloring Matter. Schmidt[29] isolated a brown amorphous substance to which he attributes the brown color. This substance is stated to be soluble in dilute alcohol and solutions of the alka-

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[21] *l. c.*

[22] Monheim obtained 4.5 per cent. of a resin resembling piperine which he designated *cubebin.* Buchner's Repert. f. d. Pharm. (1833); 44. p. 199.

Schmidt reports the presence of 2.484 per cent. in fresh cubebs and 2.576 per cent. in cubebs kept in storage for some time. *l. c.*

[23] *l. c.*

[24] Arch. d. Pharm. (1873), 202. p. 388.

[25] *l. c.*

[26] Buchner's n. Repert. f. d. Pharm. (1865), 14, p. 97.

[27] *l. .c*

[28] Schmidt observed the presence of 2.258 per cent .of indifferent resin in the fresh fruits, 2.968 per cent. in stored fruits, *l. c.*

Bernatzik obtained 3.515 per cent. of this resin, *l. c.*

[29] *l. c.*

lies. The green color of the fatty oil as observed by the same investigator is stated to be due to chlorophyll.

Ash. According to E. Schmidt,[30] the ash of the cubeb fruit is composed of the basic elements, K, Ca, Mg, and Fe in combination with the acid radicles Cl′, SO_4'', PO_4''', CO_3'' and SiO_3'', also free SiO_2.

Cubeb fruits yield about 5.5 to 6.0 per cent. of ash.[31]

Constituents of Therapeutic Importance.

The value of the oleoresin of cubeb as a therapeutic agent is very probably due to its resin content. In addition to its diuretic action, the acid resin is said to render the urine feebly antiseptic and to act as an astringent.[1] Cubebin has been shown to be physiologically inactive passing through the intestines unabsorbed.[2] The volatile oil is stated to act merely as a carminative[3] and its presence is even considered by some to be undesirable[4] owing to its irritating action.

Physical Properties

Ash. According to E. Schmidt,[30] the ash of the cubeb fruit is directed by the *United States Pharmacopœia* has a grass-green color when spread out in a thin layer on a white porcelain surface. The commercial product, however, is often brownish-green or brown in color due to the use of the ripe fruit[5] in its manufacture. In such cases, the desired green color is sometimes imparted to the preparation by the addition of copper salts.[6]

Odor: The oleoresin has a strong aromatic odor like that of the crushed cubeb fruit. In fact, the odor is so strongly aromatic that unevaporated solvent (alcohol), even when present in considerable amounts, cannot be detected by the sense of smell.

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[30] Arch. d. Pharm. (1870), 191, p. 11.

[31] Schmidt obtained only 3.36 per cent of ash, *l. c.*

Warnecke reports the yield of ash as 5.45 per cent. Pharm. Ztg. (1886), 31, p. 536.

LaWall and Bradshaw give the ash content of two samples of cubeb as 5.70 and 6.10 per cent.. respectively. Proc. A. Ph. A. (1910), 58, p. 751.

[1] Vieth, Med. Klin. (1905), p. 1276.

[2] Heffter, Arch. f. Exp. Path. u. Pharm. (1895), 35, p. 371.

[3] Heydenreich, Am. Journ. Pharm. (1868), 40, p. 42.

[4] Bernatzik, Buchner's neues Repert. (1865), 14, p. 97.

[5] See under "Drug used, its collection, preservation, etc."

[6] Bédall (1894).

Taste: The taste is bitter and somewhat spicy, like that of cubeb, only more pronounced.

Consistence: The oleoresin is, as a rule, a rather thin liquid when compared with the other members of this class of preparations. Its consistence, however, varies to a considerable extent owing to a difference in the volatile oil content.[1] Some of the preparations examined in the laboratory were so thick that they could only be poured with difficulty.

Solubility: The official preparation forms clear or slightly cloudy solutions with alcohol, acetone, ether, chloroform, carbon disulphide, and glacial acetic acid. It is almost completely soluble in petroleum ether. The solubility of the European product, which is usually prepared with a mixture consisting of equal parts of alcohol and ether, is about the same.

Specific gravity: The oleoresins prepared in the laboratory in 1916 showed a specific gravity of 0.99 + at 25° C regardless of whether the solvent employed in extracting the drug was alcohol, acetone or ether. The uniformity is attributed to the fact that particular pains were taken to evaporate the solvent under the same conditions in each case, thereby insuring approximately the same volatile oil content for each of the finished preparations. The variation in specific gravity due to a difference in volatile oil content is shown in the data given for the first four of the laboratory preparations. The commercial samples examined also show a variation due to this influence, except, in the case of the low specific gravity observed by Procter, which was stated to be due to the presence of unevaporated solvent (ether). Tables illustrating these points follow:

TABLE 49—*Specific gravities of laboratory preparations.*

Sample No.	Date	Observer	Solvent	Specific gravity
				At 76° F
1	1866	Procter	Alcohol	0.985
2	"	"	Ether	0.967
3	"	"	Benzin	0.932
				At 25° C
1	1910	DuMez & Netzel	Alcohol	0.980
2	"	"	Acetone	0.994
3	"	"	Ether	0.985
1	1916	DuMez	Alcohol	1.049 [1]
2	"	"	"	0.994
3	"	"	Acetone	0.999
4	"	"	Ether	0.993
5	"	"	Petrol. ether	0.963

[1] A thick preparation containing only 4.71 per cent. of volatile matter.

[1] See under "Chemistry of the drug and the oleoresin".

TABLE 50—*Specific gravities of commercial oleoresins.*

Sample No.	Date	Observer	Source	Specific gravity
				At 76° F.
1....	1866	Procter.....	Not given.....	0.900 (1)
				At 25° C.
1.....	1916	DuMez	Lilly & Co.....	0.968
2.....	"	"	Squibb & Sons.....	0.969
3.....	"	"	Parke, Davis & Co.....	0.971
4.....	"	"	Sharp & Dohme.....	0.975
5.....	"	"	Stearns & Co.....	1.017

[1] Contained ether.

Refractive index: The results obtained in the laboratory indicate that the refractive index of the oleoresin should be about 1.499 when determined at 25°C. The solvent employed in extracting the drug appears to have little influence on this constant, except in case petroleum ether is used, when it is slightly lower. The effect due to variation in volatile oil content is but slight as is shown in the tables which follow:

TABLE 51.—*Refractive indices of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Refractive index
				At 25° C
1.....	1910	DuMez & Netzel.....	Alcohol	1.495
2.....	"	" "	Acetone.....	1.499
3.....	"	"	Ether	1.499
1.....	1916	DuMez	Alcohol.....	1.502 (1)
2.....	"	"	Alcohol.....	1.500
3.....	"	"	Acetone	1.500
4.....	"	"	Ether.....	1.499
5.....	"	"	Petrol ether.....	1.495

(1) Low in volatile oil content.

TABLE 52—*Refractive indices of commercial oleoresins.*

Sample No.	Date	Observer	Source	Refractive index
				At 25° C
1.....	1916	DuMez	Lilly & Co.....	1.498
2.....	"	"	Squibb & Sons.....	1.499
3.....	"	"	Parke. Davis & Co.....	1.499
4.....	"	"	Sharp & Dohme.....	1.499
5.....	"	"	Stearns & Co.....	1.501

Chemical Properties.

Loss in weight on heating: An examination of the tables which follow shows that the oleoresin usually loses between 20 and 40 per cent. on heating at 100 to 110°C, the variation being due to the difference in the volatile oil content. The relatively small loss in weight observed in the case of four of the laboratory preparations is to be attributed to the removal of a part, or the whole, of the more volatile constituents of the essential oil in the process of evaporating the solvent. The comparatively great loss noted for two of the commercial samples is thought to have been due to the presence of unevaporated solvent. The results obtained in the determinations made in the laboratory as well as those reported in the literature are given in the tables which follow:

TABLE 53.—*Laboratory preparations—loss in weight on heating.*

Sample No.	Date	Observer	Solvent	Per cent of loss on drying
				At 100° C
.....	1887	Kremel	Alcohol	20.40
				At 110° C
1.....	1916	DuMez	Alcohol	23.06
2.....	"	"	Acetone	24.10
3.....	"	"	Ether	25.88
4.....	"	"	Petrol. ether	25.24
5.....	"	"	Alcohol	11.99
6.....	"	"	Acetone	9.96
7.....	"	"	Ether	8.31
8.....	"	"	Alcohol	4.71

TABLE 54—*Commercial oleoresins—loss in weight on heating.*

Sample No.	Date	Observer	Source	Per cent of loss on drying
				At 100° C
1.....	1893	Dieterich	Germany	32.70
1.....	1894	"	"	31.02
1.....	1895	"	"	20.90
1.....	1905	"	"	55.91 [1]
				At 110° C
1.....	1916	DuMez	Sharp & Dohme	30.72
2.....	"	"	Stearns & Co	31.68
3.....	"	"	Parke, Davis & Co	37.03
4.....	"	"	Lilly & Co	44.21 [1]
5.....	"	"	Squibb & Sons	61.96 [1]

[1] Probably contained unevaporated solvent (alcohol).

Ash content: The ash content of the oleoresin varies with the solvent employed in its preparation as is shown in the first of the tables which follow. The highest values were obtained for the official product, in the preparation of which alcohol was the solvent used. The camparatively low ash content obtained for the commercial samples examined, while suggesting the use of some other solvent in the manufacture of these preparations, is thought to have been due to the greater amount of volatile matter (essential oil) present. Although copper was detected in the ash of all of the commercial products, the quantities present were too small to effect the value of this constant to any considerable extent. The following tables give the ash content of the oleoresin as reported in the literature and as determined in the laboratory:

TABLE 55.—*Ash contents of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Per cent of ash
1.....	1916	DuMez	Alcohol	0.45
2.....	"	"	Acetone	0.20
3.....	"	"	Ether	0.13
4.....	"	"	Petrol, ether.....	0.07
5.....	"	"	Alcohol	0.48
6.....	"	"	Acetone	0.22
7.....	"	"	Ether	0.15
8.....	"	"	Alcohol	0.51

TABLE 56—*Ash contents of commercial oleoresins.*

Sample No.	Date	Observer	Source	Per cent. of ash	Foreign constituents
1.....	1893	Dieterich ...	Germany	0.50	
1.....	1894	"	"	0.52	
1.....	1895	"	"	0.47	
1.....	1897	"	"	0.10	
1.....	1905	"	"	0.87	
1.....	1916	DuMez	Squibb & Sons.....	0.21 [1]	Copper
2.....	"	"	Sharpe & Dohme	0.40	"
3.....	"	"	Parke, Davis & Co.....	0.35	"
4.....	"	"	Lilly & Co.....	0.29 [1]	"
5.....	"	"	Stearns & Co.....	0.37	"

[1] Unevaporated solvent (alcohol) probably present.

Acid number: The acid numbers of the oleoresins prepared in the laboratory varied from 21.8 to 26.7, depending on the nature of the solvent employed in their preparation. The num-

ber, 26.7, obtained in the case of the preparation made with alcohol agrees very well with that (26.2) obtained by Kremel for the oleoresin when prepared in a like manner. The low acid numbers obtained for the commercial samples are explained by the presence of relatively large amounts of volatile matter (generally essential oil, but unevaporated solvent in two cases) in these preparations, which has the effect of reducing the concentration of the free acids. The values obtained for this constant follow:

TABLE 57.—*Acid numbers of laboratory preparations.*

Sample No.	Date	Observer	Solvent	Acid number
1	1887	Kremel	Alcohol	26.2
2	"	"	Ether	31.2
1	1916	DuMez	Alcohol	26.7
2	"	"	Acetone	22.8
3	"	"	Ether	22.2
4	"	"	Petrol. ether	21.8

TABLE 58.—*Acid numbers of commercial oleoresins.*

Sample No.	Date	Observer	Source	Acid number
1	1916	DuMez	Lilly & Co.	12.8 (1)
2	"	"	Squibb & Sons	13.4 (1)
3	"	"	Stearns & Co.	14.4
4	"	"	Parke, Davis & Co.	15.4
5	"	"	Sharp & Dohme	18.7

(1) Probably contained unevaporated solvent (alcohol).

Saponification value: The saponification values obtained for the oleoresins prepared in the laboratory showed a slight variation due to the nature of the solvent used in extracting the drug as is shown in the first of the tables which follow. As a rule, however, the difference in the volatile oil content of the oleoresin, due to a variation in the conditions under which it has been prepared, is thought to be the principal factor influencing the value of this constant, as is also brought out in the first table. In the examination of commercial samples, the presence of unevaporated solvent must be taken into considera-

tion in this connection. The results obtained in the determination of this constant in the laboratory follow:

TABLE 59—*Saponification values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Saponification value
1	1916	DuMez	Alcohol	65.9
2	"	"	Acetone	63.7
3	"	"	Ether	63.4
4	"	"	Petrol, ether	67.0
1	"	"	Alcohol	63.9
2	"	"	Acetone	57.9
3	"	"	Ether	59.5
1	"	"	Alcohol	105.9 (1)

[1] This preparation contained a relatively small amount of volatile matter (principally essential oil). See page 1056 under "Loss in Weight on Drying".

TABLE 60.—*Saponification values of commercial oleoresins.*

Sample No.	Date	Observer	Source	Saponification value
1	1916	DuMez	Lilly & Co	48.5 (1)
2	"	"	Parke, Davis & Co	53.3
3	"	"	Squibb & Sons	49.3 (1)
4	"	"	Sharp & Dohme	55.0
5	"	"	Stearns & Co	65.9

(1) Unevaporated solvent (alcohol) probably present.

Iodine value: Further observations are necessary before a definite statement can be made as to what the iodine value of this preparation should be. Determinations made in the laboratory appear to indicate that it is influenced largely by the volatile oil content as those preparations which lost the greatest amount on drying usually gave the highest values for this constant. Apparent exceptions to this rule are to be found in the samples obtained from Lilly & Company and Squibb & Sons, respectively. In these cases, unevaporated solvent (alcohol) is thought to have been present, although, it could not be detected by the odor. The following tables show the values obtained for the preparations examined in the laboratory.

TABLE 61—*Iodine values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Iodine value
1.....	1916	DuMez.....	Alcohol.....	126.0
2.....	"	"	Acetone	131.6
3.....	"	"	Ether.....	138.5
4.....	"	"	Petrol, ether	141.8
1	"	"	Alcohol.....	130.0
2.....	"	"	Acetone	113.2
3.....	"	"	Ether.....	115.6
1.....	"	"	Alcohol.....	92.0

TABLE 62—*Iodine values of commercial oleoresins.*

Sample No.	Date	Observer	Source	Iodine value
1.....	1916	DuMez.....	Squibb & Sons.....	130.6[1]
2.....	"	"	Lilly & Co.....	136.7[1]
3.....	"	"	Parke, Davis & Co...	146.9
4..... ...	"	"	Sharp & Dohme.....	147.3
5.....	"	"	Stearns & Co.....	147.6

[1] Unevaporated solvent probably present.

Other Properties.

The oleoresin, upon long standing, forms a white deposit consisting of cubebin, indifferent resin, cubebic acid and thickened oil. As the greater part (80 per cent.)[1] of this precipitated material consists of the therapeutically inert cubebin,[2] the *United States Pharmacopœia* directs that it be removed before dispensing the preparation.

Special Qualitative Tests.

The methods which have been devised for the indentification of this oleoresin or as a test for its quality are based on the fact that characteristic color changes are produced when it is acted upon by certain acids. Sulphuric, sulphomolybdic[3] and

[1] Schmidt (1870).

[2] See under "Constituents of therapeutic importance".

[3] Dieterich, in 1897, pointed out that sulphomolybdic acid might be used in place of sulphuric acid. The resulting color, however, was stated to be a cherry-red instead of a blood-red.

hydrochloric[1] acids have been made use of in this connection, the first mentioned being the reagent most generally employed.

Attention was first called to the value of sulphuric acid in the identification of this preparation by Kremel in 1887. He, however, reported nothing definite, merely stating that a carmine-red color was produced when the "strong" acid and oleoresin were mixed. It was not until ten years later (1897), when the firm of Dieterich in Helfenberg published their method of procedure, that this test assumed a definite form. The test as carried out by this firm is typical of those in use at the present time and is as follows:

> Upon mixing 0.01 gram of the oleoresin with 3 to 5 drops of concentrated sulphuric acid, the mixture should assume an intense blood-red color.[2]

The fact that certain constituents of the cubeb fruit, namely, cubebin, the acid resin (cubebic acid) and the indifferent resin, formed red colored mixtures with sulphuric acid was noted by Schmidt in 1870. These observations have been confirmed in this laboratory in so far as they pertain to the production of a red color. It was further noted, however, that the shade of red varies with the particular constituent under consideration, the cubebin giving rise to a mixture which is brownish-red in color, whereas, the color is bright red (carmine-red) in the case of the acid or indifferent resin. As all of the above mentioned constituents are normally present in the oleoresin, the particular shade of red (blood-red) obtained in this test must be due to the blending of the colors produced by the action of the acid on the several constituents, and cannot be caused by the action of the acid on the cubebin, alone, as is usually reported in the literature.

As the shade of red obtained will naturally vary with the relative quantities of the several constituents present, this test not only serves as a means of identification, but is also of value in determining roughly the quality of the preparation as well.[3] Thus, a bright red color obtained by the action of the acid may

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[1] Test of Gluecksmann. See the following pages.

[2] The so-called false cubebs give a dirty brown color when triturated with concentrated sulphuric acid, hence, we may expect the oleoresin prepared therefrom to form a mixture of a similar color. See Pharm Ztg. (1912), 84, p. 845.

[3] Bédall (1894) observed that the oleoresins possessing a green color gave a more intense red with sulphuric acid that those which were brown in color.

be taken as an indication of the presence of relatively large amounts of the therapeutically active resins, while a dark shade of red implies that the cubebin content is exceptionally large or that the resins are present in comparatively small amounts.

The test of Gluecksmann (1912) in which hydrochloric acid is the reagent made use of, appears to be based on the presence of cubebin.[1] It is carried out as follows:

> Dissolve a small quantity (a trace) of the oleoresin in concentrated acetic acid and dilute with the latter until the solution shows scarcely any color. Heat to boiling and add 5 drops of a 35 per cent. solution of hydrochloric acid to a 5 cubic centimeter portion. A faint yellowish-brown color should appear immediately. Upon standing quietly, the color should change in 2 to 4 hours to a brownish-violet, and then to a violet blue, after which it should gradually disappear.

While the foregoing may prove to be a test of considerable worth in the identification of the oleoresin, the length of time required for its completion would appear to be a drawback to its general application.

The tests of this nature prescribed by the various pharmacopœias all involve the use of sulphuric acid. As will become apparent in the following description of these methods, the color specified differs to a considerable extent. This may be due, as already pointed out, to a variation in the relative quantities of the reacting constituents, or, as has been further observed in the laboratory, to the strength of the acid employed. A very slight dilution with water will cause the color to change from red to purple. The following are the tests prescribed by the different pharmacopoeias:

Austrian Pharmacopoeia (1906): The oleoresin should give a red color on being triturated with concentrated sulphuric acid.

French Pharmacopoeia (1908): The oleoresin should give a purple-red color with concentrated sulphuric acid.

Swiss Pharmacopoeia (1907): If 0.01 to 0.02 grams of the oleoresin are mixed with a few drops of concentrated sulphuric acid, an intense brownish-red color should be produced. Upon diluting with a little water, the color should change to a rose and upon further dilution, it should disappear.

Hungarian Pharmacopoeia (1909): A drop of concentrated sulphuric

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[1] This assumption is made in view of the fact that the closely related compounds, coniferyl alcohol and syringenin, give similar color reactions with hydrochloric acid. See Euler, *Die Pflanzenchemie* (1908), Vol. I, p. 87.

acid added to a drop of the oleoresin spread out in a thin layer on a white porcelain surface should produce a blood-red mixture.

German Pharmacopoeia (1910): If 1 cubic centimeter of a mixture of 4 parts of concentrated sulphuric acid and 1 part of water is poured over 1 drop of the oleoresin, a red color should be produced. Upon diluting the mixture with water the color should disappear.

Special Quantitative Tests.

Apparently but one attempt has been made to develope a method for the quantitative determination of the constituents of therapeutic importance in this preparation, the same having been made by Kremel in 1887. As no work of this nature was done on the oleoresin in the laboratory, and, as there is no further information on this subject in the literature, a statement cannot be made as to the value of this method. However, as a suggestion of what might be accomplished in this direction, a description of the method is included here. It is as follows:

Kremel's Method for the Estimation of Cubebic Acid (1887): Dissolve 3 to 5 grams of the oleoresin in 4 times the quantity of alcohol (90 per cent.), filter the solution and add alternately to the filtrate an alcoholic solution of calcium chloride and ammonia water until a distinct cloudiness appears. Set the liquid aside for a day or two to allow the calcium salt of cubebic acid to crystallize. Then, collect the precipitate on a filter, wash successively with alcohol (90 per cent.) and ether, dry at 100°C and weigh. Compute the weight of the cubebic acid using the formula, $C_{13}H_{12}O_7Ca$, for the calcium salt.

According to the results obtained by Kremel, the oleoresin prepared with ether shsowed a cubebic acid content of 2.35 per cent., while the same when prepared with alcohol gave 5.75 per cent. of cubebic acid.

Adulterations.

Willful adulteration of this preparation does not appear to be practiced very extensively, although, the occassional use of fixed oils[1] or salts of copper[2] for this purpose has been reported

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[1] Schneider and Suess, *Handkommentar zum Arzneibuch fuer das deutsche Reich* (1902), p. 376.

[2] Bédall (1894).

A trace of copper is usually present in the commercial preparations as a result of the use of copper utensils in their manufacture. (See under "Ash".)

in the literature. On the other hand, accidental adulteration effected through the use of ripe instead of unripe fruits in the preparation of the oleoresin is thought to be quite general. (See under "Drug used, its collection, preservation, etc.")

OLEORESIN OF GINGER

Synonyms

Aetherisches Ingwerextrakt, Nat. Stand. Disp. 1884.
Ethereal Extract of Ginger, King's Am. Disp., (1900), p. 1336.
Extractum Zingiberis aethereum, Hirsh, Univ. P. 1902, No. 1320.
Extractum Zingiberis aethereum, King's Am. Disp. (1900), P. 1336.
Gingerin, Chem. and Drugg. (1913), 82, p. 470.
Gingerine, Am. Journ. Pharm. (1898), 70 p. 466.
Oleoresina Zingiberis, U. S. P. 1910.
Oléorésine de Gingembre, U. S. Disp. 1907.
Piperoide du Gingembre, Béral, 1834.
Piperoid of Ginger, U. S. Disp. 1865.
Zingiberin, U. S. Disp. 1907.

History

The oleoresin of ginger was prepared in 1834 by Béral, a Frenchman, but was apparently first brought to the notice of American pharmacists by Proctor in 1849. It was introduced into the *United States Pharmacopœia* in 1860 and is still official at the present time. While the oleoresin has never been officially recognized abroad, a similar preparation is said to be used extensively in England under the name of gingerin.[1]

Drug Used, Its Collection, Preservation, Etc.

For this drug, the present pharmacopœial definition is as follows: "The dried rhizomes of *Zingiber officinale* Roscoe (Fam. *Zingiberaceae,)* the outer cortical layers of which are often either partially or completely removed. Preserve it in tightly-closed containers, adding a few drops of chloroform or carbon tetrachloride, from time to time, to prevent attacks by insects." The official drug has also been described in the literature under the following botanical synonyms: *Amomum Zingiber* Linné, and *Zingiber Zingiber* (Linné) Rusby.

[1] Gingerin is stated to be the extract obtained upon evaporating off the alcohol from the tincture of ginger. Chem. & Drugg. (1913), 82, p. 470.

The rhizomes as they are found on the market occur in a variety of forms characteristic of the source from which they are obtained. In view of this fact, the Pharmacopœia recognizes six different commercial varieties, namely: Jamaica ginger, African ginger, Calcutta ginger, Calicut ginger, Cochin ginger and Japanese ginger. These commercial forms differ to a considerable extent, not only through natural causes, but also through a difference in the conditions under which they are harvested and prepared for the market.

As a rule the rhizomes are dug after the stems have withered, January or February, when one or more years old. Experience has shown the oleoresin content to be the greatest at this period of the year.[1] They are then washed in boiling water to prevent germination, dried rapidly in the sun, and as such constitute, what is known as black, coated, or unscraped ginger. In other cases, after treatment with boiling water, a part or the whole of the epidermis is removed, the rhizomes dried, and bleached with sulphur fumes, chlorinated lime, milk of lime or gypsum. This constitutes the so-called, white, uncoated, scraped, race or hard ginger.[2]

In commenting on the relative values of these various forms of ginger in the preparation of the oleoresin, it should be stated, first of all, that the yield of oleoresin is influenced to the largest extent by habitat, African ginger giving the maximum yield.[3] Secondly, the extent to which the rhizomes have been decorticated is an important factor, as the outer corky layer contains none of the oleoresinous material. These factors will be more fully discussed under yield. To what degree, if at all, the process of so-called bleaching effects the yield or quality of oleoresin does not become apparent from the literature. It is thought, however, that a heavy coating of gypsum, for instance, would tend to considerably reduce the percentage of oleoresin obtainable.

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[1] Hooper, Pharm. Journ. (1912), 89, p. 391.
[2] Culbreth, *Mat. Med. and Pharmacol.* (1917), p. 130.
[3] See reference under "Yield of oleoresin".

U. S. P. Text and Comments Thereon.

The oleoresin of ginger first became official in the Pharmacopoeia of 1860. It has remained official throughout all of the subsequent editions.

1860

Oleoresina Zingiberis

Oleoresin of Ginger

Take of ginger,[1] in fine powder,[2] twelve troyounces;
Stronger Ether[3] twelve fluidounces;
Alcohol[4] a sufficient quantity.

Put the ginger into a cylindrical percolator,[5] press it firmly, and pour upon it the stronger ether.[6] When this has been absorbed by the powder, add alcohol until twelve fluidounces[7] of filtered liquid have passed. Recover from this, by distillation on a water-bath, nine fluidounces of ether,[8] and expose the residue, in a capsule, until the volatile part has evaporated.[9] Lastly keep the oleoresin in a well-stopped bottle.[10]

1870

Oleoresina Zingiberis

Oleoresin of Ginger

Take of ginger,[1] in fine powder,[2] twelve troyounces;
Stronger Ether[3] twelve fluidounces;
Alcohol[4] a sufficient quantity.

Put the ginger into a cylindrical percolator, provided with a stop-cock, and arranged with a cover and receptacle suitable for volatile liquids,[5] press it firmly, and pour upon it the stronger ether.[6] When this has been absorbed by the powder, add alcohol until twelve fluidounces of liquid have slowly passed.[7] Recover from this the greater part of the ether by distillation on a water-bath,[8] and expose the residue, in a capsule, until the volatile part has evaporated.[9] Lastly, keep the oleoresin in a well-stopped bottle.[10]

1880

Oleoresina Zingiberis

Oleoresin of Ginger

Ginger,[1] in No. 60 powder,[2] *one hundred (100) parts* 100
Stronger Ether,[3] *a sufficient quantity.*

Put the ginger into a cylindrical percolator, provided with a cover and receptacle suitable for volatile liquids,[5] press it firmly, and gradually pour stronger ether upon it, until one hundred and fifty (150) parts of the liquid have slowly passed, or until the Ginger is exhausted.[7] Recover the greater part of the ether by distillation on a water-bath,[8] and expose the residue, in a capsule, until the remaining ether has evaporated.[9]

Keep the oleoresin in a well-stopped bottle.[10]

1890

Oleoresina Zingiberis
Oleoresin of Ginger

Ginger,[1] in No. 60 powder,[2] *five hundred grammes* .....500 Gm.
Ether,[3] *a sufficient quantity.*

Put the ginger into a cylindrical glass percolator, provided with a stop-cock, and arranged with cover and receptacle suitable for volatile liquids.[5] Press the drug firmly, and percolate slowly with ether, added in successive portions, until the drug is exhausted.[7] Recover the greater part of the ether from the percolate by distillation on a water-bath,[8] and, having transferred the residue to a capsule, allow the remaining ether to evaporate spontaneously.[9]

Keep the oleoresin in a well-stoppered bottle.[10]

1900

Oleoresina Zingiberis
Oleoresin of Ginger

Ginger,[1] in No. 60 powder,[2] *five hundred grammes* .....500 Gm.
Acetone,[3] *a sufficient quantity.*

Introduce the ginger into a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and a receptacle suitable for volatile liquids.[5] Pack the powder firmly, and percolate slowly with acetone, added in successive portions, until the ginger is exhausted.[7] Recover the greater part of the acetone from the percolate by distillation on a water-bath,[8] and, having transferred the residue to a dish, allow the remaining acetone to evaporate spontaneously in a warm place.[9] Keep the oleoresin in a well-stoppered bottle.[10]

Average dose.—0.030 Gm. = 30 milligrammes (½ grain.)

1910

Oleoresina Zingiberis
Oleoresin of Ginger
Oleores. Zingib.

Ginger,[1] in No. 60 powder,[2] *five hundred grammes* .....500 Gm.
Ether,[3] *a sufficient quantity.*

Place the ginger in a cylindrical glass percolator, provided with a stop-cock and arranged with cover and a receptacle suitable for volatile liquids.[5] Pack the powder firmly, and percolate slowly with ether, added in successive portions, until the drug is exhausted.[7] Recover the greater part of the ether from the percolate by distillation, on a water-bath,[8] and, having transferred the residue to a dish, allow the remaining ether to evaporate spontaneously in a warm place.[9] Keep the oleoresin in a well-stopped bottle.[10]

Average dose.—Metric, 0.03 Gm.—Apothecaries, ½ grain.

1) For a description of the different commercial varieties of the official drug, see page 1065 under "Drug used, its collection, preservation, etc."

2) As starch, in the shape of fine granules, constitutes about 20 per cent. of the ginger rhizome, the latter can only be obtained in the form of a uniformly fine powder by reducing the other tissues to a corresponding degree of fineness. It is for this reason and for the purpose of insuring the complete breaking up of all of the small resin cells that the Pharmacopœia directs that the drug be reduced to a No. 60 powder.

3–4) Ether is the solvent which appears to be best adapted to the preparation of this oleoresin in that it completely extracts the pungent principles from the drug and yields a product containing a minimum amount of undesirable extractive matter. According to Garnett and Grier (1909) acetone, which was directed to be used by the Pharmacopœia of 1900, does not completely exhaust ginger, even when a Soxlet's apparatus is used. It is, therefore, fortunate that the present Pharmacopœia again specifies that ether be used for this purpose.

In the earlier editions of the Pharmacopœia (editions of 1860 and 1870), alcohol was directed to be used as a "follow up" solvent to replace the ether with which percolation was begun. This procedure was abandoned in 1880 for reasons which will be discussed later.

5) Since 1870, the Pharmacopœia has directed that percolation be carried out in a special form of percolater adapted to the use of volatile liquids. For a description of such forms, see Part I under "Apparatus used."

6–7) The method of extracting the drug as outlined in the earlier editions of the Pharmacopœia, the editions of 1860 and 1870, was essentially the same as suggested by Béral in 1834. See Part I, page 929. From a practical standpoint, this method possessed distinct advantages, especially at the time when it was adopted, in that a considerable saving in the cost of the preparation of the oleoresin was effected through the use of alcohol as a "follow up" solvent for replacing the relatively expensive ether. The method, however, was not entirely satisfactory as the finished product contained a considerable amount of undesirable extractive matter owing to the greater solvent properties of the alcohol. Another disadvantage lay

in the fact that a relatively large amount of volatile oil was lost in the removal of the solvent.

The present edition of the Pharmacopœia directs that the drug be completely exhausted by simple percolation with ether. Here, as in the case of the oleoresin of capsicum, the extraction of the drug with the aid of some form of continuous extraction apparatus would effect a considerable saving in solvent and without injury to the finished product.

8–9) With respect to the removal of the solvent from the percolate, the present edition of the Pharmacopœia directs that this be accomplished in greater part by distillation on a water bath and that the remainder be allowed to evaporate spontaneously in a warm place, a procedure similar to that described in the earlier editions. For reasons, identical with those given in the comments on the oleoresin of cubeb (see page 1045), it is thought that the pharmacopœial directions should include specific statements with reference to the amount of solvent to be recovered by distillation and the temperature at which the remainder is to be removed in order to insure greater uniformity in the product obtained.

10) Upon exposure to the air, a portion of the volatile oil contained in the oleoresin is altered (resinified) or lost through evaporation. The preparation should, therefore, be kept in well-stoppered bottles.

Yield

With respect to the solvents, alcohol (95 per cent.), acetone and ether, the yield of oleoresin, in the case of ginger, varies in magnitude in the order in which the solvents are mentioned. For these menstrua, a minimum yield of 2.57 per cent has been reported while the maximum yield has been stated to be as high as 11.1 per cent. When petroleum ether is the solvent used, the yield is much lower, being only about one-half that obtained in the preceding cases. In this connection, the source of the rhizomes is a factor of first importance. Thus, it has been found that Jamaica ginger usually gives the smallest yield and African ginger the highest, while Cochin ginger occupies an intermediate position in this respect. These facts will be brought out more clearly in the tables which follow.

The yield of oleoresin is further influenced by the degree to which the rhizomes have been deprived of the outer corky layer, and, in the case of bleaching, to the manner in which the latter was accomplished. With respect to this statement, the yield, in the case of the unbleached ginger, will be the greatest when decortication is complete. When the rhizomes have been bleached, in addition to being partially or wholly decorticated, the influence of the latter, may be diminished, in part at least, by the process employed in accomplishing the former. Thus, if gypsum or lime have been used for this purpose, the weight of the insoluble material in the rhizomes will be considerably increased, which will have the effect of reducing the percentage yield of oleoresin. These points are also brought out in the tables which follow.

TABLE 63.—*Yield of oleoresin as reported in the literature.*

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per cent.	
1834	Béral	.....	.....	5.20	.....	
1879	Thresh	.....	.....	3.29	.....	Jamaica ginger.
		.....	.....	4.96	.....	Cochin "
		.....	.....	8.06	.....	African "
1886	Jones	3.38	.....	3.58	.....	
1888	Siggnis	[Alcohol (sp. gr. 0.82)] 5.00	.....	.....	.....	Jamaica ginger, unbleached.
		4.80	.....	.....	.....	" " , bleached (limed)
		6.65	.....	.....	.....	East Indian ginger.
		6 57	.....	.....	.....	" " "
		6.17	.....	.....	.....	African ginger.
		7.00	.....	.....	.....	" "
1888	Trimble	.....	.....	3.97	Benzin 2.48	
1891	Riegel	5.00	.....	.....	Benzin 2.50	Jamaica ginger, unbleached.
		8.00	.....	8.00	.....	East Indian ginger, epidermis removed.
1892	Sherrard	.....	.....	3.85	.....	
		.....	.....	4.72	.....	
		.....	.....	5.20	.....	
		.....	.....	5.40	.....	
1892	Beringer	.....	5.57	.....	.....	
1893	Dyer and Gilbard	.....	.....	3.00 to 5.20	.....	Upon subsequent extraction with alcohol 0.80 to 1.50 per cent. of material was obtained.
1895	Davis	.....	.....	4.30 to 4.84	.....	Jamaica ginger.
		.....	.....	5.75 to 6.27	.....	African "
1896	Liverseege	.....	.....	5.50	Methyl alcohol 6.50	
1897	Glass and Thresh	.....	.....	5.00	.....	Jamaica ginger.
		.....	.....	4.33	.....	Cochin "
		.....	.....	6.33	.....	African "
1901	Bennet	[Alcohol (90 per cent.)] 3.94to 5.61	.....	2.57 to 6.41	.....	Jamaica ginger, whole.
		3.41to 5.67	.....	2.97 to 4.60	.....	Jamaica ginger, ground.
		4.91to 6.74	.....	.....	.....	Cochin ginger, whole.
		5.41to 6.51	.....	.....	.....	Cochin ginger, ground.
		5.14to 6.61	.....	.....	.....	African ginger, whole.
		5.14to 6.47	.....	.....	.....	African ginger, ground.
1903	Ballard	.....	.....	3.75	.....	Tahiti ginger.
		.....	.....	6.33	.....	Ivory Coast ginger.
1903	Southall Bros. & Barclay	[Alcohol (90 per cent)] 4.35		[Eth'r (Sp. gr. 0.717)] 4.76	.....	Jamaica ginger.
		4.57		6.04	.....	Cochin "
		9.93		11.09	.....	African "

TABLE 63.—*Yield of oleoresin as reported in the literature*—Continued.

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per cent	
1908	Vanderkleed	.....	.....	.....	Solvent(?) 5.58 9.55	Reported as yield of oleoresin.
1909	Vanderkleed	.....	.....	.....	Solvent(?) 3.14 to 6.91	Represents the yield from 16 samples of Jamaica ginger. Reported as oleoresin.
1909	Vanderkleed	.....	.....	.....	8.20	African ginger.
	Vanderkleed	.....	.....	.....	9.03	" "
1909	Patch	3.70 to 6.20	.....	.....	.....	
1910	Vanderkleed	.....	.....	.....	Solvent(?) 5.63	Jamaica ginger. Reported as yield of oleoresin.
		.....	.....	.....	6.31	
		.....	.....	.....	10.12	African ginger. Reported as yield of oleoresin.
1911	Vanderkleed	.....	.....	.....	3.40 to 6.60	Jamaica ginger. Reported as yield of oleoresin.
		.....	.....	.....	7.12 to 9.48	African ginger. Reported as yield of oleoresin.
1912	Vanderkleed	.....	.....	.....	3.44 to 6.64	Jamaica ginger. Reported as yield of oleoresin.
		.....	.....	.....	6.85 to 11.10	African ginger. Reported as yield of oleoresin.
1912	Patch	3.30 to 6.00	.....	.....	.....	Jamaica ginger.
1912	Hooper	6.40	.....	.....	.....	Young rhizomes harvested in December.
		8.30	.....	.....	.....	Rhizomes harvested in February.
1913	Patch	4.23	.....	.....	.....	Average yield of 9 samples of ginger.
1913	Vanderkleed	.....	.....	.....	Solvent(?) 3.10 to 5.75	Reported as yield of oleoresin. Results obtained in extracting 37 samples of Jamaica ginger.
1913	Vanderkleed	.....	.....	.....	Solvent(?) 6.85 to 9.92	Results obtained in extracting 17 samples of African ginger.
1913	Engelhardt	.....	.....	.....	Solvent(?) 2.81 to 5.24	Results obtained in extracting 8 samples of Jamaica ginger.
1914	Rippetoe	4.98	.....	2.79	.....	Jamaica ginger.
		5.50	.....	4.97	.....	" "
		6.20	.....	5.31	.....	African ginger.
		6.23	.....	5.45	.....	" "
1914	Vanderkleed	.....	.....	.....	Solvent(?) 5.06	Average yield of 3 samples of Jamaica ginger.
		.....	.....	.....	9.00	Average yield of 3 samples of African ginger.
1915	Vanderkleed	.....	.....	.....	3.93	Yield of Jamaica ginger.
		.....	.....	.....	7.99	" " African ginger.
		.....	.....	.....	8.90	" " " "

TABLE 64. — *Yield of oleoresin as obtained in the laboratory.*

Yield of oleoresin						Remarks
Date	Observer	Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per cent	
1909	DuMez & Arnold	6.60	.....	5.30	.....	Represents yield using a Soxhlet's extraction app., except in the case of alcohol.(1)
1910	DuMez & Netzel.	6.33	5.62	5.00	Benzin 2.57	Represents yield using a Soxhlet's extraction app., except in the case of alcohol.
1916	DuMez	6.28	5.49	4.92	Petrol. ether 3.15	Represents yield using a Soxhlet's extraction app., except in the case of alcohol.

[1] Jamaica ginger was the variety of the drug used in all cases. When alcohol was the solvent employed, the process of extraction was that of simple percolation.

Chemistry of the Drug and Oleoresin.

Tabulation of Constituents.

The chemistry of the constituents of ginger is still incomplete in many details, although, it has been the subject of a number of investigations.[1] In the light of our present knowledge, the following may be said to comprise the constituents of importance to the pharmacist: volatile oil, gingerol, resins, fat, wax, gum, sugar, starch and inorganic matter. Thresh[2] has identified the following in the oleoresin prepared by extracting the rhizomes with ether:

Volatile Oil	Resin	Wax
Gingerol	Fat	Ash

Occurrence and Description of Individual Constituents.

Volatile Oil.[3] The volatile oil or so-called essence of ginger is described by Thresh[4] as being a pale straw colored limpid

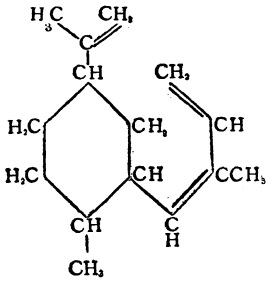
[1] Morin, Journ. de Pharm. et de Chim. (1823), 9, p. 256; Thresh, Pharm. Journ. (1879), 39, p. 171; Jones, Chem. & Drugg. (1886), 28, p. 413; Gane, Pharm. Journ. (1892), 51, p. 802; Balland, Journ. Pharm. Chim. (1903), 18, p. 248; Reich, Zeitschr. Unters. Nahr. u. Genussm. (1907), 14, p. 549.

[2] *l. c.*

[3] The description of the volatile oil as given above is for the product obtained from the rhizomes by steam distillation. The oil as it exists in the oleoresin prepared from the rhizomes by extraction with a solvent will undoubtedly differ somewhat.

[4] Pharm. Journ. (1881), 41, p. 198; Year-Book of Pharm. (1881), 18, p. 393.

fluid with a somewhat camphoraceous odor and an aromatic, but not a pungent taste. It is laevogyrate (–25 to 50°) and has a specific gravity of 0.875 to 0.886. It is soluble in strong alcohol, petroleum ether, carbon disulphide, benzene, turpentine and glacial acetic acid. The principal constituent of the oil, a sesquiterpene, gingerene or zingiberene, ($C_{15}H_{24}$) was first definitely described by von Soden and Rojahn[5] in 1900. According to Semmler and Becker,[6] it is a monocyclic butadiene having the following structure:



The former investigators also identified d-camphene and phellandrene[7] in the lower boiling fractions. In addition to these hydrocarbons, Schimmel & Company[8] have reported the presence of citral, cineol, borneol and probably geraniol, and Dodge[9] the presence of an aldehyde of the probable formula, n–$C_9H_{19}CHO$.

The volatile oil has been found to be present in the rhizomes in varying quantities depending on their age before harvesting, the methods of curing and their geographical source.[10] Ac-

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[5] Pharm. Ztg. (1900), 45, p. 414.

[6] Ber. d. deutsch. chem. Gesell. (1913), 46, p. 1814.

[7] Schimmel & Co. Semi-Ann. Rep. (1905), II, p. 38.

[8] Phellandrene and d-camphene were identified in the oil by Bertram and Walbaum in 1894. Journ. f. prakt. Chem. (1894), 49, p. 18.

[9] Chem. Abs. (1912), 6, 3, p. 2976; Orig. Com. 8th Intern. Congr. Appl. Chem. 6 p. 77.

[10] Gane reports the presence of volatile oil in ginger as follows: Jamaica 0.64 per cent., Cochin 1.35 per cent., African 1.615 per cent., Fijian 1.45 per cent. Pharm. Journ. (1892), 51, p. 802.

Thresh obtained 0.75 per cent. of oil from Jamaica ginger, 1.35 per cent. from Cochin and 1.61 per cent. from African. Pharm. Journ. (1879), 39, p.1. 191.

Haensel states that he obtained only 1.072 per cent. of volatile oil from Jamaica ginger, whereas other sorts yielded from 2 to 3 per cent. Pharm. Ztg. (1903), 48, p. 58.

Bennet found 0.20 to 0.90 per cent. of oil in Jamaica ginger, Pharm. Journ. (1901), 66, p. 522.

Reich gives the following as the volatile oil content of various sorts of

cording to Cripps and Brown a "good ginger" will yield from 2.24 to 3.48 per cent.[11]

Gingerol. Gingerol or zingiberol[12] is the constituent or mixture of constituents to which ginger is said to owe its pungency. It is a colorless, odorless, viscid fluid possessing an extreme pungency. Its exact composition has not been determined, the most recent investigations indicating that it is a mixture of phenols.[13] It is readily soluble in strong alcohol, carbon disulphide, benzol and oil of turpentine, but only slightly soluble in petroleum ether.

Gingerol is present in the rhizomes in amounts varying from 0.6 to 1.82 per cent.[14]

Resins. The resins of ginger have been isolated and described by Thresh.[15] This investigator recognizes four individuals with respect to their physical properties and their behavior toward acids and alkalies, viz: a neutral resin, an α-resin, a β-resin and a γ-resin.

The neutral resin is stated to be a black, pitch-like substance soluble in ether, alcohol, benzene and oil of turpentine, but insoluble in petroleum ether and carbon disulphide.

The α-resin is a soft, but brittle substance soluble in ether and alcohol, but insoluble in the remainder of the above mentioned solvents.

The β-resin is also soft and brittle, but is soluble in all of the above solvents.

The γ-resin is firmer in consistence and is soluble in ether, alcohol and petroleum ether.

The total resin content of the rhizomes varies to a considerable

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ginger: Cochin 1.38 per cent., Japan 1.38 per cent., Bengal 1.6 per cent., African 2.54 per cent. Zeitschr. Unters. Nahr. u. Genussm. (1907), 14, p. 549.

[11] Analyst (1909), 34, p. 519.

[12] The term *gingerol* was first used by Thresh in 1884 to designate the pungent principle of ginger. Year-Book of Pharm. (1884), 21, p. 516.

Zingiberol is evidently a modification of the above, the idea being to bring the nomenclature in closer conformity with the name of the botanical source—*Zingiberis officinale* Roscoe.

[13] Garnet and Grier, Year-Book of Pharm. (1907), 44, p. 441.

[14] Thresh obtained gingerol in the following quantities: Jamaica ginger 0.66 per cent., Cochin 0.60 per cent., African 1.45 per cent. Pharm. Journ. (1879), 39, p. 193.

Gane reports the presence of the following percentages: Jamaica 0.84 per cent., Cochin 0.60 per cent., African 1.45 per cent., Fijian 1.82 per cent. Pharm. Journ. (1892), 51, p. 802.

[15] Pharm. Journ. (1879), 39, p. 193.

extent and appears to depend principally on their geographical source. The minimum yield (1.18 per cent.) has been obtained from Jamaica ginger, the maximum yield (4.47 per cent.) from the Fijian rhizome.[16]

Fat and Wax. Little or no work has been done toward determining the composition of the fat or wax in ginger. The two substances, combined, are stated to constitute 0.70 to 1.225 per cent. of the rhizome.[17].

Ash. The qualitative examination of the ash of ginger has been undertaken by Thresh,[18] who reports the presence of the basic elements: K, Ca, Mg, Mn,[19] and Fe combined with H_2CO_3 and H_3PO_4. The ash of African ginger is stated to contain the largest amount of manganese.

The ash content[20] of the whole rhizome appears to be influenced but little by the locality from which obtained, 3.0 to5.5 per cent. being conservative limits for the usual commercial varieties. Peeling[21] appears to decrease the amount of ash while bleaching[22] (liming) increases it.

Constituents of Therapeutic Importance.

The physiological action of the oleoresin of ginger was at one time thought to be due to the resin content, but the work of Thresh[1] has shown the pungency to be the property of the phenolic constituents known collectively as gingerol. The car-

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[16] Thresh reports the total resin content of ginger as follows: Jamaica 1.18 per cent., Cochin 1.815 per cent., African 3.775 per cent., Pharm. Journ. (1879), 39, p. 173.

Gane noted the presence of the following percentages: Jamaica ginger 1.76 per cent., Cochin 1.815 per cent., African 3.775 per cent., Fijian 4.475 per cent. Pharm. Journ. (1892), 51, p. 802.

[17] The combined fat and wax present in ginger is stated by Thresh to be as follows: Jamaica 0.70 per cent., Cochin 1.205 per cent., African 1.225 per cent. *l. c.*

Gane found the following amounts: Jamaica ginger 0.92 per cent., Cochin 1.20 per cent., African 1.225 per cent., Bengal 0.86 per cent., *L. C.*

[18] Pharm. Journ. (1879), 29 pp. 174 and 193.

[19] See also Flueckiger, *Ibid.* (1872), 32, p. 208.

[20] C. Richardson. Bull. 13, Dept Agr. Washington, 1887; Gane, Pharm. Journ. (1892), 51, p. 802; Liverseege, Vierteljahresschr. Nahrungs-u. Genussm. (1896), 11, p. 353; Glass, Pharm. Journ. (1897), 58, p. 245; Bennet, *Ibid.* (1901, 66, p. 522.

[21] Winton, Ogden and Mitchell obtained 3.66 to 4.06 per cent. of ash for unpeeled and unbleached Cochin ginger, 3.36 per cent. for the same when peeled and bleached. Rep. Conn. Agr. Exp. Sta. (1898), p. 202; (1899), p. 102.

[22] Davis reports 5.20 per cent. of ash for unbleached Jamaica ginger, 6. 55 per cent. for the bleached. Pharm. Journ. (1895), 54, p. 472.

[1] Year-Book of Pharm. (1884), 21, p. 516.

minative action of the preparation must also be attributed in part to the volatile oil contained therein.

Physical Properties.

Color: The oleoresins examined in the laboratory were observed to be rather dark brown in color when spread out in thin layers on a white porcelain surface. This property, however, is reported to vary somewhat with the variety and condition of the ginger used in making the preparation. When African ginger is employed, the oleoresin is stated to be dark brown in color, whereas, uncoated Jamaica ginger is said to yield a preparation comparatively light in color.[1]

Odor: The oleoresin, when prepared according to the official process, has the full aroma of ginger, the quality of which is stated to be influenced largely by the variety of ginger used.[2]

Taste: The preparation has the sharp pungency and flavor of ginger. This property, like the odor, is stated to vary with the variety of ginger used, Jamaica ginger yielding the product with the best flavor.[3]

Consistence: The oleoresin is a thick liquid, being of about the consistence of molasses, as a rule, but varying somewhat with the variety of the ginger used in its preparation. The fluidity is said to be the greatest when prepared from Jamaica ginger and the least when made from the African variety.[4]

Solubility: The oleoresin is soluble in absolute alcohol, acetone, ether, chloroform, and glacial acetic acid. It is partially soluble in petroleum ether, the extent of its solubility depending on the solvent used in its preparation as is shown in the following table:

TABLE 65—*Solubility of the oleoresin in petroleum ether.*

Solvent used in preparing the oleoresin.	Alcohol	Acetone	Ether
Per cent. of oleoresin soluble in petrol. ether..	45.55	49.59	69.44

[1] Parrish. *Treatise on Pharmacy,* (1867), p. 233.
[2] Idris (1898).
[3] Idris (1898).
[4] Idris (1898).

As will be noticed this difference in solubility is quite pronounced and it should, therefore, serve as a ready means of identifying the solvent used in the manufacture of the preparation.

Specific gravity: At 25°C a specific gravity of 1.020 to 1.036 was found for this oleoresin when acetone or ether were employed in its preparation. This constant was observed to be slightly higher when alcohol was used as a menstruum and considerably lower (less than 1.000) when petroleum ether was employed. In the case of the commercial samples examined, a low specific gravity is to be attributed to the presence of unevaporated solvent in one instance, and in the other, it is thought to be due to an abnormally large volatile oil content. The data obtained in the examination of laboratory and commercial samples are given in the tables which follow.

TABLE 66—*Specific gravities of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Specific gravity
				At 25° C
1	1916	DuMez	Alcohol	1.041
2	"	"	Acetone	1.030
3	"	"	"	1.033
4	"	"	"	1.036
5	"	"	Ether	1.020
6	"	"	Petrol. ether	0.990

TABLE 67—*Specific gravities of commercial oleoresins.*

Sample No.	Date	Observer	Source	Specific gravity
				At 25° C
1	1916	DuMez	Squibb & Sons	0.997 [1]
2	"	"	Sharp & Dohme	1.014
3	"	"	Lilly & Co	1.024

[1] Contained ether.

Refractive index: A refractive index of about 1.517 at 25°C was observed for the preparations made in the laboratory with acetone or ether. When alcohol was employed in extracting the drug, the resulting product was found to have a slightly higher refractive index, while petroleum ether yielded an oleoresin in

which this constant was observed to be considerably lower. The low refractive index found for two of the commercial samples was very likely due to the fact that they contained twice as much volatile matter (principally essential oil) as the laboratory preparations. The effect of this influence, together with that produced by the presence of unevaporated solvent, is brought out in the following tables:

TABLE 68.—*Refractive indices of the oleoresins prepared in laboratory.*

Sample No.	Date	Observer	Solvent	Refractive index
				At 25° C
1.....	1916	DuMez	Alcohol	1.520
2.....	"	"	Acetone.....	1.517
3.....	"	"	"	1.517
4.....	"	"	"	1.518
5.....	"	"	Ether	1.517
6.....	"	"	Petrol. ether...	1.501

TABLE 69.—*Refractive indices of commercial oleoresins.*

Sample No.	Date	Observer	Source	Refractive index
				At 25° C
1.....	1916	DuMez	Squibb & Sons.....	1.501[1]
2.....	"	"	Sharp & Dohme	1.505
3.....	"	"	Lilly & Co	1.512

[1] Contained ether.

Chemical Properties.

Loss in weight on heating: The oleoresins prepared in the laboratory lost, as a rule, between 11 and 13 per cent. of their weight on heating at 110°C, whereas the loss in the case of the commercial samples was about twice as great. While this difference may have been due to the employment of different methods in the making of these preparations (a vacuum pan having probably been used in the removal of the solvent in the case of the commercial products), it is more likely the result of the presence of a greater amount of volatile oil in the drugs from which the latter were prepared. The loss in weight

as found for the preparations examined in the laboratory is given in the tables which follow.

TABLE 70—*Laboratory preparations—loss in weight on heating.*

Sample No.	Date.	Observer.	Solvent.	Per ct. of loss on heating.
				At 110° C
1.....	1916	DuMez.....	Alcohol.....	12.82
2.....	"	"	Acetone	11.92
5.....	"	"	"	7.34
6.....	"	"	"	11.54
3.....	"	"	Ether.....	11.08
4.....	"	"	Petrol. ether.....	11.50

TABLE 80. — *Commercial samples—loss in weight on heating.*

Sample No.	Date	Observer	Source	Per cent of loss on heating
				At 110° C
1.....	1916	DuMez	Lilly & Co.....	18.90
2.....	"	"	Squibb & Sons.....	21.39[1]
3.....	"	"	Sharp & Dohme.....	22.97

[1] The presence of ether could be detected by the odor.

Ash content: The ash content of the oleoresin prepared with acetone was found to be 0.28 per cent., whereas, that of the preparation made with ether was only 0.14 per cent. The values obtained for the commercial samples examined also showed this variation due to the nature of the solvent. Copper, although detected in two of these preparations (commercial oleoresins), was present in such small quantities that the results were not affected materially thereby. The following tables show the results obtained in the ash determinations made in the laboratory.

TABLE 81.—*Ash contents of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Per cent of ash
1.....	1916	DuMez	Alcohol	0.42
2.....	"	"	Acetone.....	0.30
3.....	"	"	"	0.26
4.....	"	"	"	0.28
5.....	"	"	Ether	0.14
6.....	"	"	Petrol. ether.....	0.06

TABLE 82.—*Ash contents of commercial oleoresins.*

Sample No.	Date	Observer	Source	Per cent of ash	Foreign constituents
1.....	1916	DuMez	Squibb & Sons.....	0.15 [1]	Copper
2.....	"	"	Lilly & Co	0.26	
3.....	"	"	Sharp & Dohme.....	0.27	"

[1] Contained ether.

Acid number: The acid numbers obtained for the oleoresins prepared in the laboratory were found to be fairly uniform regardless of the solvent employed in extracting the drug, except in the case of petroleum ether, when the value found was low, namely, 11.2. The values obtained for the commercial samples examined were almost identical with those obtained for the laboratory preparations, even though the former in all cases contained about twice as much volatile matter (generally essential oil, in one case, unevaporated solvent in addition) as the latter. The values obtained for this constant in the laboratory are given in the tables which follow.

TABLE 83—*Acid numbers of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Acid number
1.....	1916	DuMez.....	Alcohol.....	13.9
2.....	"	"	Acetone	14.5
3.....	"	"	"	13.8
4.....	"	"	"	13.5
5.....	"	"	Ether	13.7
6.....	"	"	Petrol. ether	11.2

TABLE 84—*Acid numbers of commercial oleoresins.*

Sample No.	Date	Observer	Source	Acid number
1.....	1916	DuMez.....	Lilly & Co.....	13.3
2.....	"	"	Squibb & Sons.....	13.8 [1]
3.....	"	"	Sharp & Dohme.....	14.0

[1] Contained ether.

Saponification value: Saponification values of 103.4 to 110.4 were obtained for the oleoresin when prepared with

acetone. For the preparation in which ether was employed as a menstruum in extracting the drug, a saponification value of 102.9 was obtained. The comparatively low values obtained for the commercial samples examined are to be accounted for by the fact that in all cases, they contained nearly twice as much volatile matter (presumably essential oil) as the laboratory preparations. The values found for this constant are given in the tables which follow.

TABLE 85—*Saponification values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Saponification value
1.....	1916	DuMez.....	Alcohol.....	119.4
2.....	"	"	Acetone.....	103.4
3.....	"	"	"	110.4
4.....	"	"	"	105.7
6.....	"	"	Ether.....	102.9
5.....	"	"	Petrol. ether.....	78.1

TABLE 86—*Saponification values of commercial oleoresins.*

Sample No.	Date	Observer	Source	Saponification value
1.....	1916	DuMez.....	Sharp & Dohme.....	94.1
2.....	"	"	Squibb & Sons.....	98.4[1]
3.....	"	"	Lilly & Co.....	89.9

[1] Contained a trace of ether.

Iodine value: Iodine values of 122.4 to 124.1 were obtained for the oleoresin when prepared with acetone. The preparations made with alcohol or ether gave values very near the same, whereas, the value of this constant was somewhat higher (126.9) when petroleum ether was the solvent employed. With respect to the commercial samples, the values found were lower in all cases. In one instance, this was due to the presence of unevaporated solvent, while, in the other cases it is to be attributed to the relatively large amount of volatile matter (essential oil) present. The iodine values found for the preparations examined in the laboratory follow.

TABLE 87.—*Iodine values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Iodine value
1	1916	DuMez	Alcohol	122.3
2	"	"	Acetone	122.4
3	"	"	"	111.5 [1]
4	"	"	Ether	124.1
5	"	"	Petrol. ether	121.1
6	"	"		126.9

[1] The drug in this instance was extracted by simple percolation.

TABLE 88.—*Iodine values of commercial oleoresins.*

Sample No.	Date	Observer	Source	Iodine value
1	1916	DuMez	Squibb & Sons	104.2[1]
2	"	"	Lilly & Co.	109.9
3			Sharp & Dohme	112.0

[1] Contained ether.

Special Qualitative Tests.

Most of the qualitative methods which have been mentioned in connection with the standardization of this preparation are of the nature of tests for the detection of adulterations. The oleoresin of capsicum[1] is the adulterant which appears to have received special attention, several methods for detecting its presence having been reported.

Tests for the Presence of the Oleoresin of Capsicum

La Wall, in 1910, pointed out the necessity of a test for the presence of the oleoresin of capsicum as he had observed that many of the commercial samples of the oleoresin of ginger used in the preparation of ginger ale extracts were adulterated with this substance. At the same time, he also described a method whereby this form of adulteration might be detected. His method is almost identical with that of Garnett and Grier published in 1907, both being based on the destruction of the

[1] While the oleoresin of capsicum *per se* may occassionally be added to the finished product, it is thought that the adulteration is usually accomplished by mixing capsicum with the ginger previous to the extraction of the oleoresin.

pungent principles (gingerol) of the oleoresin of ginger with alkalies, whereby the pungent principle (capsicin) of the oleoresin of capsicum remains unaltered. As it was subsequently found that the pungent principles of the former were not completely destroyed by this treatment, Nelson proposed a modification of the above methods, in which he makes use of manganese dioxide for completing the disintegration of these constituents. Full descriptions of these methods follow:

Method of Garnett and Grier (1907): Digest 1 gram of the oleoresin for 15 minutes on a water bath with a small quantity of caustic alkali dissolved in alcohol. Evaporate the solution to remove the alcohol and make the residue faintly acid with hydrochloric acid. Transfer the liquid to a test tube and shake it with 5 cubic centimeters of ether which have previously been used to rinse the dish. Allow the mixture to stand quietly and then taste the separated ethereal layer. If sharply pungent, adulteration with capsicum is indicated.

Method of La Wall (1910): Add 10 cubic centimeters of half-normal alcoholic potassium hydroxide solution to 1 gram of the oleoresin contained in a shallow porcelain dish and evaporate to dryness on a water bath. Dissolve the residue in 50 cubic centimeters of water and transfer the solution to a separatory funnel. Add 20 cubic centimeters of ether and shake vigorously. After allowing the mixture to stand until the ether has separated, run the latter off on a watch glass and expose it until the solvent has all evaporated. The residue should have a warm camphoraceous taste. A sharp pungent taste indicates adulteration with capsicum.

Method of Nelson (1902):[2] Add 10 cubic centimeters of double-normal alcoholic potassium hydroxide solution to one gram of the oleoresin contained in a porcelain dish and evaporate on a steam bath. Add about 0.1 gram of powdered manganese dioxide and 5 to 10 cubic centimeters of water, and continue heating for about 20 minutes, or until all of the volatile oil has been expelled. Cool, acidify with dilute sulphuric acid and extract at once with petroleum ether. Evaporate the petroleum ether solution in a small crucible, keeping the residue within as small an area as possible. When all of the solvent has evaporated, apply the tongue to the residue, being careful to keep the material on the tip. If capsicum is present, the characteristic burning sensation will soon be felt.

The latter is the method which was employed in making the test in the laboratory. In no case, however, was capsicum detected in the samples examined.

[2] Journ. Indust. and Eng. Chem. (1910), 2, p. 419.

Special Quantitative Tests.

While the matter of determining the quality of the unadulterated product has apparently received but little attention, two distinct methods have, nevertheless, been made use of in its evaluation. They are the methods of Garnett and Grier for the determination of the gingerol content, and the physiological test employed by the H. K. Mulford Company.

Methods for the Estimation of the Gingerol Content.

The only method of an analytical nature which has been suggested for the quantitative evaluation of this oleoresin is based on the fact that the pungent principles, gingerol, are more readily soluble in 60 per cent alcohol, than in petroleum ether. A description of the manner in which this assay is carried out follows.

Method of Garnett and Grier (1909): Dissolve the gingerol by boiling about 1 gram of the oleoresin with several portions of petroleum ether, filter the solutions thus obtained and remove the solvent by evaporation on a water bath. Dissolve the residue in alcohol (60 per cent.) added in three separate portions, shake the united alcoholic solutions with a small amount of petroleum ether to remove traces of fat and remove the alcohol from the hydro-alcoholic portion by evaporation. Shake the residual liquid with 3 portions of ether added successively, filter the combined shakings into a tared flask, remove the ether by evaporation on a water bath, dry at 100°C and weigh. In the final shaking out, carbon disulphide or chloroform may be used in place of the ether.

The use of this method in the laboratory has shown that it gives fairly constant results, and, as it is easily carried out, it should prove to be of practical value. The results obtained in the examination of oleoresins prepared in the laboratory and those obtained from commercial sources are given in the following tables:

TABLE 89—*Gingerol content of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Gingerol content
				Per cent.
1.....	1916	DuMez.....	Alcohol.....	27.2
2.....	"	"	Acetone	23.2
3.....	"	"	Ether.....	27.5
4.....	"	"	Petrol. ether	43.9

TABLE 90—*Gingerol content of commercial oleoresins.*

Sample No.	Date	Observer	Source	Gingerol content
				Per cent.
1.....	1916	DuMez.....	Lilly & Co.....	19.5
2.....	"	"	Sharp & Dohme.....	24.0
3.....	"	"	Squibb & Sons.....	28.2

The first of the preceding tables shows that the gingerol content varies with the solvent employed in the preparation of the oleoresin. Further, that this variation is not in inverse ratio to the yield of oleoresin obtained as might be expected, but is exceptionally low in the case of acetone due to the fact that it is a difficult matter to completely exhaust the drug when the latter is the solvent used.

The low gingerol content of two of the commercial samples as shown in the second table, points to the use of acetone in their preparation. A similar effect might, however, be produced when ether or alcohol are employed if the ginger used is of poor quality (low in gingerol content,) or if percolation is terminated before complete exhaustion of the drug has taken place. The oleoresin obtained from Squibb and Sons is stated to have been prepared with ether, which statement is confirmed by the result obtained in the determination of the gingerol content as is also shown in the second table.

Physiological Tests.

The H. K. Mulford Company reports the use of a physiological test for determining the quality of this oleoresin. As an arbitrary standard, the firm has taken a preparation which is pungent to the taste in a maximum dilution of 1 to 20,000. While there is no information, at hand to indicate what solvent was employed as the diluent, experience in the laboratory has shown that dilute alcohol (50 per cent.) may be used for this purpose. After vigorously shaking the oleoresin with alcohol, the resulting solution should preferably be filtered before applying to the tongue. Although no extensive series of experiments were made with this test in the laboratory, the results obtained would appear to indicate that the above standard is rather low as the pungency in the preparations examined was

easily perceptible in dilutions of 1 to 30,000. In view of the fact that personal idiosyncrasy must be a factor in applying this test, the use of the previously described method for the estimation of the gingerol content is thought to be more preferable for use in this connection.

Adulterations

There is no evidence to show that the oleoresin as prepared for pharmaceutical use is adulterated. La Wall,[1] however, states that the commercial article used in the manufacture of ginger ale frequently contains oleoresin of capsicum.

A trace of copper was found in most of the commercial samples examined. See under "Ash content."

OLEORESIN OF LUPULIN

Synonyms

Aetherisches Lupulinextrakt, Nat. Disp. 1879.
Extractum Lupulini, Hirsh, Univ. P. 1902, No. 1222.
Extractum Lupulini aethereum, Nat. Disp. 1879.
Oleoresina Lupulinae, U. S. P. 1860.
Oleoresina Lupulini, U. S. P. 1880.
Oléorésine de Lupuline, U. S. Disp. 1907.
Ethereal Extract of Lupulin, King's Am. Disp. (1900), p. 1333.

History

The first mention of the oleoresin of lupulin which could be found in pharmaceutical literature appeared in Procter's article, "Formulae for fluid extracts in reference to their more general adoption in the next Pharmacopœia," published in 1859. Procter's oleoresin was in reality an ethereal extract, ether having been the menstruum employed in exhausting the drug. In this connection, it is interesting to note that the extract prepared with the use of alcohol had previously been brought to the notice of the American pharmacist by Livermore in 1853, while the attention of the European pharmacist had been directed to the same by Planche as early as 1823. The oleoresin was first admitted to the *United States Pharmacopœia* in 1860, in which it remained official for more than half a century, having been

[1] LaWall (1910).

omitted from the present revised edition. It has never received recognition by any of the foreign pharmacopœias.

Drug Used, Its Collection, Preservation, Etc.

Lupulin has not been included in the late edition of the *United States Pharmacopœia.* In the preceding edition, it was defined as "the glandular trichomes separated from the fruit of *Humulus Lupulus* Linné (Fam. *Moraceae).*"

The drug, as it occurs on the market, is of varying degrees of purity due, principally, to the method of obtaining it. While some of it is probably obtained by picking the scales from the fruits and then shaking or rubbing the glands through a fine sieve, the bulk of the commercial article consists of the sweepings gathered up from the floors of the hop bins.[1] Such being the case, it is only natural to expect contamination with sand and other earthy materials. The impurities, in part, are usually removed by washing with water when the sand settles to the bottom and the lupulin is skimmed off and dried.

The glands, on storing, especially if exposed to the air, undergo a change, becoming dark brown in color and developing a rancid odor. Rabak[2] and Russell,[3] respectively, have shown one of the changes to be a conversion of the so-called soft resin into the hard. The development of the disagreeable odor has been attributed to the formation of valeric acid[4] resulting from the oxidation of one or more of the constituents. In view of the foregoing, the *British Pharmacopœia* directs that the drug be renewed annually and rejected as soon as it becomes dark in color or developes a cheesy odor.

In this connection, it should also be stated that hops are often sulphured previous to storing. To what extent, if any, this treatment affects the quality of the lupulin obtained therefrom and later the oleoresin, does not appear to have been determined.

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[1] Flueckiger, *Pharmakognoise des Pflanzenreichs* (1891), p. 255.
[2] Bull. No. 271, U. S. Dept. of Agric. (1913), p. 13.
[3] Bull. No. 282, U. S. Dept of Agric. (1915), p. 9.
[4] Bungener, Pharm. Journ. (1884), 43, p. 1008.

U. S. P. Text and Comments Thereon.

The oleoresin, which was official in the *United States Pharmacopœia* from 1860 to 1900, has been omitted from the last edition (edition of 1910).

1864

Oleoresina Lupulinae

Oleoresin of Lupulin

Take of Lupulin [1] twelve troyounces; Ether [2] a sufficient quantity.

Put the lupulin into a narrow cylindrical percolator, press it firmly, and gradually pour ether upon it until thirty fluidounces of filtered liquid have passed.[4] Recover from this, by distillation on a water-bath, eighteen fluidounces of ether,[5] and expose the residue, in a capsule, until the remaining ether has evaporated.[6] Lastly, keep the oleoresin in a wide-mouthed bottle, well stopped.[7]

1870

Oleoresina Lupulinae

Oleoresin of Lapulin

Take of Lupulin [1] twelve troyounces; Ether [2] a sufficient quantity.

Put the lupulin into a narrow cylindrical percolator, provided with a stop-cock, and arranged with cover and receptacle suitable for volatile liquids,[3] press it firmly, and gradually pour ether upon it, until twenty fluidounces of liquid have slowly passed.[4] Recover the greater part of the ether by distillation on a water-bath,[5] and expose the residue in a capsule, until the remaining ether has evaporated.[6] Lastly, keep the oleoresin in a wide-mouthed bottle, well stopped.[7]

1880

Oleoresina Lupulini

Oleoresin of Lupulin

[Oleoresina Lupulinae, Pharm., 1870]

Lupulin,[1] *one hundred parts* ....100. Stronger Ether[2], *a sufficient quantity.*

Put the lupulin into a narrow cylindrical percolator, provided with a cover and receptacle suitable for volatile liquids,[3] press it firmly, and gradually pour stronger ether upon it, until one hundred and fifty (150) parts of liquid have slowly passed.[4] Recover the greater part of the ether by distillation on a water-bath,[5] and expose the residue, in a capsule, until the remaining ether has evaporated.[6]

Keep the oleoresin in a well-stopped, wide-mouthed bottle.[7]

1890

Oleoresina Lupulini
Oleoresin of Lupulin

Lupulin,[1] *one hundred grammes*..... 100 Gm.
Ether,[2] *a sufficient quantity.*

Put the lupulin into a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and receptacle suitable for volatile liquids.[3] Press the drug very lightly, and percolate slowly with ether, added in successive portions, until the drug is exhausted.[4] Recover the greater part of the ether from the percolate by distillation on a water-bath,[5] and, having transferred the residue to a capsule, allow the remaining ether to evaporate spontaneously.[6]

Keep the product in a well-stoppered bottle,[7]

1900

Oleoresina Lupulini
Oleoresin of Lupulin

Lupulin,[1] *five hundred grammes*..... 500 Gm.
Acetone,[2] *a sufficient quantity.*

Introduce the lupulin into a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and a receptacle suitable for volatile liquids.[3] Press the powder very lightly, and percolate slowly with acetone, added in successive portions, until the lupulin is exhausted.[4] Recover the greater part of the acetone from the percolate by distillation on a water-bath,[5] and, having transferred the residue to a dish, allow the remaining acetone to evaporate spontaneously in a warm place.[6] Keep the oleoresin in a well-stoppered bottle.[7]

Average dose.—0.200 = Gm. 200 milligrammes (3 grains).

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1) For description of the drug, see page 1088 under "Drug used, its collection, preservation, etc."

2) The solvents which have been used for the purpose of extracting lupulin are ether, acetone and alcohol. Of these, the first two have been recognized at different times by the Pharmacopœia, acetone being the solvent which was directed to be used by the edition of 1900, whereas ether was specified in previous editions.

With respect to the relative values of the above, from a therapeutical standpoint, a statement cannot be made owing to the lack of specific information on the subject. From a pharmaceutical standpoint, however, ether and acetone, re-

spectively possess an advantage over alcohol in that they extract less inert material and yield products which are softer in consistence and conform more closely in their general properties to the other members of this class of preparations. The products obtained, even when using acetone or ether, are, however, more of the nature of an extract than an oleoresin.

A better solvent for use in this connection would appear to be petroleum ether. While, it has apparently never received consideration for this purpose, it appears to be particularly well adapted to the same in that it completely extracts the valuable constituents of the drug (see soft resins, page 1095) with but little of the inert material and yields a product of such consistence that it can be poured.

3) For a description of the various forms of percolation conforming to the pharmacopœial specifications for use in this connection, see Part I under "Apparatus used."

4) The various editions of the Pharmacopœia in which this preparation has been official have directed that the material composing the oleoresin be extracted from the drug by simple percolation. In the earlier editions, percolation was directed to be continued until a certain definite amount of percolate was obtained, whereas, the pharmacopœias of 1890 and 1900 required that the operation be continued until the drug was exhausted. In either case, the quantity of solvent required is considerably greater than that which is necessary to completely exhaust the drug when some form of continuous extractor is used. Since the quality of the finished product is the same in both cases, it is thought that the later method of extraction is to be preferred.

5–6) Owing to the fact that certain constituents of the oleoresin are prone to undergo changes when the latter is exposed to the air (see page 1088 under "Drug used, its collection, preservation, etc."), the pharmacopœial directions, that the last portions be allowed to evaporate spontaneously, are unfortunate. It is thought that a better procedure would be to evaporate the solvent completely at the temperature of the water bath, thereby considerably shortening the time of exposure.

8) For the reasons just mentioned, the finished product should be kept in well-stoppered bottles.

Yield

The yield of oleoresin to ether is usually given in the text-books and treatises on pharmacy as 50 to 60 per cent., while a yield of 32.49 to 70.8 per cent. has been reported in the journals. The irregularity in the quality of the lupulin as ordinarily found on the market very likely accounts for this variation. The drug, when of good quality should give a yield of at least 60 per cent. The following tables show the variation in the yield as reported in the literature, also, the results obtained in the laboratory.

TABLE 91—*Yield of oleoresin as reported in the literature.*

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per ct.	
1853	Livermore	66.00				
1888	Trimble			60.59	Benzin 7.04	
1892	Beringer		71.00	70.80		
1892	Sherrard			66.50		
1907	Van der Harst			52.00		
				65.00		
1908	Dohme and Engelhardt			56.00		
1909	Parson			54.00		
				60.10		
				66.70		
1909	Bernegau			34.00 to 65.80		Results obtained in the extraction of 10 samples of lupulin.
1909	Dohme & Engelhardt			47.00		
				50.00		
				53.00		
1911	Bernegau			57.70		
				58.90		
				62.10		
1911	Francis			60.0+		Seven of 8 samples yielded more than 60 per cent. of extractive to ether.
1913	Gane			44.94 to 65.60		Results obtained in the extraction of 4 samples of lupulin.
1913	Patch			63.96 to 77.82		Results obtained in the extraction of 53 samples of lupulin.
1913	Engelhardt			Below 60.00		Eight of 12 samples of lupulin extracted gave below 60 per cent. of ether soluble matter.
1914	Rippetoe			32.49		
				55.18		
				57.06		
1915	Glickman			44.20		
				54.70		
				55.00		
				55.30		
				55.50		
				57.10		
				58.60		
				68.20		
				69.20		

TABLE 92—*Yield of oleoresin as obtained in the laboratory.*

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Benzin	
		Per ct.	Per ct.	Per ct.	Per cent	
1910	DuMez & Netzel.	78.13	68.42	66.71	14.46	Represents the yield obtained using a Soxhlet's extraction app., except in the case of alcohol.

Chemistry of the Drug and Oleoresin

Tabulation of Constituents

The chemistry of lupulin[1] *per se* has received comparatively little attention, although, a very considerable knowledge concerning its constituents has been gained through the work of the brewing chemists and others[2] on hops. The isolation of the

[1] The following have reported more or less complete analyses of lupulin: Ives, Silliman's Am. Journ. of Science (1820), 2, p. 303; Payen, Pelletan and Chevalier, Journ. de Pharm. et de Chim. (1822), 8, p. 209; Personne, *Ibid.* (1854), 59, p. 329; Chapman, The Hop and its Constituents. The Brewing Review, London, (1905).

[2] Power, Tutin and Rogerson, who have completed one of the most recent as well as extensive pieces of work on the constituents of the hop, have isolated the following substances:

I Volatile oil

II Alcoholic Extract soluble in water:
1. Choline ($C_5H_{15}O_2N$.)
2. l-Asparagine ($C_4H_8O_3N_2$.)
3. Potassium nitrate
4. Tannin
5. Sugar forming a d-phenylhydrazone, m. p. 208.
6. Amorphous bitter material.
7. Volatile base having a coniine-like odor.

III Alcoholic extract insoluble in water:
1. Hentriacontane ($C_{31}H_{64}$.)
2. Ceryl alcohol ($C_{27}H_{56}O$.)
3. Phytosterol ($C_{27}H_{46}O$.)
4. A phytosterolin, phytosterol glucoside ($C_{33}H_{56}O_6$.)
5. Volatile fatty acids: formic, acetic, butyric, valeric, β-isopropylacrylic ($C_6H_{10}O_2$), and nonoic.
6. Saturated and unsaturated non-volatile acids: palmitic, stearic, cerotic, an isomeride of arachidic ($C_{20}H_{40}O_2$), cluytinic and linolic.
7. A new bitter crystalline phenolic substance, humulol ($C_{17}H_{18}O_3$.)
8. A new tasteless crystalline phenolic substance, xanthohumol ($C_{13}H_{14}O_3$.)

Journ. Chem. Soc. (1913), 103, p. 1267.

following constituents of pharmaceutical interests has been reported: Volatile oil, resin, wax, alkaloids and inorganic substances. Chapman[3] gives the composition of the ethereal extract as follows:

α-resin	18.06 per cent.
β-resin	67.74 " "
Wax	0.28 " "
Other constituents (fat, oil, γ-resin, etc.)	13.64 " "
Ash	0.27 " "

Occurrence and Description of Individual Constituents.

Volatile Oil.[4] The volatile oil obtained by distillation with steam is a pale yellow, or colorless, mobile liquid possessing a fragrant and characteristic odor, and a slightly burning taste. It is almost insoluble in water, to which, however, it imparts its odor, and only slightly soluble in dilute alcohol. It is soluble in ether, petroleum ether, chloroform and the other volatile oil solvents. The specific gravity at 20°C is 0.8357 to 0.8776, and the specific rotatory power, $[\alpha]_D^{20}$, is 0.20 to 0.58.[5]

According to Chapman,[6] the oil is composed of the terpene, myrcene, ($C_{10}H_{16}$), 40 to 50 per cent; inactive linalool, a fraction of 1 per cent; linalyl isononoate, a fraction of 1 per cent; the sesquiterpene, humulene[7] ($C_{15}H_{24}$), about 40 per cent; and probably some ether of geraniol with a small amount of a diterpene. Rabak,[8] who has more recently completed an investigation of the constituents of the oil, states that, in addition to myrcene and humulene, the oil contains the heptoic, octoic and nonoic acid esters of myrcenol with traces of free fatty acids and probably some free alcohols.

As much as 2 per cent of volatile oil has been obtained from lupulin by steam distillation.[9]

Resin. The chemical constitution of the so-called "hop resins" is still an unsolved problem, the literature being replete

[3] *Ibid*, p. 81. The hop and its constituents. The Brewing Review, London (1905).

[4] The following references are to the earlier literature on the volatile oil: Payen, Pelletan and Chevalier and Personne, l. c.; Wagner, Journ. f. prakt. Chem. (1853), 58, p. 351; Ossipon, *Ibid.* (1886), 142, p. 238.

[5] Chapman, *l. c.*

[6] *Ibid.*

[7] E. Deussen, who has determined the constitution of humulene, finds it to be 1-caryophyllene. Journ. f. prakt. Chem. (1911), 83, p. 483.

[8] Journ. Agric. Research (1914), 2, p. 115.

[9] Payen, Pelletan and Chevalier, *l. c.* See also Semmler, *l. c.*

with vague and contradictory statements.[10] For practical purposes, the classification of Hayduck[11] appears to be the most useful. This investigator distinguishes three resins, which he designates α, β and γ, according to their solubility in petroleum ether and their behavior toward a solution of lead acetate. The α- and β-resins are soluble in petroleum ether, and are further known as the "soft resins," being of a soft consistence at the ordinary temperature. The γ-resin is insoluble in petroleum ether, but soluble in ether or alcohol. It is also known as the "hard resin."

The soft resins are supposed to contain the valuable bitter substances present in hops. From these resins, Lintner and Schnell[12] isolated two crystalline bitter substances of an acid nature. One of these, $C_{15}H_{24}O_4$, they proposed naming "humulon;"[13] the other, they have designated "lupulic acid."[14]

According to Chapman, the total resins constitute more than 55 per cent. of the lupulin.[15]

Wax. According to Lermer[16] the wax is insoluble in 90 per cent alcohol and can be obtained by treating the ethereal extract with this solvent. He identified it as myricyl palmitate. As Power, Tutin and Rogerson[17] report the presence of ceryl alcohol and cerotic acid in hops, it is quite probable that ceryl cerotate is also a constituent of the wax.

Alkaloids. Choline[18] ($C_5H_{15}O_2N$) is the only base occurring in lupulin, the identity of which has been established. There is, however, considerable evidence of the presence of a volatile

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[10] The theory advanced by Seyffert (Zeitschr. ges. Brauw. (1896), 19, p. 1 namely, that the hop resins are mixtures of substances in a progressive state of change is probably correct. Confirmation of this theory is to be found in the work of Russell who states that a portion of the "soft resin"' is converted into the "hard resin" upon keeping the hops in storage. U. S. Dept. Agric., Bull. No. 282 (1915), p. 9.

[11] Wochenschr. f. Brau. (1887), 4, p. 397; *Ibid.* (1888), 5, p. 937.

[12] Zeitschr. ges. Brauw. (1904), 27, p. 666.

[13] "Humulon" is very likely identical with the "hop-bitter acid" of H. Bungener, (Bull. Soc. Chim. (1886), 45, p. 487), and the "α-lupulic acid" of Barth. Zeitschr. ges. Brauw. (1900), 23, pp. 509, 537, 554, 572 and 594.

[14] "Lupulic acid" corresponds to the "β-lupulic acid" of Barth, *l. c.*

[15] *l. c.*

[16] Dingler's Polytech. Journ. (1863), 169, p. 54.

[17] *l. c.*

[18] Griess and Harrow have shown that hops contain not over 0.02 per cent. of choline. Ber. der deutsch. chem. Ges. (1885), 18, p. 717.

alkaloid possessing a coniine-like odor. Griessmayer,[19] who first noted its presence, gave it the name "lupuline."

In 1885, Williamson[20] reported the isolation of a crystalline alkaloid from wild American hops. He gave it the name "hopeine," and assigned to it the formula, $C_{18}H_{20}NO_4$. H_2O. Ladenburg,[21] who examined a sample of the material thought it to be a mixture of morphine and a more soluble base.[22] As further work[23] along this line has failed to confirm the findings of Williamson, the presence of a crystalline alkaloid must be considered doubtful.

Ash. Analyses of the ash of lupulin have apparently not been reported to date. However, Wehmer[1] states that Ca, Cl and SiO_2 are present in the ash from all parts of the hop plant, and, as Na, Mg, Fe, Al, and H_3PO_4 were identified in the ash of the oleoresins examined in the laboratory, it is quite probable that the constituents of lupulin ash are identical with those present in the ash of hops.[2]

There is a great variation in the quantity of ash obtained from commercial samples of lupulin owing to contamination with sand and other earthy matter. Barth[3] gives the yield of ash as 9.5 to 24.4 per cent, while Flueckiger[4] states that a good sample of lupulin should give about 7.0 per cent. According to Keller,[5] lupulin, washed free from all earthy matter, yielded only 2.37 per cent. of ash.

Constituents of Therapeutic Importance.

There appears to be considerable doubt at the present time as to the value of the oleoresin of lupulin as a therapeutic agent. The presence of the soluble bitter principles is said to

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[19] Dingler's Polytech. Journ. (1874), 212, p. 67. See also Power, Tutin and Rogerson, *l. c.*

[20] Pharm. Ztg. (1885), 30 p. 620.

[21] Ber. der deutsch. Chem. Ges. (1886), 19, p. 783.

[22] Williamson, in a second publication, agrees with the findings of Ladenburg and assigns the name hopeine to the more soluble base. Chem. Zeit. (1886), 10, p. 491.

[23] Greshoff could not obtain a crystalline alkaloid from lupulin. Dingler's Polytech. Journ. (1887), 266, p. 316.

[1] Wehmer, *Die Pflanzenstoffe.* Jena (1911), p. 160.

[2] Richardson, in an examination of hop ash, identified the elements, Na, K, Ca, Mg. Al and Fe, and the acids, H_3PO_4, H_2CO_3 and H_2SiO_3. Wochenschr. Brau. (1898), 15, p. 160.

[3] Zeitschr. ges. Brauw. (1900), 23, p. 509.

[4] Flueckiger, *Pharmakognosie des Pflanzenreiches.* Berlin (1891), p. 257.

[5] Pharm. Ztg. (1889), 34, p. 533.

impart to it the properties of a simple bitter.[1] The somewhat general belief that the oleoresin is a mild sedative does not appear to be well founded and is probably based on the doubtful report that hops contain an alkaloid (hopeine) resembling morphine in physiological action.[2]

Physical Properties

Color: When spread out in a thin layer on a white porcelain surface, the color of the oleoresin was observed to be a dark brown resembling very much that of the oleoresin of ginger.

Odor: The preparation when made with acetone or ether has the peculiar odor of lupulin. The odor of the commercial product, however, is often quite different. In some cases it is disagreeable and resembles valeric acid,[3] while in other cases it is pleasant and suggests the presence of the ethyl esters of the lower fatty acids.[4]

Taste: The taste is bitter and somewhat aromatic resembling that of lupulin.

Consistence: The oleoresin, when prepared according to the directions of the *United States Pharmacopœia* of 1900 is of the consistence of a very soft extract. On standing in partially filled containers, it becomes firmer as a result of the conversion of a part of the soft resin into hard resin.

Solubility: The official preparation is freely soluble in alcohol (95 per cent.), acetone, ether, chloroform and glacial acetic acid. It is partially soluble in petroleum ether, the extent of its solubility depending on the age of the oleoresin (if stored in partially filled containers) or on the age of the drug from which the latter is prepared.[5] It is also slightly soluble in hot water to which it imparts a bitter taste.

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[1] Potter, *Mat. Med., Pharm. & Therap.* (1903), p. 339.

[2] Pharm. Ztg. (1885), 30, p. 620.

[3] This is due to the use of old deteriorated drug in the preparation of the oleoresin or to the storing of the latter under improper conditions. See under "Drug used, its collection, preparation, etc."

[4] The agreeable fruity odor sometimes noticed is thought to be due to the presence of ethyl esters of the lower fatty acids formed as a result of the extraction of old deterioratd drug with alcohol.

[5] On aging under ordinary conditions, the soft resin present in the drug or oleoresin is converted, in part, into hard resin. As only the former is soluble in petroleum ether, old oleoresins, or those prepared from old drug, are usually less soluble in this solvent than the preparations freshly made from unaltered drug. See under "Drug used, its collection, preservation, etc."

Specific gravity: The oleoresin has the highest specific gravity of any of the preparations of this class, specific gravities of 1.065 and 1.067 having been observed for the same when made with ether and acetone, respectively. Alcohol yields a product of somewhat greater density, whereas the use of petroleum ether gives an oleoresin of low specific gravity. The important factors influencing the specific gravity of this oleoresin, aside from the effect produced by the use of different solvents in its preparation, are thought to be the condition of the drug[1] when extracted and the presence of unevaporated solvent in the finished product. The results obtained in the examination of laboratory and commercial samples are given in the following tables.

TABLE 93—*Specific gravities of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Specific gravity
				At 25° C
1	1910	DuMez & Netzel	Alcohol	1.089
2	"	" "	Acetone	1.067
3	"	" "	Ether	1.065
4	"	" "	Benzin	1.037

TABLE 94—*Specific gravities of commercial oleoresins.*

Sample No.	Date	Observer	Source	Specific gravity
				At 25° C
1	1916	DuMez	Sharp & Dohme	1.065
2	"	"	Squibb & Sons	1.083 [1]
3	"	"	Lilly & Co.	1.086

[1] The preparation had a slight odor of ether.

Refractive index: The refractive index of the oleoresin, when prepared with acetone, was found to be 1.516 at 25°C, which agrees fairly well with that obtained for the sample from Sharp and Dohme. The low refractive index observed for the sample from Lilly and Co. is thought to be due to the presence of un-

[1] See under the caption "Chemistry of the drug and oleoresin".

evaporated solvent (probably alcohol). The results obtained in the determinations made in the laboratory are given in the tables which follow:

TABLE 95—*Refractive index of the oleoresin prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Refractive index
				At 25° C
1	1916	DuMez	Acetone	1.5163

TABLE 96—*Refractive indices of commercial oleoresins.*

Sample No.	Date	Observer	Source	Refractive index
				At 25° C
1	1916	DuMez	Lilly & Co.	1.496
2	"	"	Sharp & Dohme	1.519

Chemical Properties.

Loss in weight on heating: A loss in weight of 9.59 to 15.63 per cent. was observed for the laboratory preparations when heated at 110°C. Except in the case of the oleoresin which contained unevaporated solvent (alcohol), the loss did not exceed 10.32 per cent. Results of a similar magnitude were obtained for the commercial samples examined as is shown in the tables which follow.

TABLE 97—*Laboratory preparations—loss in weight on heating.*

Sample No.	Date	Observer	Solvent	Per cent. of of loss on heatlng
				At 110° C
1	1916	DuMez	Alcohol	15.63[1]
2	"	"	Acetone	9.59
3	"	"	Ether	10.32
4	"	"	Benzin	10.08

[1] Contained unevaporated solvent.

TABLE 98—*Commercial oleoresins—loss in weight on heating.*

Sample No.	Date	Observer	Source	Per cent. of loss on heating
				At 110° C
1.....	1916	DuMez.....	Sharp & Dohme.....	7.22
2.....	"	"	Squibb & Sons.....	9.46
3.....	"	"	Lilly & Co.....	20.68

[1] Contained ether.
[2] Probably contained unevaporated solvent (alcohol).

Ash content: The ash contents, in the case of the oleoresins prepared in the laboratory, were found to be 0.93, 1.46 and 1.82, depending on whether ether, acetone or alcohol was employed in their preparation. A somewhat similar variation in the amount of ash obtained for the commercial samples examined is, therefore, taken to be an indication of the indiscriminate use of the above mentioned solvents in their manufacture, instead of acetone as was directed to be employed by the 1900 edition of the *United States Pharmacopœia.* The slightly higher values obtained for the commercial samples may have been due to the copper, which was found to be present in considerable amounts. The results obtained in the ash determinations made in the laboratory follow:

TABLE 99—*Ash contents of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Per cent. of ash
1.....	1916	DuMez.....	Alcohol.....	1.62
2.....	"	"	Acetone.....	1.46
3.....	"	"	Ether.....	0.93
4.....	"	"	Petrol. ether.....	0.08

TABLE 100—*Ash contents of commercial oleoresins.*

Sample No.	Date	Observer	Source	Per cent. of ash	Foreign constituents
1.....	1916	DuMez.	Squibb & Sons.....	0.87[1]	Copper
2.....	"	"	Lilly & Co.....	1.53[2]	"
3.....	"	"	Sharp & Dohme.....	1.71	

[1] Contained ether.
[2] Probably contained unevaporated solvent (alcohol).

Acid number: The acid numbers given in the first of the tables which follow are those obtained for preparations which had stood in the laboratory for six years previous to being examined. As the acidity of the oleoresin very likely increases on ageing, when kept under ordinary conditions, due to the oxidation of some of its constituents, it is thought that a somewhat lower value is to be expected for this constant in the case of the freshly made preparation. The relatively low value found for the oleoresin prepared with alcohol was due to the presence of unevaporated solvent, which not only acts as a diluent, but also combines to some extent with the acids present forming esters, the latter imparting a fruity odor to the preparation. Viewed in the light of the foregoing statements, the acid numbers obtained for the commercial samples indicate that two of them were very probably old preparations and that the third (the sample obtained from Lilly & Co.) contained unevaporated solvent (alcohol). The results obtained in the determination of this constant in the laboratory follow.

TABLE 101—*Acid numbers of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Acid number
1.....	1916	DuMez.....	Alcohol.....	62.9 [1]
2.....	"	"	Acetone	84.1
3.....	"	"	Ether	80.1
4.....	"	"	Benzin.....	79.7

[1] Contained unevaporated solvent.

TABLE 102—*Acid numbers of commercial oleoresins.*

Sample No.	Date	Observer	Source	Acid number
1.....	1916	DuMez.....	Lilly & Co.....	61.7
2.....	"	"	Sharpe & Dohme.....	85.5
3.....	"	"	Squibb & Sons.....	78.4[1]

[1] Contained ether.

Saponification value: Saponification values ranging from 223.4 to 239.6 were obtained for the oleoresins prepared in the laboratory, the variation being due, very likely, to the nature of the solvent employed in extracting the drug. The values found

for the commercial preparations were somewhat lower, due, in two cases, to the presence of unevaporated solvent. In the third instance, the low saponification value obtained was very probably due to a difference in the quality of the lupulin from which the oleoresin was extracted. The results obtained in the examination of laboratory and commercial preparations follow.

TABLE 103—*Saponification values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Saponification value
1	1916	DuMez	Alcohol	223.4 [1]
2	"	"	Acetone	239.6
3	"	"	Ether	230.8
4	"	"	Benzin	227.4

[1] Contained ether.

TABLE 104—*Saponification values of commercial oleoresins.*

Sample No.	Date	Observer	Source	Saponification value
1	1916	DuMez	Lilly & Co.	158.8[1]
2	"	"	Sharp & Dohme	220.0
3	"	"	Squibb & Sons	223.3[2]

[2] Probably contained unevaporated solvent (alcohol).
[2] Contained ether.

Iodine value: The oleoresin, when prepared with acetone, ether, or benzin, was found to have an iodine value varying from 94.7 to 96.2. When alcohol was the solvent employed in its preparation, the value obtained was considerably lower, namely, 82.05. A comparison of thse values with those found for the commercial samples indicates that alcohol is sometimes used in their preparation. The extremely low value obtained for the oleoresin of Lilly & Co. is to be attributed to the presence of unevaporated solvent (alcohol) as well as to the effect produced by its use as a menstruum. The iodine values obtained for the preparations examined in the laboratory are given in the tables which follow.

TABLE 105—*Iodine values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Iodine value
1.....	1916	DuMez.....	Alcohol.....	82.05[1]
2.....	"	"	Acetone.....	96.2
3.....	"	"	Ether.....	94.7
4.....	"	"	Benzin.....	95.7

[1] Alcohol was present.

TABLE 106—*Iodine values of commercial oleoresins.*

Sample No.	Date	Observer	Source	Iodine value
1.....	1916	DuMez.....	Lilly & Co.....	68.7[1]
2.....	"	"	Sharp & Dohme.....	92.9
3.....	"	"	Squibb & Sons.....	91.5[2]

[1] Alcohol was probably present.
[2] The odor of ether was noticeable.

Adulterations.

Adulteration effected through the use of old deteriorated drug in the manufacture of this preparation has been noted. See under "Drug used, its collection, preservation, etc."

The presence of copper was detected in all of the commercial samples examined. See under "Ash content."

OLEORESIN OF PARSLEY FRUIT

Synonyms

Aetherisches Petersilieextrakt. Culbreth, Mat. Med. (1917), p. 428.
Green Apiol,[1] Brit. Pharm. Cod. 1907.
Oil of Parsley, Parrish. Treat. on Phar. (1867), p. 757.
Oleoresina Petroselini, U. S. P. 1910.
Oléorésine de Persil, Culbreth, Mat. Med. (1917), p. 428.
Liquid Apiol, Brit. Pharm. Cod. 1907.

[1] "Green apiol" is stated to be an alcoholic extract of the parsley fruit; "yellow apiol," the product obtained on treating this extract with animal charcoal, or upon saponifying the same with lead oxide; and "white apiol" the volatile oil obtained on distilling the extract with steam. Brit. and Col. Drugg. (1910), 58, p. 235.

The compound, $C_{12}H_{14}O_4$, spoken of in chemical literature as apiol is known commercially as crystalline apiol. Brit. Pharm. Cod. (1907), p. 112.

History.

The oleoresin of parsley appears to have come into existence through the attempts which were made to discover a simple method for the preparation of the so-called "apiol" of Homolle and Joret,[1] which was first brought to the attention of the pharmacist in 1855. The first mention of the oleoresin, insofar as could be determined with the information at hand, is to be found in Parrish's Treatise on Pharmacy published in 1867. Since that time, the preparation, or one of a similar nature, has been on the market under the name of "green apiol" or "liquid apiol," but was never given official recognition until the appearance of the present edition of the *United States Pharmacopoeia.*

Drug Used, Its Collection, Preservation, Etc.

In the present edition of the *United States Pharmacopoeia,* parsley fruit is defined as follows: "The dried ripe fruit of *Petroselinum sativum* Hoffman (Fam. *Umbelliferae),* without the presence or admixture of more than 5 per cent. of foreign seeds or other matter. Preserve Parsley Fruit carefully in tightly-closed containers protected from light." The plant from which the fruit is obtained has also been known under the following botanical synonyms: *Carum Petroselinum* Benth. and Hook., and *Apium Petroselinum* Linné.

Parsley is an annual herb commonly cultivated in the gardens of Europe and America. The fruit ripens in the fall, when it is gathered, dried and preserved for domestic use or shipped to market. The fruit as found in the market shows no marked difference in appearance regardless of its source. However, it is known to differ in its chemical composition. Thus, the fruits grown in Germany contain apiol as the principal constituent of therapeutic importance, whereas, those grown in France contain myristicin.[2] The volatile oil content also appears to vary with the source as Flueckiger[3] states that the

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[1] The "apiol" of Homolle and Joret is stated to be the product which remains unsaponified when the ether or chloroform soluble portion of the alcoholic extract of parsley fruit is heated with litharge. Journ. de Pharm. et de Chim. (1855), 28, p. 212.

[2] See under "Chemistry of parsley fruit".

[3] *Pharmakognosie des Pflanzenreichs* (1891), p. 938.

fruits grown in Norway have an exceptionally strong odor. Both of the foregoing variations in the composition of the drug would naturally be imparted in an increased degree to the oleoresins prepared therefrom. As the chemistry of the American fruit does not appear to have been studied, its value in this connection cannot be said to be definitely established. There is good reason, however, to believe that the oleoresins made in this country, in part at least, are prepared from home grown fruits.[1]

The large amount of fixed and volatile oils present in these fruits requires that they be preserved in tightly closed containers protected from the light.

U. S. P. Text and Comments Thereon.

The oleoresin was given official recognition for the first time by being admitted to the late edition of the *United States Pharmacopœia* (edition of 1910).

1910

Oleoresina Petroselini

Oleoresin of Parsley Fruit

Oleores. Petrosel.—Liquid Apiol

Parsley Fruit,[1] in No. 60 powder,[2] *five hundred grammes* ....500 Gm.
Ether,[3] *a sufficient quantity.*

Place the parsley fruit in a cylindrical glass percolator provided with a stop-cock and arranged with a cover and a receptacle suitable for volatile liquids.[4] Pack the powder firmly, and percolate slowly with ether, added in successive portions until the drug is exhausted.[5] Recover the greater portion of the ether by distillation on a water-bath,[6] and, having transferred the residue to a dish, remove the remaining ether by spontaneous evaporation in a warm place, stirring frequently.[7] Allow the oleoresin to stand without agitation for four or five days, decant the clear liquid portion from any solid residue,[8] and preserve it in well-stoppered bottles.[9]

Average Dose.—Metric, 0.5 mil—Apothecaries, 8 minims.

1) For a description of the drug, see page 1104 under "Drug used, its collection, preservation, etc."

2) The Pharmacopœia directs that the fruit be reduced to a

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[1] Joseph K. Janks in his book on spices states that parsley is being grown in this country. Jos. K. Janks, Spices, New York (1915), p. 69.
Culbreth on page 428 of the 1917 edition of his work on Materia Medica also refers to the cultivation of parsley in the United States.

No. 60 power for percolation. Owing to the large fatty oil content, this degree of fineness is difficult to attain, and, as experiments conducted in the laboratory indicate that a No. 40 powder is equally satisfactory for this purpose, it appears that a change to this effect in the pharmacopœial directions is desirable.

3) Ether is the solvent directed by the Pharmacopœia to be used for the extraction of the substances constituting the oleoresin. Observations made in the laboratory indicate that other solvents may also be employed for this purpose without in any way detracting from the value of the finished product. Thus, acetone and petroleum ether were found to yield products almost identical with that obtained by the use of ether. The latter is to be preferred to benzin as suggested by Beringer (1892) since its composition is more constant. Alcohol which is used commercially in the preparation of some of the so-called liquid apiols dissolves a considerable amount of coloring matter and other inert substances and, therefore, yields a product of inferior quality.

4) For a description of the various forms of percolators which have been designed to meet the specifications of the Pharmacopœia, see Part I under "Apparatus used".

5) The pharmacopœial directions governing the extraction of the oleoresinous material are to slowly percolate the drug with ether, added in successive portions, until complete exhaustion has been effected. Here again, the use of some form of continuous extraction apparatus would appear to be an improvement over the present method.

6–7) For comments on this step in the pharmacopœial method of preparation, see under comments on the oleoresin of cubeb.

8) Upon the complete removal of the solvent from the percolate, the residual oily liquid deposits a small amount of waxy matter which the Pharmacopœia directs shall be removed by decantation. When either is the solvent used in extracting the drug, this deposit amounts to less than 1 per cent of the oleoresin, while the percentage is somewhat greater, about 1.5 per cent when acetone is used. The deposit resulting when benzin was the solvent employed was found by Beringer to be equal to about 3 per cent.

9) The oleoresin should be kept in well-stoppered bottles as

it loses volatile oil upon exposure to the air, and as the glycerides are prone to undergo partial decomposition due to the action of the moisture and oxygen.

Yield.

The information at hand is not sufficient to permit of a statement being made as to what the average yield of oleoresin should be in this case. The results obtained in the laboratory and those reported by Beringer show that it is at least 24 per cent., when ether or acetone are the solvents employed in extracting the drug, whereas those reported by Vanderkleed would appear to indicate that the yield is much lower. The available information of this nature is given in the following tables:

TABLE 107—*Yield of oleoresin as reported in the literature.*

Date	Observer	Yield of Oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Other Solvents	
		Per cent.	Per cent.	Per cent.	Per cent.	
1892..	Beringer.....	.....	24.0	.....	Benzin 19.3	The total yield of extractive matter to benzin is given as 22.3 per cent, which includes 3 per cent. of deposited wax.
1913..	Vanderkleed	.....	.....	.....	Solvent (?) 11.40	Reported as yield of oleoresin.
	"	.....	.....	.....	13.04	
	"	.....	.....	.....	14.70	

TABLE 108—*Yield of oleoresin as obtained in the laboratory.*

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per ct.	
1916	DuMez.....	.....	28.89	29.17	.....	Represents the yield using a Soxhlet's Extraction App.

Chemistry of the Drug and Oleoresin.

Enumeration of Constituents.

The following are the known constituents of parsley fruit which may be considered of pharmaceutical interest; volatile oil, fatty oil, apiin, and inorganic substances. While analyses of the oleoresin have not been reported, the first two named constituents of the fruit, together with a small amount of inorganic matter, very likely represent this preparation when made by extracting the drug with ether, as apiin is stated to be insoluble in this solvent.

Occurrence and Description of Individual Constituents.

Volatile Oil.[1] The volatile oil of parsley fruit is described as a colorless or yellowish, thick liquid having a specific gravity of 1.03 to 1.10 at 15°C. The angle of rotation in a 100 millimeter tube is given as -5° to -10°. It is soluble in alcohol, ether, chloroform and petroleum ether. On cooling or shaking with water, it precipitates apiol.[2]

The composition of the oil varies with the locality in which the fruit is grown. The principal constituent of the oil distilled from the fruit grown in Germany is apiol. Myristicin is present only in very small quantities.[3] It is stated that the apiol content is often so great that the oil is a semi-solid at ordinary temperatures. In the French oil, myristicin predominates, while apiol, together with allyltetramethoxybenzene, is present in small amount.[4] The constitution of these compounds is represented by the following formulas:

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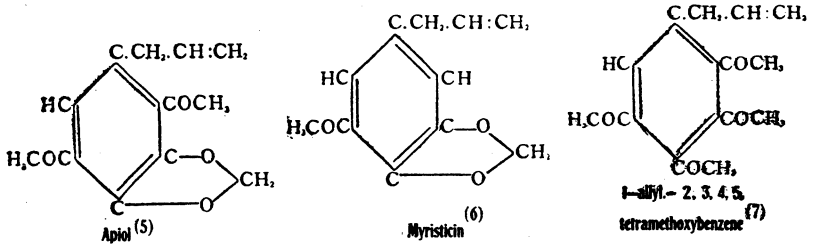
[1] The following list comprises the more important references to the earlier literature on the volatile oil: Bley, Trommsdorff's neues Journ. (1827), 14, p. 134; Bolle, Arch. der Pharm. (1829), 29, p. 168; Blanchet and Sell, Ann. der Chem. (1833), 6, p. 301; Loewig and Weidmann, *Ibid.* (1839), 32, p. 283; von Gerichten, Ber. der. deutsch. chem. Ges. (1876), 9, pp. 258 and 1477.

[2] Schimmel & Co., Ber. (1906), p. 95.

[3] Thoms, Ber. der deutsch. chem. Ges. (1903), 36, p. 3451; *Ibid* (1908), 41, p. 2753; Chevalier, Bull. sci. pharmacologique (1910), 17, p. 128; Chem. Abs. (1911), 5, p. 1490.

[4] *Ibid.* Also, Bignami and Testoni, Gaz. Chim. ital. (1900), 30, p. 240.



Apiol is a crystalline solid possessing in a strong degree the odor of parsley. Its melting point is 30°C and the boiling point 294°C.[8] Eykman[9] gives the specific gravity at 14°C as 1.176, and the refractive index $[n]_D$ as 1.538. It is soluble in alcohol, ether, chloroform and oils. It also dissolves in concentrated sulphuric acid, the solution formed being blood-red in color.

Myristicin is a liquid possessing but little odor. It does not solidify even when cooled to a comparatively low temperature. Semmler[10] gives the specific gravity as 1.141 at 25°C. Its solubility is similar to that of apiol.

In addition to the foregoing, Thoms[11] reports the presence of the following in both, the German and French oils: l-pinene, phenols and palmitic acid.

Semmler[12] reports the volatile oil content of parsley fruit to be 2 to 6 per cent.

Fatty Oil.[13] The fatty oil of parsley fruit is a greenish yellow mobile liquid. It is soluble in a mixture of alcohol and ether, in ether, chloroform and carbon disulphide. A sample from Schimmel & Co., examined by von Gerichten and Koehler,[14]

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[5] Eykman. Ber. der. deutsch. chem. Ges. (1890), 23, p. 862; Thoms, *Ibid.* 1903, 36, p. 174.

[6] Thoms. Chem. Ztg. (1903). 27, p. 938.

[7] Thoms. Ber. der. deutsch. chem. Ges. (1908), 41, p. 2761.

[8] Ciamician and Silber, *Ibid.* (1888), 21, p. 1632.

[9] *l. c.*

[10] Semmler, *Die aetherische Oele* (1907), 4, p. 168.

[11] *Arbeiten aus d. Pharm. Inst., Univ. Berlin* (1909), 6, p. 190.

[12] Semmler. *Die aetherische Oele* (1907), 4, p. 173.

[13] Grimme obtained 16.7 per cent. of a red-brown oil having the following properties: specific gravity at 15° C, 0.9243; refractive index at 35° C, 1.4778; saponification value, 176.5; iodine value, 109.6; acid value, 3.4; unsaponifiable matter, 2.18 per cent. He was unable to obtain a test for the presence of phytosterin in the unsaponifiable residue. Pharm, Centralh. (1911), 52, p. 663.

[14] Ber. der. deutsch. chem. Ges. (1909), 42, p. 1638.

showed the following properties: specific gravity at 15°C, 0.972; refractive index at 40°C, 1.4624; saponification value, 190.9; iodine value, 80.07.

The saponifiable portion of the oil was found to be composed of the glyceryl esters of oleic, palmitic, stearic and petroselinic acids. The latter is stated to be isomeric with oleic acid. From the unsaponifiable residue, Matthes and Heintz[15] isolated a hydrocarbon, $C_{20}H_{42}$, to which they gave the name petrosilan; also, myricyl alcohol and a mixture giving a test for phytosterin.

The average fatty oil content of the fruit is probably about 20 per cent.[16]

Apiin.[17] Apiin ($C_{27}H_{32}O_{16}$) is a glucoside. Its melting point is stated to be 228°C. On hydrolysis, it yields a sugar and apigenin (trioxyflavon) $C_{15}H_{10}O_5$. It is soluble in hot alcohol or water, insoluble in ether, and therefore, it is not likely to be present in the oleoresin.

Ash. Avaliable information concerning the constituents of the ash of parsley fruit is limited to the anaylsis of Rump,[18] who reports the presence of the basic elements, K, Ca, Mg and Fe in combination with the acids, HCl, H_2SO_4, H_3PO_4, H_2CO_3 and H_2SiO_3, also, some free SiO_2.

The ash content[19] of parsley fruit is about 6.50 to 7.00 per cent. Commercial samples sometimes show a higher percentage of ash due to contamination with foreign matter.[20]

Constituents of Therapeutic Importance.

The oleoresin of parsley fruit is said to be used chiefly as an emmenagogue. Such being the case, its therapeutical value is undoubtedly due to the volatile oil which it contains as both apiol[1] and myristicin,[2] constituents of the essential oil, have

[15] Ber. der. pharm. Ges. (1909), 19, p. 325.

[16] Rump. obtained 22 per cent. of fatty oil. Buchner's Repert. f. d. Pharm. (1836), 6, p. 6. Grimme gives the yield as 16.7 per cent. l. c.

[17] von Gerichten, Ber. der deutsch. chem. Ges. (1876), 9, p. 1121.

[18] Buchner's Repert. f. d. Pharm. (1836), 56, p. 26.

[19] Rump gives the ash content as 6.5 per cent. *Ibid.* Warnecke reports the percentage of ash as 7.04. Pharm. Ztg. (1886), 31, p. 536.

[20] La Wall and Bradshaw report two commercial samples of parsley fruit yielding 6.61 and 9.10 per cent. of ash, respectively. Proc. A. Ph. A. (1910), 58, p. 752.

[1] Heffter, Arch. f. exp. Path. u. Pharmak. (1895), 35, p. 365. Chevalier Bull. Sci. pharmacologique, 17, pp. 128–131.

[2] Juerss, Schimmel & Co., Ber. (1904), p. 159.

been shown to be severe intestinal irritants. The activity of the volatile oil may be further accounted for by the presence of terpenes as these compounds are also known to be irritants.[3]

Physical Properties

Color: When spread out in a thin layer on a while porcelain surface, the oleoresin was observed to be greenish-yellow in color. The so-called fluid apiols of commerce, preparations made with alcohol, are of a comparatively deep green color.

Odor: The oleoresin has the agreeable aromatic odor of parsley.

Taste: The taste is spicy like that of the drug from which it is prepared.

Consistence: The oleoresin is a rather thin liquid, being of about the consistence of olive oil.

Solubility: The official preparation is soluble in acetone, ether, chloroform, carbon disulphide and petroleum ether. It is almost insoluble in alcohol or water.

Specific gravity: The specific gravities of the oleoresins prepared in the laboratory were found to be 0.937 and 0.940 at 25°C. In the making of these preparations ether and acetone, respectively, were employed as menstrua for extracting the drug. The specific gravity of the only commercial sample, conforming in its general properties to the official product, was observed to be about the same, i. e. 0.943. In the case of the other commercial products, the greater density is thought to be due to the use of alcohol in their preparation.[1] The results for the determinations made in the laboratory follow.

TABLE 109—*Specific gravities of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Specific gravity
				At 25° C
1.....	1916	Du Mez.....	Acetone.....	0.940
2.....	"	.....	Ether.....	0.937

[3] Kehrer, Arch. f. Gyn. (1910), 90, p. 169.

[1] This statement is also based on the dark green color of the preparations and the fact that alcohol is the solvent mentiond in the literature in connection with the preparation of the so-called fluid apiols. See under "History" of the oleoresin.

TABLE 110—*Specific gravities of commercial oleoresins.*

Sample No.	Date	Observer	Source	Specific gravity
				At 25° C
1.....	1916	DuMez.....	Sharp & Dohme.....	0.943
2.....	"	"	Squibb & Sons.....	0.984[1]
3.....	"	"	Merck & Co.....	1.008[2]

[1] Apiol, fluid,—Squibb.
[2] Apiol, fluid, green,—Merck.

Refractive index: Observations made in the laboratory indicate that the oleoresin should have a refractive index of about 1.477 at 25°C, when ether or acetone are employed in the extraction of the drug. A result almost identical with the preceding was obtained for the only commercial sample examined. The refractive indices observed in the case of the so-called liquid apiols were somewhat higher, due very likely to the use of alcohol in their preparation. The data given in the following tables illustrate these points.

TABLE 111—*Refractive indices of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Refractive index
				At 25° C
1	1916	DuMez.....	Acetone.....	1.477
2	"	"	Ether.....	1.477

TABLE 112—*Refractive indices of commercial oleoresins.*

Sample No.	Date	Observer	Source	Refractive index
				At 25° C
1	1916	DuMez.....	Sharp & Dohme.....	1.475
2	"	"	Squibb & Sons.....	1.486[1]
3	"	"	Merck & Co.....	1.1488[2]

[1] Apiol, Fluid—Squibb.
[2] Apiol, Fluid, Green,—Merck.

Chemical Properties.

Loss in weight on heating: The oleoresins prepared in the laboratory, using ether and acetone as menstrua for exhausting the drug, lost 7.87 and 7.92 per cent. of their weight, respectively, on heating at 110°C. In the case of the only commercial sample examined, the loss was about one-half as great due very likely to a smaller amount of volatile matter (essential oil) being contained in the drug from which the latter was prepared. The results obtained are given in the tables which follow.

TABLE 113—*Laboratory preparations—loss in weight on heating.*

Sample No.	Date	Observer	Solvent	Per cent. of loss on heating
				At 100° C
1.....	1916	DuMez.....	Acetone..	7.92
2.....	"	"	Ether.....	7.87

TABLE 114—*Commercial oleoresins—loss in weight on heating.*

Sample No.	Date	Observer	Source	Per cent. of loss on heating
				At 110° C
1.....	1916	DuMez..	Sharp & Dohme.....	3.35

Ash content: The results obtained in the determination of the ash content of the oleoresins examined in the laboratory are given in the tables which follow. Aside from the fact that the amount of ash obtained varied with the solvent used in the making of the preparations, the only items of importance brought out by these results are that ether was evidently employed in the manufacture of the commercial product and that the latter contained copper.

TABLE 115—*Ash contents of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Per cent. of ash
1.....	1916	DuMez.....	Acetone.....	0.18
2.....	"	"	Ether.....	0.09

TABLE 116—*Ash contents of commercial oleoresins.*

Sample No.	Date	Observer	Source	Per cent. of ash	Foreign constituents
1.....	1916	DuMez... ...	Sharp & Dohme.....	0.09	Copper

Acid number: The acid numbers obtained for the oleoresins prepared with acetone and ether were found to be 9.3 and 9.2, respectively, indicating that the difference in the nature of the two solvents has but little influence on the value of this constant. The high value found for the sample obtained from Sharp & Dohme is thought to be due to the hydrolysis of some of the glycerides, and, therefore, to indicate an old preparation, or one that has been prepared from old deteriorated drug. The acid numbers obtained for the oleoresins examined, also those found for the so-called liquid apiols, are given in the tables which follow.

TABLE 117—*Acid numbers of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Acid number
1	1916	DuMez.....	Acetone.....	9.3
2	"	"	Ether.....	9.2

TABLE 118—*Acid numbers of commercial preparations.*

Sample No.	Date	Observer	Source	Acid number
1	1916	DuMez.....	Merck & Co.....	12.1[1]
2	"	"	Sharp & Dohme	50.5
3	"	"	Squibb & Sons.....	58.5[2]

[1] Apiol, Fluid, Green.

Saponification value: The saponification values of the oleoresins prepared in the laboratory, using ether and acetone as menstrua for extracting the drug, were found to be 158.5 and 165.6, respectively. The high value (181.6) obtained for Sharp

& Dohme's preparation is thought to be due to the presence of a relatively large amount of the glyceride of petroselinic acid, which is stated by von Gerichten to have a saponification value of 191.2. See under "Chemistry of the drug and oleoresin." Tables showing the saponification values of the preparations examined in the laboratory follow. For comparison with the foregoing data, the values obtained for the so-called liquid apiols have also been included in these tables.

TABLE 119—*Saponification values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Saponification value
1.....	1916	DuMez.....	Acetone.....	165.6
2.....	"	"	Ether.....	158.5

TABLE 120—*Saponification values of commercial preparations.*

Sample No.	Date	Observer	Source	Saponification value
1.....	1916	DuMez.....	Merck & Co.....	108.5[1]
2.....	"	"	Squibb & Sons.....	126.7[2]
3.....	"	"	Sharp & Dohme.....	181.6

[1] Apiol, Fluid, Green,—Merck.
[2] Apiol, Fluid,—Squibb.

Iodine value: The iodine values as found for the oleoresins prepared in the laboratory are given in the first of the tables which follow. It will be observed that there is a considerable difference in these values due to the nature of the solvent employed in extracting the drug. The low iodine value observed for the preparation made by Sharp & Dohme is to be attributed to the partial oxidation of the unsaturated glycerides. For comparison, the iodine values of two samples of so-called "liquid apiols"(preparations made with alcohol) have been included in the tables which follow.

TABLE 121—*Iodine values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Iodine value
1.....	1916	DuMez.....	Acetone	132.5
2.....	"	"	Ether	122.9

TABLE 122—*Iodine values of commercial preparations.*

Sample No.	Date	Observer	Source	Iodine value
1.....	1916	DuMez.....	Sharp & Dohme.....	110.6
2.....	"	"	Squibb & Sons.....	123.3 [1]
3.....	"	"	Merck & Co.....	130.2 [2]

[1] Labeled "Apiol-Fluid."
[2] Labeled Apiol, Fluid, Green.

Adulterations.

A trace of copper was found to be present in the commercial samples examined. See under "Ash content."

OLEORESIN OF PEPPER

Synonyms

Aetherisches Pfefferextrakt, Nat. Disp. 1884.
Ethereal Extract of Black Pepper, King's Am. Disp. 1900.
Extractum Piperis, Hirsh, Univ. P. 1902, No. 1244.
Extractum Piperis Fluidum, U. S. P. 1850.
Fluid Extract of Black Pepper, U. S. P. 1850.
Oil of Black Pepper, King's Am. Disp. 1900.
Oleoresina Piperis, U. S. P. 1900.
Oléorésine de Poivre noir, U. S. Disp. 1907.

History.

The oleoresin of pepper appears to have been first obtained as a by-product[1] in the preparation of piperine. Thus, Dr. Meli in France as early as 1825, reported having obtained the so-called "oil of black pepper" as a residue on separating the piperine from the alcoholic extract of the drug. The first notice of its use as a therapeutic agent apparently came from

[1] Jourdan, *Univ. P.* (1832), p. 346

America as Carpenter, in 1829, in an article on Peruvian bark, refers to its use by Dr. Chapman of Philadelphia in connection with the administration of quinine. The oleoresin prepared with ether became official in the *United States Pharmacopœia* in 1850 under the title *Extractum Piperis Fluidum.* In the 1860 edition, the name was changed to *Oleoresina Piperis,* under which title, it is still official at the present time. Neither this preparation nor one of a similar nature has ever been given official recognition abroad.

Drug Used, Its Collection, Preservation, Etc.

According to the present edition of the *United States Pharmacopoeia,* the drug recognized is "the dried, unripe fruit of *Piper nigrum* Linné (Fam. *Piperaceae),* without the presence or admixture of more than two per cent of stems or other foreign matter." It has also occassionally been referred to under the botanical synonyms, *Piper trioicum* Roxb.

As becomes apparent from the foregoing, only the unripe fruits should be used. As the fruit reaches maturity, the chlorophyll content diminishes and it becomes less pungent.[1] A variation in the chlorophyll would naturally effect the properties of the oleoresin prepared therefrom, while a difference in piperine content would have no significance in this connection as only a small portion of the total piperine (to which pepper owes its pungency)[2] remains in solution in the oleoresin, the greater part being precipitated upon the ermoval of the solvent.

Pepper, as it occurs on the market, consists of a number of commercial varieties, viz: Malabar, Cochin, Penang, Singapore, Siam and others.[3] The quality of these varieties is ordinarily governed by weight, the Malabar being the heaviest. The Penang, however, is stated to be the most pungent. The manner in which either of these qualities effect the oleoresin does not appear to have been determined. While the Pharmacopoeia makes no provisions for the preservation of this drug, its volatile oil content necessitates the use of closed containers.

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[1] Flueckiger. *Pharmakognosie des Pflanzenreiches* (1891), p. 913.
[2] Kayser, Chem. Centralb. (1888), 59, p. 261.
[3] Jos. K. Janks, *Spices, New York,* (1915), p. 10.

U. S. P. Text and Comments Thereon.

The oleoresin of pepper has been official in the *United States Pharmacopœia* since 1850, when it was recognized under the title of *Extractum Piperis Fluidum.*

1850

Extractum Piperis Fluidum

Fluid Extract of Black Pepper

Take of Black Pepper,[1] in powder,[2] a pound;
Ether,[3] *a sufficient quantity.*

Put the powder into a percolator,[4] and pour ether gradually upon it until two pints of filtered liquor are obtained.[5] From this distill off, by means of a water-bath, at a gentle heat, apint and a half of ether,[6] and expose the residue in a shallow vessel, until the whole of the ether has evaporated,[7] and the deposition of piperin in crystals, has ceased. Lastly, separate the piperin by expression through a cloth,[8] and keep the liquid portion.

1860

Oleoresina Piperis

Oleoresin of Black Pepper

Extractum Piperis Fluidum, *Pharm.*, 1850

Take of Black Pepper,[1] in fine powder,[2] twelve troyounces;
Ether,[3] *a sufficient quantity.*

Put the Black Pepper into a cylindrical percolator,[4] press it firmly, and gradually pour ether upon it until twenty-four fluidounces of filtered liquid have passed.[5] Recover from this, by distillation on a water-bath, eighteen fluidounces of ether,[6] and expose the residue, in a capsule, until the remaining ether has evaporated,[7] and the deposition of piperin in crystals has ceased. Lastly, separate the oleoresin from the piperin by expression through a muslin strainer,[8] and keep it in a well-stopped bottle.[9]

1870

Oleoresina Piperis

Oleoresin of Black Pepper

Take of Black Pepper,[1] in fine powder,[2] twelve troyounces;
Ether,[3] *a sufficient quantity.*

Put the Black Pepper into a cylindrical percolator provided with a stop-cock, and arranged with cover and receptacle suitable for volatile liquids,[4] press it firmly, and gradually pour ether upon it, until twenty fluid ounces of liquid have slowly passed.[5] Recover the greater part of the ether by distillation on a water-bath,[6] and expose the residue, in a capsule, until the remaining ether has evaporated,[7] and the deposition of piperin in crystals has ceased. Lastly, separate the oleoresin from the piperin by expression through a muslin strainer,[8] and keep it in a well-stopped bottle.[9]

1880

Oleoresina Piperis

Oleoresin of Pepper

Pepper,[1] in No. 60 powder,[2] *one hundred parts* 100
Stronger Ether,[3] *a sufficient quantity.*

Put the pepper into a cylindrical percolator, provided with a cover and receptacle suitable for volatile liquids,[4] press it firmly, and gradually pour stronger ether upon it, until one hundred and fifty (150) parts of liquid have slowly passed.[5] Recover the greater part of the ether by distillation on a water-bath,[6] and expose the residue, in a capsule, until the remaining ether has evaporated,[7] and the deposition of piperine, in crystals, has ceased. Lastly, separate the oleoresin from the piperine by expression through a muslin strainer.[8] Keep the oleoresin in a well-stopped bottle.[9]

1890

Oleoresina Piperis

Oleoresin of Pepper

Pepper,[1] in No. 60 powder,[2] *five hundred grammes*.....500 Gm.
Ether,[3] *a sufficient quantity.*

Put the pepper into a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and receptacle for volatile liquids.[4] Press the drug firmly, and percolate slowly with ether, added in successive portions, until the drug is exhausted.[5] Recover the greater part of the ether from the percolate by distillation on a water-bath,[6] and, having transferred the residue to a capsule, set this aside until the remaining ether has evaporated,[7] and the deposition of crystals of piperine has ceased. Lastly, separate the oleoresin from the piperin by expression through a muslin strainer.[8]

Keep the oleoresin in a well-stoppered bottle.[9]

1900

Oleoresina Piperis

Oleoresin of Pepper

Pepper,[1] in No. 40 powder,[2] *five hundred grammes* .....500. Gm.
Acetone,[3] *a sufficient quantity.*

Introduce the pepper into a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and a receptacle for volatile liquids.[4] Pack the powder firmly, and percolate slowly with acetone, added in successive portions, until the pepper is exhausted.[5] Recover the greater part of the acetone from the percolate by distillation on a water-bath,[6] and, having transferred the residue to a dish, set this aside in a warm place, until the remaining acetone has evaporated,[7] and the deposition of crystals of piperin has ceased. Lastly, separate the oleoresin from the piperin by straining through purified cotton.[8] Keep the oleoresin in a well-stoppered bottle.[9]

Average dose.—0.030 Gm. = 30 milligrammes (½ grain).

1910

Oleoresina Piperis

Oleoresin of Pepper

Oleores. Piper.

Pepper,[1] in No. 40 powder,[2] *five hundred grammes* .....500. Gm.
Ether,[3] *a sufficient quantity.*

Place the pepper in a cylindrical glass percolator, provided with a stop-cock, and arranged with a cover and a receptacle for volatile liquids.[4] Pack the powder firmly, and percolate slowly with ether, added in successive portions until the drug is exhausted.[5] Recover the greater part of the ether from the percolate by distillation on a water-bath,[6] and, having transferred the residue to a dish, set this aside in a warm place until the remaining ether has evaporated,[7] and the deposition of piperine has ceased. Lastly, separate the oleoresin from the piperine by straining through purified cotton.[8] Keep the oleoresin in a well-stopped bottle.[9]

Average Dose.—Metric, 0.03 Gm.—Apothecaries, ½ grain.

1) For a description of the official drug, see page 1117 under "Drug used, its collection, preservation, etc."

2) The last two editions of the Pharmacopœia have specified that the drug be in the form of a No. 40 powder for percolation. Previous editions, with the exception of that of 1850, in which the degree of fineness was not stated, required that a fine

powder (No. 60) be used for this purpose. The coarser powder possesses the advantages of being more readily produced and of being better adapted to the rapid exhaustion of the drug.

3) The solvents which have been experimented with in the preparation of this oleoresin are alcohol, ether, acetone, benzin and petroleum ether. Of these, ether has proven to be the most satisfactory and is the solvent specified for this purpose by the present Pharmacopœia. Acetone, which was directed to be used by the Pharmacopœia of 1900, like alcohol, is unsatisfactory as the large amount of extractive matter obtained interferes with the separation of the piperine. Benzin or petroleum ether, on the other hand, dissolves piperine but slightly and, therefore, yield a product low in piperine content. See tables on page 1134.

4) For a description of percolators adapted to the use of volatile liquids, as specified for use in this connection by the Pharmacopœia, see Part I under "Apparatus used."

5) With respect to the manner of exhausting the drug, it is thought that the process of continuous extraction would be a distinct improvement over the present pharmacopœial method. The reasons for this statement have already been given in the comments of the preceding oleoresins and need not be repeated here.

6–7) As this oleoresin does not appear to undergo any noticeable changes upon exposure to the air, except to lose a small amount of volatile oil, the conditions under which the solvent is removed from the percolate are not as important as in the case of the other oleoresins. The time necessary to complete the preparation, however, can be considerably shortened if the operation is completed at the temperature of the water bath, for which reason, this procedure is thought to be justified.

8) The Pharmacopœia directs that the mixture obtained on evaporating the solvent from the percolate be allowed to stand until the deposition of the piperine is complete and that the latter then be separated from the liquid portion by straining through purified cotton. The object to be attained in allowing the piperine to deposit is not understood as it has been found in actual practice that the liquid portion does not sep-

arate as a rule, but that the whole sets to form a semi-solid mass owing to the large amount of piperine present. The means by which the separation of the piperine was accomplished in the laboratory appears to be more rational and is as follows: the mixture was heated on the water bath until the portion constituting the oleoresin was quite fluid when it was filtered through cotton with the aid of a suction pump. The piperine which deposited from the filtered oleoresin on cooling was finally separated by decantation.

9) As the oleoresin loses volatile oil on exposure to the air, it should be kept in well-stoppered bottles.

Yield.

The yield of oleoresin to acetone or ether is about 4.5 to 6.5 per cent. With petroleum ether, a yield of 3.2 per cent. was obtained in the laboratory. Aside from the effect which the solvent has upon the amount of the oleoresin obtained, the temperature at which the piperin is separated is a factor to be considered. The higher the temperature at which this is accomplished, the greater the amount of piperine remaining in solution and the greater the yield of finished product, and *visa versa.*

In the tables which follow, the yield of total extract is frequently reported as oleoresin. These reports should not be confused with those pertaining to the official preparation, which consists of the liquid portion only, the precipitated piperine and other insoluble material having been removed. Data of this kind have been included here for the sake of comparison with results of a like nature obtained in the laboratory and in order to point out the erroneousness of such reports.

TABLE 123—*Yield of oleoresin as reported in the literature.*

Date	Observer	Yield of oleoresin to-				
		Alcohol	Acetone	Ether	Other solvents	
		Per ct.	Per ct.	Per ct.	Per cent.	
1888	Trimble			8.79	Benzin 2.80	Represents total yield of extractive matter.
1892	Beringer		9.97			
			5.93	5.00 to 6.70		Yield of oleoresin.
	Sherrard			8.84		Reported as yield of oleoresin. (1)
				9.64		
1903	Ballard			5.50		Pepper from the Indies. Total extract.
				8.70		Pepper from Guadeloupe. Total extract.
				10.15		Pepper from the coast of Dahomey. Total extract.
1913	Patch			10.04		Represents total yield of extract.
				10.87		
				12.88		
"	Engelhardt				Solvent (?) 9.20	Reported as yield of oleoresin. (1)
					10.60	
					11.00	
					12.50	

(1) Undoubtedly represents total extract.

TABLE 124—*Yield of oleoresin as obtained in the laboratory.*

Date	Observer	Yield of oleoresin to—				Remarks
		Alcohol	Acetone	Ether	Petrol. ether.	
		Per ct.	Per ct.	Per ct.	Per ct.	
1916	Du Mez	11.10	10.65	10.42	7.14	Represents total extract.
"	"	5.32	5.09	4.44	3.20	Represents the portion decanted and washed from the deposited piperine.

Chemistry of the Drug and Oleoresin.

Tabulation of Constituents.

The chemistry of black pepper has been the subject of a number of investigations[1] conducted during the past century. As a result of these investigations, the presence of the following substances of pharmaceutical interest has been established: volatile oil, piperine, resin, starch, coloring matter and inorganic constituents. In addition to the foregoing, the presence of fatty oil, piperidine and methyl pyrroline has been reported. The following are stated by Kayser and others[2] to be present in the oleoresin when prepared with ether:

Resin	Volatile Oil
Coloring Matter	Fatty Oil
Ash	Piperine

Occurrence of Description of Individual Constituents.

Volatile Oil:[3] According to the report of Schimmel and Company,[4] the volatile oil of pepper is a colorless or yellowish-green liquid, having a phellandrene-like odor. At 15°C, the specific gravity is given as 0.88 to 0.905 and the angle of rotation in a 100 millimeter tube as -5° 2′ to + 2° 27′. It is stated to be soluble in 15 parts of alcohol (90 per cent).

Early attempts to determine the composition of the oil were made by Dumas,[5] and Soubeiran and Capitaine.[6] In 1887, Eberhardt[7] isolated a l-terpene which he failed, however, to

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[1] Among those who have reported more or less complete analyses of pepper the following may be mentioned: Pelletier, Ann. de Chim. et de Phys. (1821), 16, p. 337; Luca, Tschenb. f. Scheidekünstl. u. Apoth. (1822), 43, p. 81; H. Röttger, Arch. f. Hygiene (1886), 4, p. 183; Richardson, U. S. Dept. of Agric. Bull. No. 13, (1887), p. 206; Johnstone, Chem. News (1888), 58, p. 235; Kayser, Chem. Centralb. (1888), 59, p.261; Weigle, Apoth. Ztg. (1893), 8, p. 468; Hebebrand, Zeitschr. Unters. Nahr. u. Genussm. (1896), p. 345; Winton, Ogden and Mitchell, Ann. Rep. Conn. Exp. Sta. (1898), p. 198; Balland, Journ. de Pharm. et de Chim. (1903), 157, p. 296.

[2] Kayser, Weigle, Balland, *l. c.*

[3] The description of the oil as here given is for that obtained from the fruit by distillation with steam.

[4] Schimmel & Co., Semi-Ann. Rep., Oct. 1893, p. 34.

[5] Ann. d. Chem. (1835), 15, p. 159; Journ. f. prakt. Chem. (1835), 4, p. 434.

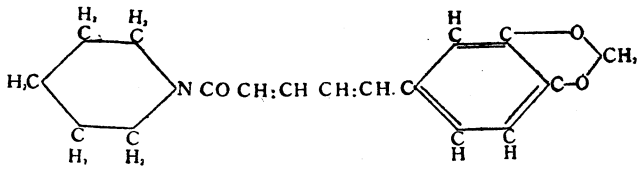
[6] Journ. de Pharm. et de Chim. (1840), 26, p. 83.

[7] Arch. der Pharm. (1887), 225, p. 515.

identify. Schimmel and Company[8] have reported the presence of phellandrene and cadinene.

From 0.70 to 2.2 per cent. of volatile oil has been obtained from the fruits by steam distillation.[9]

Piperine.[10] Piperine ($C_{17}H_{19}NO_3$) was first isolated by Oersted in 1819.[11] It is a weak base crystallizing from alcohol in colorless, shining, four sided prisms, the melting point of which is 128 to 129°C. It is slightly soluble in boiling water, readily soluble in alcohol, ether, chloroform, benzene and volatile oils, slightly soluble in petroleum ether. When acted upon by solutions of the alkalies, it is hydrolyzed breaking down into piperidine and piperic acid. Its constitution is represented by the following structural formula:[12]



The quantity of piperine present in the fruit of black pepper as obtained on the market varies to a considerable extent. This variation is very probably due in greater part to natural causes, such as the age of the fruit before harvesting, climatic conditions under which grown, *et cetera.*[13] The yield is variously stated as being from 4.05 to 13.02 per cent.[14]

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[8] *l. c.*

[9] A yield of 0.7 to 1.69 per cent. of volatile oil is reported by C. H. Richardson *l. c.* W. Johnstone obtained 0.98 to 1.87 per cent. Analyst (1889), 14, p. 41. G. Teyxeira and B. Ferrucio give the yield as 1.4 per cent. Bull. Chim. Pharm. (1900), 38, p. 534; Chem. Centralb. (1900), 71, p. 736. Schimmel & Co. (l. c.) report the yield as 1.3 to 2.2 per cent.

[10] Rochleder, Ann. d. Chem. (1845), 54, p. 255; Babo and Keller, Journ. f. prakt. Chem. (1857), 72, p. 53; Rugheimer, Ber. d. deutsch. chem. Ges. (1882), 15, p. 1390.

[11] Schweitz. Med. Journ. (1819), 29, p. 80; Buchner, Repert. f. die Pharm. (1820), 10, p. 127.

[12] Ladenburg and Scholtz, Ber. d. deutsch. chem. Ges. (1894), 27, p. 2958.

[13] Caseneuve and Caillot report the piperine content to be as follows: Sumatra, 8.10 per cent; Singapore, 9.15 per cent; Penang, 5.24 per cent. *l. c.* G. Graff gives the following percentages of ether soluble nitrogenous matter as piperine: Java, 5.85 to 9.5 per cent.; Lampong, 5.13 to 7.09 per cent.; Penang, 9.12 to 9.42 per cent.; Saigon, 6.16 per cent.; Singapore, 11.08 per cent. Zeitschr. f. öffentl. Chem. (1908), 14, p. 425.

[14] W. Johnstone obtained 5.21 to 13.03 per cent of piperine from nine samples of black pepper, *l. c.*

C. Heisch gives the yield as 4.05 to 9.38 per cent. Analyst (1886), 11. p. 186.

F. Stevenson reports the presence of 7.14 per cent. or piperine. *Ibid.* 12, p. 144.

Resin. The presence of 1.25 to 2.08 per cent. of resin in black pepper has been reported.[15] Buchheim,[16] the only investigator who appears to have attempted to isolate the same in sufficient purity to determine its composition, states that it is a condensation product of piperidine with an acid, to which he gives the name *Chavicinsaure.* He assigns the name *Chavicin* to this compound, and describes it as a yellowish-brown mass soluble in alcohol, ether, petroleum ether and the other common solvents.

Coloring Matter. The green coloring matter in pepper is stated to be chlorophyll.[17] The brown coloring matter observed in the ethereal or alcoholic extracts has not been identified.

Fatty Oil.[18] The presence of a fatty oil in black pepper must be considered doubtful at the present time. Hirsch[19] states that a microscopical examination of the fruit revealed the presence of a fatty oil in the endosperm. Kayser,[20] Weigle,[21] and others mention fatty oil as one of the constituents. None of these investigators, however, appear to have isolated the oil in a pure state or to have described it in detail. Ditzler,[22] who made this matter the subject of a special investigation, concluded that glycerides were absent. Likewise, Gerock[23] could obtain no fat from white pepper.

Piperidine.[24] Piperidine has been named as a constituent of black pepper by Johnstone,[25] who found the average content of nine samples to be 0.56 per cent. Kayser[26] disputes the findings of Johnstone and states that the base obtained by distillation is ammonia.

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[15] Teyxeira and Ferrucio give the resin content as 1.25 per cent., F. Stevenson as 1.44 per cent. *l. c.*

F. Blyth reports the presence of 1.7 to 2.08 per cent. ***Foods, Their Composition and Analysis*** (1903), p. 496.

[16] Buchner's n. Repert. f. Pharm. (1876), 25 p. 335; Pharm. Journ. 1876, 36, p. 315.

[17] Arthur Meyer, *Das Chlorophyllkorn,* Leipzig (1883), p. 2.

[18] In the literature on food chemistry, the non-volatile ether extract is usually spoken of as fat or fatty oil. See Winton, Ogden and Mitchell, *l. c.*

[19] Flueckiger, ***Pharmakognosie des Pflanzenreiches*** (1891), p. 914.

[20] *l. c.*

[21] *l. c.*

[22] Arch. d. Pharm. (1886), 224, p. 103.

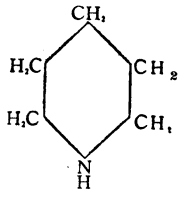
[23] *Ibid.*

[24] As piperidine is one of the products obtained when piperine is hydrolysed, it is quite probable that it is not a normal constituent of the fruit but is formed when the powdered material is subjected to distillation.

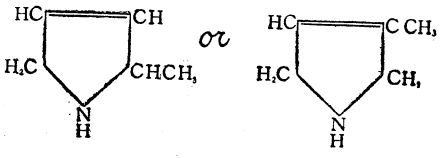
[25] *l. c.*

[26] *l. c.*

Piperidine is a colorless limpid liquid having a specific gravity of 0.8591 at 25°C, and boiling at 106.3°C.[27] It is stated to have an odor resembling both, that of ammonia and pepper. It is a powerful base behaving generally like ammonia in its action on the metallic bases. It is soluble in all proportions in alcohol or water. It has the following structural formula.[28].



Methyl-Pyrroline. Pictet and Court[29] report the occurrence of 0.01 per cent of methyl-pyrroline in black pepper obtained from Singapore. The exact constitution has not been determined, but the authors are of the opinion that it is a C-methyl pyrroline represented by one of the following formulas:



Ash. The basic elements, K, Na, Mg, Ca, Fe and Mn, combined with the acids, HCl, H_3PO_4, H_2SO_4, H_2SiO_3 are the components of the ash of black pepper as determined by Rottger[30] and others.[31]

The average ash content of black pepper is stated by Blyth[32]

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[27] Perkin, Chem. Soc. Journ. (1889), 55, p. 699.

[28] Hofmann, Ber. der. deutsch. chem. Ges. (1879), 12, p. 985; Koenigs, *Ibid.*, p. 2341; Ladenburg, *Ibid.* (1885), 18, pp. 2956 and 3100.

[29] Pictet states that he was able to isolate pyrrolidine and N-methyl pyrroline from various leaves by steam distillation after treatment with sodium carbonate. He is of the opinion that the methyl pyrrolines undergo rearrangement forming pyridine and quinoline rings, thus giving rise to the more complex alkaloids. Arch. Sci. Phys. Nat. (1905), 19, p. 329; Ber. d. deutsch. chem. Ges. (1907), 40, p. 3771.

[30] Arch. Hyg. (1886), 4, p. 183. „

[31] Blyth, Chem. News (1874), 30, p. 170.

[32] *Ibid.*

to be 4.845 per cent. As high as 8.99 per cent. has been reported.[33].

Constituents of Therapeutic Importance

The oleoresin of pepper is said to be used chiefly in the South, where it is administered with quinine in the treatment of "intermittent fever." Its value in this connection is accounted for by the presence of piperine which has been shown to be an active antiperiodic.[1] Piperdine and methyl pyrroline, if present, would impart similar properties,[2] while the composition of the contained volatile oil would indicate a carminative action.

Physical Properties

Color: The color of the oleoresin, when the latter was spread out in a thin layer on a white porcelain surface, was observed to be a greenish-brown, closely resembling that of the oleoresin of cubeb when prepared from the ripe fruits. The so-called oil of black pepper, sometimes sold as a substitute for the official oleoresin, is stated to be considerably darker in color due to the removal of the greater part of the volatile oil.

Odor: The odor, while slight, resembles that of ground pepper.

Taste: The taste is sharp and spicy, the sharpness becoming more noticeable after the oleoresin has been retained in the mouth for a short time.

Consistence: The oleoresin is a thick, sticky liquid which can only be poured with difficulty. The fluidity is greatly increased by heating the preparation on a water bath.

Solubility: The oleoresin is completely soluble in alcohol, ether, acetone, chloroform, carbon disulphide and glacial acetic acid. It is only partially soluble in petroleum ether and is insoluble in water.

Specific gravity: The specific gravity of the oleoresin is fairly constant, only, when similar conditions with respect to

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[33] Heish reports the ash content of 8 samples of black pepper to be from 4.35 to 8.99 per cent. Analyst (1886), 11, p. 186. Others who have reported on the ash content of pepper are Bergman, Zeitschr. f. Analyt. Chem. (1882), 21, p. 535, and von Raumer, Zeitschr. angew. Chem. (1893), p. 453.

[1] Wood, *Therapeutics, Principles and Practice,* (1908), p. 482.

[2] Tunnicliffe and Rosenheim, Centralbl. f. Physiol. (1902), 16, p. 93.

temperature have been observed during the separation of the precipitated piperine. A comparatively slight difference in temperature causes a considerable variation in the amount of the latter constituent retained in solution, which results in a corresponding variation in the specific gravity of the finished product. This effect is further noticed in connection with the menstruum employed in extracting the drug, e. g. petroleum ether which is a poor solvent for piperine yields an oleoresin relatively low in specific gravity. With respect to the commercial samples examined, a low specific gravity was, in one instance, found to be due to the presence of unevaporated solvent. The tables which follow show the specific gravity of the samples examined in the laboratory.

TABLE 125—*Specific gravities of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Specific gravity
				At 25° C
1.....	1916	DuMez.....	Alcohol.....	1.069
2.....	"	"	Acetone	1.083
3.....	"	"	Ether.....	1.056
4.....	"	"	Petrol, ether	0.981

TABLE 126—*Specific gravities of commercial oleoresins.*

Sample No.	Date	Observer	Source	Specific gravity
				At 25° C
1.....	1916	DuMez.....	Squibb & Sons.....	0.985[1]
2.....	"	"	Sharp & Dohme.....	1.061

[1] The odor of ether was very noticeable.

Refractive index: The refractive index of this preparation as observed in the laboratory was not constant, varying from 1.521 to 1.696. From an inspection of the first of the tables which follow, it would appear that this variation was a result of the influence of the solvent employed in extracting the drug. While the solvent undoubtedly exerts an influence in this connection, it does so indirectly, that is, through its effect on the piperine content.[1] The latter, however, is also influenced by

[1] See discussions under "Piperine content" and "Yield of oleoresin," respectively.

the temperature at which the preparation is finished—the temperature at which the liquid oily portion, which constitutes the official oleoresin, is separated from the deposited material, including the excess of piperine. In the case of commercial samples, the piperine content and, therefore, the refractive index may also be affected by the presence of unevaporated solvent. The results obtained in the laboratory in the determination of this property are given in the tables which follow.

TABLE 127—*Refractive indices of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Refractive index
				At 25° C
1.....	1916	DuMez.....	Alcohol.....	1.559
2.....	"	"	Acetone	1.696
3.....	"	"	Ether.....	1.562
4.....	"	"	Petrol, ether....	1.521

TABLE 128—*Refractive indices of commercial oleoresins.*

Sample No.	Date	Observer	Source	Refractive index
				At 25° C
1.....	1916	DuMez.....	Squibb & Sons.....	1.501(a)
2.....	"	"	Sharp & Dohme.....	1.560

(a) Contained ether.

Chemical Properties.

Loss in weight on heating: A loss in weight varying from 9.49 to 11.52 per cent. was obtained for the laboratory preparations, when heated at 110°C, showing that the nature of the solvent employed in extracting the drug has but little influence on this property. With respect to the commercial samples examined, the loss was much greater, being as high as 32.64 per cent. in one case. The comparatively great loss in the latter instance was due to the presence of unevaporated solvent (ether.) The results obtained in the determination of this constant in the laboratory follow.

TABLE 129—*Laboratory preparations—loss in weight on heating.*

Sample No.	Date	Observer	Solvent	Per cent. of loss on heating
				At 110° C
1	1916	DuMez	Alcohol	10.34
2	"	"	Acetone	11.52
3	"	"	Ether	10.91
4	"	"	Petrol, ether	9.49

TABLE 130—*Commercial oleoresins—loss in weight on heating.*

Sample No.	Date	Observer	Source	Per cent. of loss on heating
				At 110° C
1	1916	DuMez	Sharp & Dohme	17.52
2	"	"	Squibb & Sons	32.64[1]

[1] Unevaporated solvent (ether) was present.

Ash content: The ash determinations made on the oleoresins prepared in the laboratory show that the solvent employed in their preparation is the chief factor influencing the results obtained. The official product, in the making of which ether was the solvent used, yielded 0.11 per cent. of ash, which was about the percentage yield obtained for one of the commercial samples examined. The other commercial oleoresin gave 0.29 per cent. of ash indicating the use of acetone in its preparation. Both samples contained copper, apparently, however, in quantities too small to noticeably affect the weight of the ash. The results of the determinations made in the laboratory follow:

TABLE 131—*Ash contents of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Per cent of ash
1	1916	DuMez	Alcohol	0.43
2	"	"	Acetone	0.32
3	"	"	Ether	0.11
4	"	"	Petrol, ether	0.05

TABLE 132—*Ash content of commercial oleoresins.*

Sample No.	Date	Observer	Source	Per cent. of ash	Foreign constituents
1.....	1916	DuMez.....	Squibb & Sons...	0.12 (a)	Copper
2.....	"	"	Sharp & Dohme..	0.29	"

[1] Contained ether.

Acid number: The acid number of the oleoresin when prepared with alcohol, acetone or ether was found to be about 19. In the case of the two commercial samples examined, however, the values obtained differed to a considerable extent, being 19.2 in one instance and 27.5 in the other. As the preparation represented by the first number contained considerable unevaporated solvent, this difference can be accounted for in part. The high values obtained for the commercial samples are thought to be due to their relatively low piperine content or to a partial decomposition of the resin. The values obtained for this constant in the laboratory follow.

TABLE 133—*Acid numbers of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Acid number
1	1916	DuMez.....	Alcohol	19.2
2	"	"	Acetone	19.0
3	"	"	Ether	18.9
4	"	"	Petrol.-ether.....	15.1

TABLE 134—*Acid numbers of commercial oleoresins.*

Sample No.	Date	Observer	Source	Acid number
1	1916	DuMez.....	Squibb & Sons	19.2 (a)
2	"	"	Sharp & Dohme.....	27.5

(a) Contained ether.

Saponification value: As will be observed in an inspection of the first of the tables which follow, the saponification value of the oleoresin varies with the solvent employed in its preparation. This appears to be due principally to the effect which the nature of the solvent has upon the piperine content of the

finished product, e. g. the piperine content of the preparation made with acetone was found to be 54.36 per cent and the saponification value 88.6, while the oleoresin when prepared with petroleum ether, contained only 15.06 per cent. of piperine and gave a saponification value of 109.5. Other influences, besides the nature of the solvent, affecting the piperine content may likewise produce a variation in the saponification value, e. g. the temperature at which the preparation is made and the presence of unevaporated solvent in the finished product. The latter may also have a direct influence. The saponification values as found for the oleoresins examined in the laboratory are given in the following tables.

TABLE 135—*Saponification values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Saponification value
1.....	1916	DuMez.....	Alcohol.....	63.7
2.....	"	"	Acetone.....	74 9
3.....	"	"	Ether.....	83 5
4.....	"	"	Petrol, ether.....	86.8

TABLE 136—*Saponification values of commercial oleoresins.*

Sample No.	Date	Observer	Source	Saponification value
1.....	1916	DuMez.....	Sharp & Dohme.....	66.3
2.....	"	"	Squibb & Sons.....	73.7(a)

(a) Contained ether.

Iodine value: Iodine values ranging from 88.6 to 95.4 were obtained for this oleoresin when acetone, alcohol or ether were the solvents employed in its preparation. This variation is due to the difference in the piperine content of these oleoresins as a result of operating under different conditions of temperature when preparing the same, as well as to the nature of the solvent. In addition to these influences, the presence of unevaporated solvent must also be taken into consideration in the case of the commercial samples, as is indicated by the values given in the following tables.

TABLE 137—*Iodine values of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Iodine value
1	1916	DuMez.	Alcohol	90.0
2	"	"	Acetone	88.6
3	"	"	Ether	95.4
4	"	"	Petrol.-ether	109.5

TABLE 138—*Iodine values of commercial oleoresins.*

Sample No.	Date	Observer	Scource	Iodine value
1	1916	DuMez	Sharp & Dohme	83.7
2	"	"	Squibb & Sons	89.9 (a)

(a) Contained ether.

Special Quantitative Tests.

At the present time, there does not appear to be a method in use for the evaluation of this oleoresin. As its therapeutic properties are due, in greater part at least, to its piperine content,[1] a quantitative method for the estimation of this constituent appears to offer the best means of determining its quality.

Method for the Estimation of the Piperine Content.

In the laboratory, the amount of piperine present was computed from the nitrogen content of the oleoresin, the latter being determined by the Gunning-Arnold[2] method. The results obtained are given in the following tables:

TABLE 139—*Piperine content of oleoresins prepared in the laboratory.*

Sample No.	Date	Observer	Solvent	Piperine content
				Per cent.
1	1916	DuMez	Alcohol	47.0
2	"	"	Acetone	54.3
3	"	"	Ether	51.3
4	"	"	Petrol, ether	15.1

[1] See under "Constituents of therapeutic importance."
[2] Bull. No. 107, Bur. of Chem. (1912), p. 162.

TABLE 140—*Piperine content of commercial samples.*

Sample No.	Date	Observer	Source	Piperine content
				Per cent.
1.....	1916	DuMez.....	Sharp & Dohme.....	27.3
2.....	"	"	Squibb & Sons.....	33.8

The laboratory samples were prepared and tested during the warm months of summer, which accounts for the high piperine content. A very considerable amount of the latter precipitated out during the colder months which followed. It is, therefore, thought that the results obtained in the case of the commercial products are the more typical.

Adulterations.

Copper was found to be present in all of the commercial samples examined. See under "Ash content."

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Planche 1823

Von den pharmaceutischen Zubereitungen des Lupulins. Mag. f. Pharm., 1, p. 183. [Trommsdorff's n. Journ. d. Pharm., 7, p. 345.]

A method for preparing the alcoholic tincture of lupulin is given. It is further stated that an extract similar in all respects to the resin said to have been isolated by Ives results when the alcohol is removed from the tincture by evaporation.

Geiger, Ph. L. 1824

Versuche über die chemische Zusammensetzung der Wurzel des maennlichen Farrenkrauts, *Polypodium (Aspidium, Nephrodium) Filix Mas.*

Mag. f. Pharm., 7, p. 38.

The article is a review of Morin's analysis of the rhizome of male fern with a note pointing out that Morin was not the first investigator to make such an analysis, but that Gebhardt had already published an analysis of the same in 1821 in an inaugural dissertation delivered at Kiel. Gebhardt is stated to have used ether for extracting the "oil."

Morin 1824

Sur la composition chimique de la racine de fougère mâle, *Polypodium filix mas* Linn.

Journ. de Pharm. et de Chim., 10, p. 223. [Mag. f. Pharm., 7, p. 38.]

In making a chemical examination of the male fern rhizomes, the author used the method of selective solvents. Upon extracting with ether, as the first solvent, and subsequently evaporating of the ether, a thick green fatty oil was obtained. The author considers this fatty substance the active principle.

Meli 1825

Neue Erfahrungen und Beobachtungen ueber die Art, das Alkaloid und das scharfe Oel des Pfeffers zu gewinnen.

Trommsdorff's n. J. d. Pharm., 11, p. 174. [Bull. de scien. math., phys. et chim., 1825, p. 191.]

It is stated that more than an ounce and a half of piperine and about four ounces of a sharp tasting oil were obtained from three pounds of black pepper by extraction with alcohol.

Peschier, Ch. 1825

Oel des maennlichen Farrenkrauts *(Aspidium Filix Mas)*, ein sehr vorzuegliches und sicheres Mittel gegen den Bandwurm.

Biblioth. univers., Nov. 1825, p. 205. [Mag. f. Pharm., 13, p. 188.]

The so called oil, *Huile de Fougère Mâle,* is directed to be prepared by extracting the powdered male fern rhizomes with ether and subsequently removing the ether by warming gently.

Buchner, A. 1826

Extractum Filicis maris resinosum.

Repert f. d. Pharm., 23, p. 433.

The preparation of this extract by means of alcohol instead of ether is recommended. The product thus obtained is spoken of as an *Extractum resinosum.* The *Huile de Fougère* of Peschier is spoken of as the *harzhaltiges Oel.* A chemical analysis of the extract is also given.

von Esenbeck, Nees 1826

Farrnkrautwurzelextrakt.

Arch. d. Pharm., 19, p. 153.

The extract is reported to have been prepared by the process of maceration, ether being the solvent employed. Four ounces of rhizomes gathered in August gave 108 grains of extract.

———————— 1827

Verhandlungen des pharmaceutischen Vereins in Wuertemberg. Repert. f. d. Pharm., 26, p. 441.

Zeller is stated to have prepared the *Extractum radicis Filicis maris resinosum* according to the method suggested by Buchner; extraction with alcohol. The extract obtained in this manner from rhizomes gathered in September amounted to 30 per cent. of the air dried drug.

Batso, V. 1827

Dissertatio inaugur. chemica de Aspidio filice mare Quam cons. et auctor. praes et direct. etc., pro summis in scient. et arte chemica honor. et doct. laurrite cappess. in univers. vindobon. publ. erudit, disq. submittit Valentinus Batso, N. H. Debreczino Bibariensis p. 37, 8. Vindobonae, typis Antonii Pichler. 1826. [Trommsdorff's n. Journ. d. Pharm., 14, 2, p. 249.]

In addition to oil, resin and fatty wax, the author finds a free acid and an alkaloid in the ethereal extract of male fern. He calls the acid *Acidum filiceum* and the alkaloid *Filicina*. He attributes the activity of the extract to these two substances.

Brandes, R. 1827

Ueber das Extractum oleo-resinosum Filicis.
Arch d. Pharm., 21, p. 253.

The physical properties of the extracts obtained by extracting male fern rhizome with ether and with *Liquor anodynus*, respectively, are described.

Buchner, A. 1827

Zur medicinischen und chemischen Geschichte der Filix mas.
Repert. f. d. Pharm., 27, p. 337.

The author speaks of the ethereal extract of male fern as the *Extractum oleoso-resinosum Filicis maris*. It is stated to contain a volatile oil, a green fatty oil, a fatty wax, a brown resin and a volatile acid (probably acetic acid.)

Van Dyk 1827

Ueber das Oleum Filicis maris.
Arch. d. Pharm., 22, p. 141.

Two ounces of powdered male fern rhizome gave 70 grains of ethereal extract, while 8 ounces of the rhizome yielded 3 ounces of extractive matter

to alcohol. The extract prepared with ether is stated to be dark olive-green in color and of the consistence of honey, that prepared with alcohol greenish-brown in color and much thicker.

Geiger, Ph. L. 1827

Analytische Versuche mit der Wurzel des maennlichen Farrenkrauts und Darstellung des Oels *(Ol. Filicis Maris)* aus derselben.

Mag. f. Pharm., 17, p. 78.

The ethereal extract when prepared from green rhizome, by extraction with ether in a *Realsche Presse* is said to be a yellowish-green oily substance.

An analysis of this extract showed the presence of 30 per cent. of resinous material soluble in alcohol, 50 per cent. of a fixed oil and a considerable amount of volatile substances.

Tilloy 1827

Bereitungsart des Oels des maennlichen Farrenkrauts.

Journ. de Chim. med., 3, p. 154. [Geiger's Mag. f. Pharm., 18, p. 157.]

The so-called oil of male fern is directed to be prepared by extracting the rhizome with alcohol. The alcoholic liquor thus obtained is treated with lead subacetate, filtered, and the solvent removed by distillation. The resulting oil is further purified by dissolving in ether and evaporating.

Dublanc, H. 1828

Extrait oléorésineux de Cubebe.

Journ. de Pharm. et de Chim., 14, p. 41.

The author's method for preparing the oleoresinous extract consists in distilling off the volatile oil with water, exhausting the dried marc with alcohol, evaporating off the alcohol, and mixing the residue so obtained with the volatile oil.

Meylink 1828

Ueber das Extractum oleo resinosum Filicis.

Arch. d. Pharm., 25, p. 243.

Two ounces of the powdered male fern rhizome are reported to have yielded 58 grains of a dark green, oily extract to ether.

Oberdoerffer 1826

Ueber die Darstellung des Cubeben Extracts.

Arch. d. Apoth. Ver., 24, p. 178.

In the method of preparation, the oil is first obtained by steam distillation, the marc remaining in the still, after drying, is then extracted with

alcohol. The residue remaining after removing the alcohol by evaporation is mixed with the volatile oil, this mixture constituting the so-called extract.

Peschier, Ch. 1828

Ueber mehrere schon frueher erschienene Analysen der Farrenkrautwurzel *(Aspidium filix mas L.)* und ueber die Gewinnung seines harzigen Oels.

Trommsdorff's n. Journ. d. Pharm., 17, p. 5.

The vermifuge properties of male fern are said to be due to its oléorésine *(oelharz)* content. This the author prepares by extracting the drug with ether and subsequently evaporating the solvent. (p. 8.) It is further stated that this oleosésine remains perfectly homogenous after months if prepared from freshly gathered rhizomes, but deposits a white granular substance when old rhizomes are used (p. 9.)

According to the author's analysis the oleoresine consists of a volatile aromatic oil, a fatty oil, resin, stearin, green and red coloring materials, acetic and gallic acids.

Winkler, F. L. 1828

Einige Worte ueber die Bereitung des Ol. Filic. Maris.

Geiger's Mag. f. Pharm., 22, p. 48.

The "oil" extracted with ether is said to be a mixture of oil, resin and oxidized tannin. Twelve ounces of rhizomes gathered in February yielded 15 drachms of extract. Two drachms of this extract yielded 43 grains of fatty oil.

Allard 1829

Note sur l'huile de fougère.

Journ. de Pharm. et de Chim., 21, p. 292.

The powdered rhizome of the male fern is directed to be extracted with alcohol and the alcoholic extract after evaporating off the solvent, washed with water. The extract is then further purified by solution in ether and subsequent evaporation.

Carpenter, G. W. 1829

Observations and Experiments on Peruvian Bark.

Silliman's Am. Journ., 16, p. 28. [Buchner's Repert f. d. Pharm., 34, p. 446.]

In the discussion of the therapeutic uses of the various constituents of Peruvian bark, it is stated that Dr. Chapman of Philadelphia prescribed piperin and oil of pepper in combination with quinine. The oil of pepper is said to be the more active therapeutically, one drop of oil being equivalent to three grains of piperin (p. 39.)

Haendess 1829

Ueber Ol. filicis maris.

Arch. d. Pharm., 28, p. 212.

Four ounces of powdered male fern rhizomes gave 170 grains of ethereal extract. Upon treating this ethereal extract with alcohol, 20 grains were dissolved leaving a residue of 150 grains. The extract first obtained was of a brownish color, after treating with alcohol it assumed a beautiful green color.

Voget 1829

Notiz ueber Ol. filicis maris.

Arch. d. Pharm., 30, p. 104.

According to the author's method of preparing the *Oleum filicis maris*, the powdered male fern rhizome is first extracted with water. After drying the drug is then extracted with ether. Twenty-eight grains of a brownish-green extract were obtained from 9 drachms of the marc.

Schuppmann 1830

Extractum resinosum Seminis Cynae.

Buchner's Repert. f. d. Pharm., 35, p. 430.

The extract is directed to be prepared by macerating 4 ounces of the coarsly powdered seed with 16 ounces of ether for 3 or 4 days, decanting the liquid portion and evaporating to remove the solvent.

Béral 1834

Du principe du gingembre, et formules de plusieurs composes pharmaceutiques dont il est la base medicamenteuse.

Journ. de Chim. med., Pharm. et Tox., 10, p. 289.

The product obtained by extracting ginger with ether is designated *Piperoide du Gingembre.* It is directed to be prepared by extracting in a percolator 4 ounces of ginger with 6 ounces of ether, the rate of flow being so regulated that the operation will consume not less than 2 hours. It is stated that 5 scruples of *piperoide* were obtained in this manner, and that 6 scruples can be obtained if the residual ether is forced out by subsequent percolation with alcohol (40°). The *piperoide* is reported to be soluble in ether, anhydrous alcohol and oils.

_____ 1838

Extrait Oléo-Résineaux de Cubebe,

Journ. de Chim. med., Pharm. et Tox., 14, p. 366.

It is stated that Hausman prepared the oleoresinous extract of cubebs by macerating the powdered drug with ether (625 grams of ether to 250 grams of drug), then decanting and evaporating the ethereal solution to remove the solvent.

Hornung 1844

Pharmaceutisch-Chemische Mittheilungen.

Arch. d. Pharm., 89, p. 34.

Three ounces of fresh, powdered rhizomes of male fern, treated with 3 ounces of ether in a *Verdraengungsapparat*, are reported to have yielded 2 drachms of extract.

Luck, E. 1845

Ueber einige Bestandtheile der Radicis Filicis.

Ann. d. Chem., 54, p. 119.

Upon standing, the ethereal extract deposits a granular substance which can be obtained quite pure by pouring off the supernatant oily layer and washing the deposit rapidly with ether. The washed precipitate, dissolved in ether, crystallizes, upon evaporation, in rhombic leaflets, m. p. 160°C, insoluble in alcohol or water. The crystals were not obtained in a sufficient degree of purity to determine their chemical constitution.

Procter, Wm., Jr. 1846

On the Ethereal Extract of Cubebs.

Am. Journ. Pharm., 18, p. 167. [Pharm. Journ., 6, p. 319.]

At Dr. Goddard's request, Procter prepared a "true oleoresin" of cubebs by extracting the drug with ether. This method is regarded by him as a great improvement over the method of Soubeiran.

Bell 1846

Oleoresinous Extract of Cubebs.

Pharm. Journ., 6, p. 319.

The report includes a reprint of Procter's paper on the ethereal extract of cubebs and remarks by Ure, at whose request the preparation was made and by whom it is stated to have been used with success. A yield of 15 to 20 per cent. of oleoresin was obtained.

Procter, Wm., Jr. 1849

Remarks on oleoresinous ethereal extracts, their preparation and the advantages they offer to the medical practitioner.

Am. Journ. Pharm., 21, p. 114.

A method for the preparation of the following ethereal extracts is given: capsicum, chenopodium, semen contra, ginger, cardamom and pellitory. (p. 116.) Several forms of apparatus, including a tin percolator, Mohr's apparatus for extracting with ether and Gilbertson's diplacement apparatus are also described as being useful in this connection.

Bock, H. 1851

Analyse der Wurzel und des Wedels von Filix mas.

Arch. d. Pharm., 115, p. 257. [Am. Journ. Pharm., 24, p. 61.]

The powdered rhizomes were extracted with ether, specific gravity 0.720. By this means, 2000 grains of the powder are reported to have yielded 257.4 grains of an oily extract which was found to be composed of volatile oil, tannic acid, resin, fatty oil stearin and chlorophyll.

The author recommends preparing the oleoresin from fresh rhizomes as he states that the greater part of the volatile oil is lost upon drying and the fatty oil tends to become rancid.

Lucke, E. 1851

Ueber einige Bestandtheile der Wurzel von Aspidium Filix mas.

Jahrb. f. prakt. Pharm., 22, p. 130. [Arch. d. Pharm., 119, p. 178; Journ. de Pharm. et de Chim., 54, p. 476.]

A crystalline substance resembling the *Filicin* obtained by Trommsdorff eight years previous was isolated from the ethereal extract. The author calls it *Filixsaeure* and assigns it the formula $C_{20}H_{15}O_9$. It is further stated that extracts prepared with ether contain no tannic acid or sugar, but filix acid, pteritannic acid and fatty oil are present. Upon being saponified, the oil yielded *Filixolinsaeure* ($C_{42}H_{40}O_4 + HO$) and *Filosmylsaeure*.

Von der Marck, W. 1852

Ueber Verfaelschung der Radicis Filicis maris.

Arch. d. Pharm., 120, p. 87.

The botanical characteristics of other than the official species are enumerated and the manner in which they differ from those of male fern pointed out.

With respect to the male fern rhizomes, the author gives the following information: rhizomes gathered in September are the most active as they contain the greatest amount of oil. In the preparation of the extract, only that portion of the rhizome having borne fronds in the year collected, should be taken. The following results were obtained using different parts of the rhizome:

1.) Extract from portion of rhizome which had borne fronds the previous year. Yield 7.8% of a brownish-green extract.
2.) Extract from portion bearing fronds during year collected. Yield 8.2% of a beautiful green extract.
3.) Extract from portion which will develop fronds the coming year. Yield, 8.5% of a beautiful green extract.

Schuck, F. 1852

Ueber Cubebin

Buchner's n. Repert. f. d. Pharm., 1, p. 213. [Jahresb. d. Pharm., 12, p. 34.]

Cubebin is stated to be slowly deposited from the ethereal extract of cubeb upon standing. The extract prepared from 17 ounces of cubeb gave 15 grains of cubebin.

Bakes, W. C. 1853

Extract of Capsicum.

Am. Journ. Pharm., 25, p. 513.

The extract was prepared at the request of a physician. Dilute alcohol was employed for exhausting the drug. Eight ounces of Capsicum yielded two ounces of extract.

It is stated that a simple ointment which acts as a rubiafacient in 20 minutes may be prepared by mixing one drachm of this extract with 1 ounce of simple cerate.

Livermore 1853

Extract of Lupulin.

Am. Journ. Pharm., 25, p. 294.

The extract is directed to be prepared by maceration, using alcohol as the solvent. Sixty-six per cent. of extractive matter was obtained by this treatment.

Garot and Schaeuffele 1857

Rapport sur le produit oléo-résineux de cubebe obtenu a l'aide du sulfure de carbone.

Journ. de Pharm. et de Chim., 65, p. 368.

The article is on the experimental preparation of the oleoresin of cubebs with carbon disulphide. This solvent is proven to be worthless for this purpose on account of the large amount necessary for extracting the drug and on account of the difficulty in removing it by evaporation.

Landerer, X.

Ueber Cubebinum.

Arch. d. Pharm., 139, p. 302.

The so-called cubebin was obtained in the preparation of *Extractum Cubebarum oleoso-resinosum,* for which a mixture of ether and alcohol was used. Upon standing in a cool place, needle-like crystals adhering in groups were noticed. These crystals were soluble in warm alcohol and gave a carmine red color with sulphuric acid.

Procter, Wm., Jr. 1859

Formulae for the fluid extracts in reference to their more general adoption in the next pharmacopœia.

Proc. A. Ph. A., 8, p. 265. [Am. Journ. Pharm., 31, p. 548.]

It is suggested that the preparations made by extracting drugs with ether be designated as *Oleoresinae* in the next pharmacopœia. Methods for preparing the following oleoresins are described: "*Oleoresina Cardamoni, Oleoresina Carophylli, Oleoresina Cubebae, Oleoresina Filicis maris, Oleoresina Lupulinae, Oleoresina Piperis Nigri, Oleoresina Pyrethri, Oleoresina Sabinae, Oleoresina xanthoxyli* and *Oleoresina Zingiberis.*"

Girtle 1863

Extractum Cubebarum oleoresinosum.

Pharm. Centralh., 3, p. 608. [Canstatt's Jahresber., 23, p. 178.]

The preparation is an aqueous-alcoholic-ethereal extract with which the volatile oil, previously obtained by distillation, has been incorporated. It is said to represent the therapeutic properties of the entire drug. It is also stated that this preparation is not identical with the *Extr. Cub. oleoso-resinosum* of Landerer (1857.)

Parrish, E. 1864

On Capsicum.

Proc. A. Ph. A., 12, p. 262. [Jahresb. f. Pharm. 1, p. 68.]

In discussing the constituents of capsicum, Parrish refers to the ethereal extract as the oleoresin.

Bernatzik, W. 1865

Chemische Untersuchung der Cubeben mit besonderer Berueecksichtigung der Wirkungsweise ihrer wesentlichen Bestandtheile.

Buchner's Repert. f. d. Pharm., 14, p. 97. [Arch. Pharm., 179, p. 123.]

The article is a comprehensive discussion of the constituents of cubebs and their physiological and therapeutic action.

Based on the results of clinical experiments, it was concluded that the desired therapeutic principle is the resinous constituent and that the volatile oil, cubeb camphor and cubebin are practically of no therapeutic value. A method for preparing the *Extractum Cubebarum resinosum,* in which cubebs freed from the volatile oil are extracted with alcohol, is given (p. 139.)

Procter, Wm., Jr. 1866

Note on Oleoresina Cubebae.

Am. Journ. Pharm., 38, p. 210. [Pharm. Journ., 25, p. 620.]

The author reports the results obtained in the extraction of cubebs with ether, alcohol and benzine. The yield of oleoresin obtained was as follows: ether, 21.9 per cent., alcohol, 27 per cent., benzine, 16.5 per cent. (p. 212). The use of benzine in the preparation of this oleoresin is not recommended as it does not extract the cubebin completely.

Rittenhouse, H. N. 1866

On Substitutes for Ether and Alcohol in the Preparation of the Official Oleoresins.

Proc. A. Ph. A., 14, p. 208. [Am. Journ. Pharm., 38, p. 24.]

The feasibility of displacing the ether remaining in the exhausted drug with benzine, glycerine or water is discussed. From experiments conducted along this line, it was concluded that benzine would be the most preferable for this purpose. A working formula in which benzine is used to this end is described. Cubebs and ginger were the drugs employed in the experiments.

Paul, C. 1867

Sur l'extrait oléorésineux de cubebe.

Journ. de Pharm., et de Chim., 84, p. 197.

The extract is directed to be prepared by treating the powdered drug successively with water, alcohol and ether. The extract so prepared is said to contain all of the medicinal principles of the original drug.

Pile 1867

On the preparation of Oleoresins with benzine.

Proc. A. Ph. A., 15, p. 94.

One pound of cubebs percolated with 2 pounds of light benzine, specific gravity 86°, Beaumé, is stated to have yielded a trifle over 5 per cent. of oleoresin of a pale ash color.

It is further stated that neither benzine nor ether completely exhaust ginger, but that alcohol is a much better solvent for this purpose.

Heydenreich, F. V. 1868

On Cubebin and the Diuretic Principle of Cubebs.

Am. Journ. Pharm., 40, p. 42.

Eighty ounces of cubebs yielded, when extracted with ether, 19 ounces of oleoresin or nearly 24 per cent.

The results obtained in the administration of cubebin, the volatile oil and the soft resin are given.

Rump, C. 1869

Extractum Lupulini aethereum.

Arch. d. Pharm., 189, p. 232. [Jahresb. d. Pharm., 4, p. 39.]

The extract of lupulin is directed to be prepared by macerating the fresh drug with ether, decanting and evaporating the ethereal solution to the consistence of a thin syrup.

Squibb, E. 1869

Report of the Committee on the Pharmacopœia.

Proc. A. Ph. A., 17, p. 298.

The process of repercolation is stated to be well adopted to the preparation of the oleoresins and that it materially lessens their cost.

Lefort, M. J. 1870

Mémoire sur les extraits sulfocarboniques, et sur leur emploi dans la preparation des huiles medicinales.

Journ. de Pharm., 90, pp. 102–110.

In considering the methods of medicating oils, the author proposes preparing the extract of the leaves of *Conium maculatum* by exhausting the drug with carbon disulphide and subsequently removing the solvent by evaporation.

Hager, 1871

Zur Bereitung des Extractum Filicis aethereum.

Pharm. Centralh., 12, p. 457. [Am. Journ. Pharm., 44, p. 104.]

It is stated that, if the rhizomes are dried over burned lime previous to extraction, and anhydrous ether (Sp. gr. below 0.723) used as the extracting solvent, the oleoresin does not deposit on standing but remains perfectly clear.

Maisch, J. M. 1872

On the use of Petroleum-Benzine in Making Oleoresins.

Am. Journ. Pharm. 44, p. 208. [Pharm. Journ., 31, p. 968; Proc. A. Ph. A., 21, p. 138; Year-Book of Pharm., 10, p. 328.]

Petroleum benzine, sp. gr. 0.700, is stated to have been used to advantage in the preparation of the oleoresins of capsicum, cubeb and ginger, but the author regards the use of this solvent in the place of ether as inadmissable until it has been proven that the proximate principles not extracted by the benzine are medicinally inert.

Buchheim 1873

Fructus Capsici.

Vierteljahresschr, f. prakt. Pharm., 22, p. 507.

[Proc. A. Ph. A., 22, p. 106.]

The *capsicin* sold by the firm of E. Merck is stated to be the ethereal extract of the capsicum fruit.

Remington, J. P. 1873

On the Use of Petroleum Benzin for Extracting Oleoresinous Drugs.

Proc. A. Ph. A., 21, p. 592.

It is stated that benzin does not extract all of the diuretic principles from buchu and that its use for extracting the oleoresinous drugs is limited on account of its inflammability and great volatility.

Patterson, J. 1875

Aspidium marginale, Wildenow.

Am. Journ. Pharm., 47, p. 292.

The ethereal extract compared very favorably in appearance, taste and color with the best German oleoresin of male fern which could be obtained upon the market. An acid resembling the filicic acid of Luck was isolated therefrom.

Kruse 1876

Versuch einer vergleichenden Analyse der in den Monaten April, Juli und October 1874, in der Umgegend Wolmars gesammelten Radicis filics maris.

Arch. d. Pharm., 209, p. 24.

The results obtained in the analyses of rhizomes gathered during the months of April, July and October are tabulated. The rhizomes gathered in April and October were found to have a more intensive green color and stronger odor than those gathered in July. The rhizomes gathered in April and July yielded a yellow colored extract while those gathered in October gave a beautiful green colored product.

Griffin, L. F. 1877

Preparations of Cubebs.

Am. Journ. Pharm., 49, p. 552.

The author found that cubebs yielded 16.5 per cent. of oil and resin to gasoline, while the wax and cubebin were not extracted. He, therefore, concludes that gasoline is adapted to the making of a good oleoresin of cubebs.

Wolff, L. 1877

On the use of Petroleum Benzin in Pharmacy.

Am. Journ. Pharm., 49, p. 1.

It is stated that benzin does not extract any of the pungent resins from ginger, no cubebic acid from cubebs, no piperin from pepper, and no santonin or resin from wormseed.

Cressler, C. H. 1878

On Aspidium marginale, Swartz.

Am. Journ. Pharm., 50, p. 290.

The author prepared an oleoresin from what he thought was male fern, but later proved to be *Aspidium marginale.* According to his report, it proved effective in expelling tapeworm.

Rohn, E. 1878

Recovering Ether in the Preparation of the Ethereal Extracts.

Schweiz. Worchenschr. f. Chem. u. Pharm., —, p. — [Year-Book Pharm., 16, p. 250.]

The author recommends mixing the exhausted drug with water and then heating the mixture over a direct flame up to 60° C, when the ether remaining in the marc distills over. In this manner three kilos of ether are stated to have been recovered from eight to ten kilos of male fern used in the preparation of the extract.

Kennedy, 1879

Aspidium marginale.

Am. Journ. Pharm., 51, p. 382.

Favorable results in the expulsion of taenia by the administration of oleoresin of *Aspidium marginale* are reported.

Thresh 1879

Proximate Analysis of the Rhizome (Dried and Decorticated) of Zingiber Officinalis and Comparative Examination of Typical Specimens of Commercial Gingers.

Pharm. Journ., 39, pp. 171 and 191.

The yield of ether extract is given as follows: Jamaica ginger, 3.29 per cent., Cochin, 4.965 per cent., African, 8.065 per cent. It is further stated that twice as much ether is required to exhaust the African ginger as it is necessary in the case of the other sorts (p. 191.)

Bowman, J. 1881

Aspidium rigidum.

Am. Journ. Pharm., 53, p. 389. [Pharm. Journ. 12, p. 263.]

A crystalline substance thought to be identical with the *Filixsaeure* of Luck was obtained from the ethereal extract of *Aspidium rigidum.*

Seifert, O. 1881

Einiges ueber Bandwurmkuren.

Wien. Med. Wochenschr., 31, p. 1364. [Centralb. f. klin. Med. 3, p. 1884.]

The author contends that the extract should be prepared from the peeled fresh drug gathered in May or October as drying causes the loss of a greater part of the volatile oil. The ether should not be evaporated until just before the extract is to be dispensed.

Maisch, J. M. 1883

Comparison of Galenical Preparations of the United States and German Pharmacopœias.

Am. Journ. Pharm., 55, p. 398.

In the preparation of oleoresin of cubebs, the German Pharmacopœia directs that a mixture of equal parts of ether and alcohol be used as a menstruum, while the *U. S. Pharmacopœia* directs that ether alone be used. In the preparation of oleoresin of aspidium, the solvents are the same (ether) but the German Pharmacopœia directs that the oleoresin be prepared by maceration instead of percolation as in the *U. S. Pharmacopœia.*

Krämer 1884

Extractum filicis maris.

Pharm. Centralh., 25, p. 578.

The fresh rhizomes gathered in May or October, are directed to be extracted with ether containing a little alcohol. The tincture thus obtained is to be preserved in a cool place and the oleoresin prepared therefrom immediately before dispensing.

Berenger-Feraud 1886

Valeur taenifuge de la fougère de Normandy.

Journ. de Pharm. et de Chim., 14, p. 321. [Arch. d. Pharm., 224, p. 134.]

The author states that the rhizomes gathered in Normandy have scarcely any action while those gathered in the Vosges or Jura mountains are very active as taeniafuges.

Jones, E. W. 1886

Amount of Starch in Ginger.

Chem. & Drugg., 28, p. 413. [Arch. d. Pharm., 224, p. 769.]

The yield of ethereal extract is given as 3.58 per cent., of alcoholic extract as 3.38 per cent.

1887

Extractum Cubebarum aethereum.

Gehe & Co. Handels -Ber. Sept., 1887, p. 50.

It is stated that, upon long standing, the extract of cubebs deposits a crystalline substance. The firm, therefore, cannot guarantee that the extract will remain clear.

Kremel, A. 1887

Notizen zur Pruefung der Arzneimittel.

Pharm. Post, 20, p. 521. [Archiv. d. Pharm., 225, p. 880.]

Methods for the identification and evaluation of the ethereal extract of cubebs are presented. The chemical constants of both the alcoholic and ethereal extracts are tabulated (p. 522.) Analytical data on the alcoholic and ethereal extract of male fern are also given (p. 523.)

Lippincott, C. P. 1887

What Are the uses of Benzine and the Lighter Petroleum Products in Pharmacy?

Proc. Penn. Pharm. Assoc., 10, p. 156.

The six official oleoresins were prepared using "benzole" as the exhausting menstruum.

Keefer, C. D. 1888

Aspidium marginale, Willdenow.

Am. Journ. Pharm., 60, p. 230.

The author states that the ethereal extract of the rhizomes of *Aspidium marginale* contains 0.61 per cent. of resin, and chlorophyll. *Filicic acid* could not be identified.

Siggnis, F. M. 1888

Comparative value of commercial gingers.

Am. Journ. Pharm., 60, p. 278.

The following percentages of resin were obtained on extracting ginger with alcohol, sp. gr. 0.820.

Jamaica, unbleached	5.0	per cent.
Jamaica, bleached	4.8	" "
East Indian	6.65	" "
East Indian	6.57	" "
African	6.17	" "
African	7.00	" "

Trimble, H. 1888
The Comparative Extractive Powers of Ether and Benzin.
Proc. Penn. Pharm. Assoc., 11, p. 60.

The following percentages of oleoresin were obtained on extraction with ether: aspidium, 6.51 per cent; capsicum, 19.5 per cent; cubebs, 21.26 per cent; lupulin, 60.59 per cent; pepper, 7.89 per cent. and ginger, 3.07 per cent. The same drugs yielded to benzin 5.9, 18.5, 16.65, 7.04, 2.8 and 2.48 per cent., respectively.

Greenwalt, W. G. 1889
Oleoresin of Male Fern.
Am. Journ. Pharm., 61, p. 169. [Proc. A. Ph. A., 37, p. 379.]

The sediment deposited by the *ethereal oil of male fern* was found by actual test to be as active as the supernatant oil; experiment is thus said to help out the statement (U. S. P. 1880) that the granular deposit should be thoroughly mixed with the liquid portion before being used.

Minner, L. A. 1890
Oleum Peponis.
Am. Jour. Pharm., 62, p. 274. [Proc. A. Ph. A., 38, p. 323.]

The pumpkin seeds comminuted with pumice stone are directed to be extracted with ether. Such a preparation is stated to have proved to be an effective taenifuge, whereas *Oleum Peponis* was ineffective.

Dieterich 1891
Extracta.
Helfenberger Ann., 1891, p. 29.

One sample of extract of male fern examined showed a "moisture content" of 2.7 per cent. and gave 0.40 per cent. of ash.

Kuersten, R. 1891
Ueber Rhizoma Pannae, *Aspidium athamanticum* Kunze.
Arch. d. Pharm., 229, p. 258.

The author found no filix acid in the ethereal extract, but a substance *Pannasaeure* having the formula $C_{11}H_{14}O_4$. A fatty and volatile oil were also isolated. The extract was found to be as active as the extract of male fern in the expulsion of tape worm.

Poulsson, E. 1891

Ueber den giftigen und bandwurmtreibenden Bestandtheil des aetherischen Filixextractes.

Arch. f. exper. Path. u. Pharm., 29, p. 1.

Filix acid is stated to occur in two forms, amorphous and crystalline. The first is reported to be therapeutically active, the latter is not. The crystalline acid is thought to be an anhydride or lactone of the amorphous acid. The author gives the name *Filicin* to the crystalline acid.

Rayman 1891

Wirkung des Extractum Filicis aethereum.

Pharm. Post, 24, p. 933.

It is stated that the extract of male fern is not well borne when taken internally if the ether has not been completely removed.

Reuter, Ludwig 1891

Ueber die Beziehungen des Filixsaeuregehaltes zur Wirkung des Extractum Filicis aethereum.

Pharm. Ztg., 36, p. 245. [Pharm. Post, 24, p. 511; Am. Journ. Pharm., 63, p. 288.]

It is stated that, in 14 out of 15 cases, prompt action was obtained using an extract which showed no deposit of filix acid and which left no residue of filix acid after treating with petroleum ether. On the other hand extracts which were rich in a deposit of filix acid also showed prompt action. Professor Kobert is cited as stating that the Russian extract is about ten times as active as the German extract and twenty times as active as the French extract.

Riegel, S. J. 1891

Ginger and its Oleoresin.

Am. Journ. Pharm., 63, p. 531. [Year-Book of Pharm., 29, p. 168.]

Unbleached Jamaica ginger and East Indian ginger (having epidermis removed) yielded 5 and 8 per cent., respectively, of oleoresin to alcohol. The unbleached Jamaica ginger gave 2.5 per cent. of extractive matter to benzin and the East Indian ginger gave 8 per cent of oleoresin to ether. All of the foregoing oleoresins were found to be completely soluble in alcohol and chloroform.

1892

Extractum Alcannae aethereum.

Gehe & Co., Handels-Ber. Apr. 1892, p. 46.

The ether extract of alkanet root is stated to be completely soluble in oil which is said not to be true of all commercial alkanet extracts.

Beringer, G. M. 1892

Oleoresins.

Am. Journ. Pharm., 64, p. 145. [Proc. A. Ph. A., 40, p. 474; Pharm. Centralh., 33, p. 314; Jahresb. d. Pharm., 27, p. 589.]

The author presents experimental data to show that acetone might be used to advantage in the preparation of the official oleoresins. He especially recommends the use of this solvent in the preparation of the oleoresin of ginger. The yield of oleoresin, using acetone as the extracting solvent for the various drugs, is reported to be as follows: aspidium, 18 per cent; capsicum, 18 per cent. (25 per cent. when the drug was completely exhausted); cubebs, 21.75 to 25 per cent; lupulin, 71 per cent; pepper, 5.93 per cent; ginger, 5.57 per cent; and parsley seed 24 per cent.

Dieterich 1892

Extracta spissa et sicca.

Helfenberger Ann., 1892, p. 44.

Three lots of extract of male fern gave 1.50, 2.10 and 1.50 per cent., respectively, of "moisture" and showed an ash content of 0.55, 0.55 and 0.55 per cent., respectively.

Kobert 1892

Ueber die wirksamen Bestandtheile im Rhizoma Filicis maris.

Pharm. Post, 25, p. 1325. [Apoth.-Ztg., 8, p. 77; Chem. Centralb., 64, p. 269; Arch. d. Pharm., 231; p. 350, Pharm. Ztg., 38, p. 64.]

The author states that the volatile oil of male fern is therapeutically active and that Poulsson's statement based on the work of Carlbohm, Liebig and Rulle, that the activity is due to filix acid alone is erroneous. He cites as an example the activity of *Aspidium athamanticum* Kunze, which contains no traces of filix acid but contains the volatile oil.

Sherrad, C. C. 1892

Value of Oleoresinous Drugs.

Chem. and Drugg., 40, p. 523. [Year-Book Pharm., 29, p. 157.]

The yield of oleoresin obtained using ether as a menstrum is reported to be as follows:

Capsicum, 4 samples, 15.5, 17.4, 18.3 and 18.4 per cent; cubebs, 9 samples, 16.4, 18.8, 21.06, 21.9, 23, 24.7, 24.8, and 24.8 per cent; ginger, 4 samples, 3.85, 4.72, 5.2, and 5.4 per cent; lupulin, 1 sample, 66.5 per cent; crude whole male fern rhizomes, 2 samples, 9.27 and 9.87 per cent; peeled male fern rhizomes, 3 samples, 7.1, 7.26 and 8.9 per cent.

Weppen and Lueders 1892

Ueber Extractum Filicis.

Apoth.-Ztg., 7, p. 514. [Pharm. Ztg., 38, 922; Pharm. Post, 25, p. 1173.]

It is stated that the extract prepared according to the *D. A. III* should have a yellowish-green color but not a deep green color. Preparations having a deep green color probably have chlorophyll or copper salts added to them. Copper can best be detected by dissolving the ash in hydrochloric acid and making the usual tests for the metal.

Two samples (commercial) of a deep green color were found to contain 0.056 and 0.044 per cent. of copper, respectively.

1893

Extractum Filicis aethereum.

Gehe & Co., Handels-Ber., Apr., 1893, p. 43.

The condition of the season in which the rhizomes are harvested is stated to have a marked effect on the color of the extract. Sometimes the genuine extract is very dark green in color, especially in dry seasons.

Beckurts and Peters. 1893

Extractum Filicis.

Apoth.-Ztg., 8, p. 549.

Upon examination, two beautiful green samples of the commercial extract were found to contain 0.135 and 0.044 per cent. of copper, respectively, evidently added for the purpose of coloring the product. An extract prepared by the author was yellowish green in color and contained no copper. A warning is issued against the use of copper utensils in the preparation of the extract.

Dieterich 1893

Extracta spissa et sicca.

Helfenberger Ann., 1893, p. 38.

One sample of extract of cubeb showed a "moisture" content of 32.7 per cent. and gave 0.50 per cent of ash (p. 39).

Three samples of extract of male fern contained 1.15, 1.60 and 1.75 per cent. of "moisture" and gave 0.50, 0.50 and 0.50 per cent. of ash, respectively (p. 39).

Dyer and Gibbard 1893

Determination between Genuine and Exhausted Ginger.

Analyst, 18, p. 197. [Proc. A. Ph. A., 42, p. 936.]

The ether extract of genuine ginger is stated to be 3.0 to 5.2 per cent. After exhausting with ether, alcohol was found to yield 0.8 to 1.5 per cent. additional extractive matter.

Bedall 1894

Extractum Cubebarum Aethereum.

Pharm. Ztg., 39, p. 49.

The author states that the extracts having a green color give a more intensive reaction for cubebin than those having a brownish color. This does not apply when the green color is due to the presence of salts of copper.

Dieterich 1894

Extracta spissa et sicca.

Helfenberger Ann., 1894, p. 72.

Three samples of extract of male fern were found to contain 3.65, 2.32 and 1.90 per cent., respectively, of "moisture." The same samples gave 0.55, 0.42 and 0.50 per cent., respectively, of ash.

Emmanuel, L. 1894

Do Drugs Supplied by the Jobber Comply with Pharmacopœial Requisition. If Not, Who is Responsible, The Jobber or the Retailer?

Am. Journ., Pharm. 56, p. 358.

A sample of powdered cubebs obtained from an Eastern firm yielded 18 per cent. of a brown oleoresin. This was reported to the seller who replied: "the *U. S. Pharmacopoeia* specifies the unripe fruit, but this is rarely found in the market, the regular article of commerce being the ripe fruit which contains less chlorophyll." p. 360.

Hell & Co.

Zur Kritik über Extract-Vorschriften und ueber fabrikmaessig dargestellte Extracte.

Pharm. Post, 27, pp. 168–171. [Journ. de Pharm. et de Chim., 139, p. 493.]

Copper is stated to be a natural constituent of the male fern rhizome. Duplicate analyses of a sample of the rhizomes carefully powdered in an iron mortar, and incinerated in a porcelain dish showed 0.0144 and 0.0148 per cent. of copper, respectively. An ethereal extract prepared in the company's laboratory showed 0.033 per cent. of the metal and a commercial sample of the extract gave 1.96 per cent. Likewise, a commercial sample of extract of cubeb was found to contain 0.40 per cent. of copper.

Poulsson, E. 1894

Beitraege zur Toxicologie der Farnkräuter.
Pharm. Post, 27, p. 238.

Two new acid substances $C_{34}H_{38}O_{14}$ and $C_{34}H_{40}O_{14}$ are reported to have been isolated from the rhizomes of *Polystichum spinulosum*. They were found to be toxic.

_____ 1895

Extractum Orleanae aethereum.
Gehe & Co., Handels-Ber. Apr., 1895, p. 53.

It is stated that good "bixinreiche" orlean species are rare. The extract is said to be used for coloring "Genussmitteln."

_____ 1895

Extractum Cubebarum aethereum liquidium.
Gehe & Co., Handels-Ber., Apr., 1895, p. 53.

A note concerning the precipitation of resin.

Bourquelot, Em. 1895

Réactions d'identité de quelquess medicaments galéniques officinaux.
Journ. de Pharm. et de Chim., 140, p. 361.

The *Extrait de Cubebe* of the French Codex is semi-liquid, that of the German and Austrian pharmacopœias of the consistence of fresh honey. To identify the oleoresin, a small quantity is placed in a white porcelain dish and a few drops of concentrated sulphuric acid are added. The genuine oleoresin gives a purple-red color immediately.

Davis, R. G. 1895

Ginger.
Am. Journ. Pharm. 67, p. 597. [Proc. A. Ph. A., 44, p. 538.]

The yield of oleoresin obtained from ginger by the official process was found to be as follows:

Jamaica ginger, whole rhizome, bleached, 4.53 to 4.62 per cent; Jamaica ginger, whole rhizome, unbleached, 2.82 to 4.41 per cent; Jamaica ginger, powdered unbleached, 4.48 per cent; Races ginger, powered, bleached, 4.09 to 5.40 per cent; Races ginger, whole rhizome, bleached, 4.02 to 5.75 per cent; African ginger, whole rhizome, 5.75 per cent; African ginger, powdered, 6.27 per cent.

Dieterich 1895

Extracta spissa et sicca.

Helfenberger Ann., 1895, p. 17.

One sample of extract of cubebs contained 20.90 per cent. of "moisture" and showed an ash content of 0.47 per cent. (p. 17).

A sample of extract of male fern showed a "moisture" content of 1.75 per cent. and gave 0.50 per cent. of ash (p. 18.)

Hyers, P. 1895

Fluid Extract of Cubeb.

Am. Journ. Pharm., 67, p. 519.

The following percentages of oleoresin are reported to have been yielded by cubebs to different solvents: ether, 22.45 per cent; alcohol, 14.48 per cent; acetone, 18.48 per cent; petroleum ether, 13.47 per cent.

——————— 1896

Extractum Filicis Ph. G. III.

Caesar and Loretz, Geschaefts-Ber., Sept. 1896, p. 46.

The firm attributes the uniform activity of their extract of male fern to the fact that the rhizomes are obtained from the same locality each year, that they are collected in the autumn and, after carefully garbling, are immediately made into extract.

Fromme's method for estimating the filix acid content of the extract is given.

Alpers, W. C. 1896

Oleoresin Capsicum.

Merck's Rep. 5, p. 593.

The author states that he obtained a yield of 19 per cent. of oleoresin after removing the fat by filteration instead of 5 per cent. as usually given in the text-books.

Bocchi, I. 1896

Methoden zur Feststellung der Identitaet und der Guete des aetherischen Filixextraktes.

Boll. Chim. farm., 1896, p. 449. [Apoth-Ztg., 11, pp. 597 and 837; Pharm. Ztg., 41, p. 596.]

Reactions for the identification of filix acid, and a method for the evaluation of the extract of male fern are given.

Daccomo and Scoccianti 1896

Die Bestimmung des Gehaltes an Filixsaeure im kaeuflichen Extractum Filicis.

Boll. Chim. farm., 5, p. 129. [Pharm., Ztg., 39, p. 280; Jahresb. d. Pharm., 31, p. 583; Apoth.-Ztg., 11, p. 174; Proc. A. Ph. A., 44, p. 433.]

The filix acid content of a number of samples of extract of male fern (self prepared and commercial) was found to vary from 11.86 to 42.53 per cent., when assayed according to the method devised by the authors. The average yield of extract obtained is given as 10 per cent.

The quantity and quality of the extract is stated to be influenced by the locality in which the rhizomes are grown, the moisture content of the drug when extracted, and the solvent. Ether, specific gravity, 0.720, is stated to be the most suitable menstruum for this purpose. Ether, specific gravity, 0.756, yielded 17 per cent. of a brownish colored extract of a tarry consistence. The presence of alcohol is said to retard the complete extraction of the filix acid.

Dieterich 1896

Extracta spissa et sicca.

Helfenberger Ann., 1896, p. 33.

One sample of extract of male fern contained 1.62 per cent. of "moisture" and gave 0.45 per cent. of ash.

Kraft, P. 1896

Ueber die Wertbestimmung von Extractum Filicis und eine neue Bestimmungsmethode der Filixsaeure.

Schweiz. Wochenschr. f. Chem. u. Pharm., 34, p. 217. [Zeitschr, d. Allg. Oesterr, Apoth. Ver. 34, p. 798; Zeitschr. f. Anal. Chem., 39, p. 531.]

It is stated that the method of Daccomo and Scoccianti for the evaluation of the extract of male fern does not give the filix acid content but the total acid content. Extracts examined by the author's method gave from 0.4 to 10.0 per cent. of filix acid.

A new constituent which the author calls *Filixwachs* was isolated from the extract.

Liverseege 1896

The Effect of Solvents on the Analytical Character of Ginger.

Pharm. Journ., 57, p. 112. [Apoth.-Ztg., 11, p. 639.]

The ethereal extract of ginger is stated to amount to 5.5 per cent. The yield to methyl alcohol is given as 6.5 per cent.

———————— 1897

Extractum Filicis, Ph. G. III.

Caesar and Loretz, Geschaefts-Ber., Sept. 1897, p. 62. [Pharm. Centralh., 38, p. 34.]

Investigations carried on by the firm showed that the best time for harvesting the rhizomes of male fern is from the middle of September to the end of October. Rhizomes collected in the spring yielded an extract low in filix acid content.

The consistence of the abstract is said to be dependent upon variations in the rhizomes, thus rhizomes rich in wax give an extract which is not fluid at ordinary temperatures.

Fromme's improved method for estimating the filix acid is given.

———————— 1897

Extractum Filicis aethereum, P G.. III.

Gehe & Co., Handels-Ber., Apr. 1897, p. 60.

The results obtained in the assay of male fern extracts by the methods of Daccomo and Scoccianti, Bocchi, and Fromme are tabulated.

Boehm, R. 1897

Beitraege zur Kenntniss der Filixsaeuregruppe.

Archiv. f. exp. Path. u. Pharm., 38, p. 35.

In addition to the volatile oil, fixed oil and filix acid, Boehm isolated four acid substances from the extract of male fern, viz: aspidin ($C_{28}H_{32}O_7$), flavaspidic acid ($C_{23}H_{28}O_8$), albaspidin ($C_{22}H_{28}O_7$) and aspidinol ($C_{12}H_{16}O_4$).

Candussio 1897

Ueber die Bereitung des Extractum Filicis aethereum.

Pharm. Post, 30, p. 7.

The author is impressed with the low cost of the commercial extract of male fern as compared with the cost when prepared by the apothecary himself. The examination of a number of samples from the best German houses showed a low filix acid content when estimated according to the method of Daccomo and Scoccianti. They were all of a beautiful green color, however.

Dieterich 1897

Extracta spissa et sicca.

Helfenberger Ann., 1897, p. 244. [Apoth.-Ztg., 13, p. 788; Pharm. Centralh., 39, p. 775.]

Two samples of extract of male fern, D. A. III, lost 4.5 and 4.72 per cent., respectively, on drying at 100°C; and gave 0.43 and 0.52 per cent. of ash, respectively.

Dieterich contends that a standard, which does not take into consideration

the volatile oil as well as the filix acid, is worthless, as the former is also active as a taenifuge. Old extracts which are inactive show the normal amount of filix acid. The diminution in activity is said to be due to the loss of the volatile oil by resinification and evaporation (p. 248.)

Dieterich **1897**

Extractum Filicis aetherum, D. A. III.
Extractum Cubebarum aethereum.
Erstes Dezennium d. Helfenberger Ann., 1886–1895, p. 322.

Eighteen samples of extract of male fern examined during 10 years showed a loss upon drying at 100°C of from 0.60 to 9.73 per cent. The same samples showed an ash content varying from 0.40 to 0.63 per cent.

Four samples of ethereal extract of cubeb showed a loss upon drying at 100°C of 20.13 to 32.7 per cent., and gave 0.10 to 0.52 per cent. of ash.

Glass and Thresh **1897**

Commercial Gingers and Essence of Ginger.
Pharm. Journ., 58, p. 245. [Am. Journ. Pharm., 69, p. 320.]

Jamaica ginger was found to yield 6.0 per cent of extractive matter to ether; Cochin, 4.33 per cent; African, 6.33 per cent.

Lauren, W. **1897**

Extractum Filicis spinulosae.
Finska Laekaresaellsk. Handl., 1897, p. 9. [Pharm. Centralh., 39, p. 975.]

The ethereal extract prepared from the rhizomes of *Aspidium spinulosum* is stated to be as active a taeniafuge as that prepared from *Aspidium filix mas.*

Madsen, H. P. **1897**

Meddelelser fra Vesterbro Apotheke Laboratorium.
Arch. f. Pharm. og Chem., 54, p. 269. [Jahresb. d. Pharm., 32, p. 591; Apoth.-Ztg., 12, p. 461.]

Extracts of male fern from Denmark, Germany, Bohemia, central Russia and Livonia were tested quantitatively for filix acid according to Fromme's method. Those from Bohemia and central Russia gave from 0.71 to 0.97 per cent. of filix acid; two samples from Germany gave 5.58 and 9.58 per cent., respectively; an extract from Wolmar in Livonia gave 13.07 per cent; the extracts from Denmark, with two exceptions (6.07 and 8.25 per cent.), gave below 2 per cent. (p. 277.)

———————— 1898

Extractum Filicis aetherum.

Gehe & Co., Handels-Ber., 1898, p. 68. [Pharm. Centralh., 39, p. 298.]

In the analyses of 11 extracts obtained during different years, 6 were found to contain aspidin, 0.2 to 3.0 per cent., but no filix acid; 4 samples contained filix acid but no aspidin; 1 sample showed a trace of aspidin and a small quantity of filix acid.

———————— 1898

Zur Arzneiform und Werthbestimmung des Filixextracts.

Pharm. Centralh., 39, p. 873.

The dilution of the extract of male fern with castor oil to a definite filix acid content is discussed.

———————— 1898

Extractum Filiçis, Ph. G. III.

Caesar and Loretz, Geschaefts-Ber., Sept. 1898, p. 72.

A continuation of the firm's investigations concerning the influence of time of harvesting upon the quality of the male fern rhizomes has shown that they do not contain the maximum amount of active constituents until the month of August. They, therefore, conclude that the rhizomes should only be harvested in the months of August, September and October.

It is further reported that analyses of the extracts recently prepared show that the present year's (1898) crop of rhizomes is, on the whole, lower in crude filicin content than that of the preceding year (1897.)

Bellingrodt, Fr. 1898

Ueber Rhizoma und Extractum Filicis.

Apoth.-Ztg., 13, p. 869.

The crude, and purified filicin content of 8 different extracts of male fern prepared by the author from rhizomes obtained from different sources are given. Similar data in the examination of 9 commercial extracts are also reported.

Dieterich, K. 1898

Zur Wertbestimmung und Arzneiform des Filixextraktes.

Apoth.-Ztg., 13, p. 788.

The addition of castor oil to the extract of male fern in sufficient quantity to bring the filix acid content to a definite standard is recommended.

Duesterbehn, F. 1898

Rhizoma und Extractum Filicis in therapeutischer, chemischer und toxicologischer Beziehung.

Apoth.-Ztg., 13, pp. 713, 720, 729 and 734.

The article is principally a review of the literature on the extract of male fern and its constituents.

Lefils 1898

Zur Herstellung von Filixextract.

Pharm. Centralh., 39, p. 901. [Zeitschr. d. oest. Apoth. Ver., 37, p. 167; Pharm. Ztg., 43, p. 939.]

The author advises the mixing of the powdered rhizomes with castor oil before preparing the extract as he is of the opinion that this procedure will retard the evaporation of the volatil oil and the precipitation of the crystalline filix acid.

Idris, T. H. 1898

Notes on Extract of Ginger.

Am. Journ. Pharm., 70, p. 466.

The alcoholic extract of ginger known as *gingerine* does not contain all of the aromatic principles of the rhizome, as most of the essential oil is lost on removing the alcohol upon evaporation. Acetone boiling at 58°C was found to be the most suitable solvent for extracting ginger. The acetone extract is a dark brown substance of treacly consistence, intensely pungent and at the same time possessing the full aroma of ginger, the quality of which largely depends on the variety of ginger used.

Miehle, Feodor 1898

Eine empfehlenswerte Form der Verordung von Extractum Filicis.

Apoth.-Ztg., 13, 777. [Pharm. Centralh., 39, p. 873.]

The author recommends diluting the extract with castor oil in order to make a standard preparation containing a definite amount of filix acid. He advises the introduction of such a preparation into the D. A. IV, under the name *Extractum Filicis oleatum.*

Plzak, F. 1898

Extractum Filicis.

Pharm. Centralh., 39, p. 687. [Jahresb. d. Pharm., 33, p. 547.]

The author found 6.48 per cent of filix acid in the extract of male fern by the Kraft method, 6.0 per cent. by Fromme's old method and 5.2 per cent. by Fromme's improved method.

Winton, Ogden and Mitchell 1898

Capsicum.

Rep. of Conn. Agr. Exp. Sta. (1898), p. 200.

The amount of extractive matter obtained with ether from different samples of red peppers is given as follows: Chilli colorado, 15.81 per cent; peppers from Natal, 16.85 per cent; from Nepaul, 21.31 per cent; and from Zanzibar, 16.19 per cent.

———————— 1899

Recently Introduced Remedies.

Am. Drugg. & Pharm. Rec., 34, p. 129.

It is stated that the *extract of Filix Spinulosa* is an ethereal extract of the rhizome of *Aspidium spinulosum* and that it has been recommended as a substitute for the preparation made from *Aspidium Filix-mas.*

———————— 1899

Extractum Filicis, Ph. G. III.

Caesar and Loretz, Geschaefts-Ber., Sept. 1899, p. 73.

A table showing the crude filicin and filix acid content of extracts prepared from 15 of the better samples of male fern rhizomes obtained from different sources in Germany is given.

Results showing the difference in extractive power of ether, sp. gr. 0.720 and ether, sp. gr. 0.728 are also given.

Hausmann, A. 1899

Ueber Extractum Filicis aethereum.

Arch. d. Pharm., 237, p. 544.

The examination of 21 commercial extracts of male fern obtained from various sources showed that aspidin was a constituent of 4 of them. As aspidin is said to be found only in *Aspidium spinulosum,* the author infers that the rhizomes of this species have been used to adulterate the official drug.

A method for the detection of aspidin is given.

———————— 1900

Extractum Filicis.

Caesar and Loretz, Geschaefts-Ber., Sept. 1900, p. 77.

A table showing the crude and purified filicin content of 12 samples of extract of male fern is given

Attention is also called to the greater tendency of the extract, prepared with ether, specific gravity 0.728, to deposit than that prepared with ether, specific gravity 0.720. The deposited material is reported to have been identified by Boehm as filix acid and a wax-like substance.

———————— 1900

Extractum Filicis aethereum.

Gehe & Co., Handels-Ber., Apr. 1900, p. 63.

The constituents of the extracts of *Aspidium filix mas, A. filix femina* and *A. spinulosum* are discussed.

Maish, H. C. C. 1900

Oleoresins. Economical preparation.

P. C. P., Alumni Report, March, 1900, p. 49. [Proc. A. Ph. A., 48, p. 495.]

Maish advises the use of the Soxhlet extraction apparatus for preparing the oleoresins on a small scale.

Patch, E. L. 1900

Answere to queries issued by the Scientific Section of the American Pharmaceutical Association.

Proc. A. Ph. A., 48, p. 199.

The commercial oleoresins frequently show the presence of acetone, p. 205.

———————— 1901

Extractum Filicis.

Caesar and Loretz, Geschaefts-Ber., Sept. 1901, p. 68.

The crude and purified filicin contents of 8 batches of extract of male fern prepared during the year are presented in tabular form.

Bennet 1901

Report on Commercial Ginger.

Pharm. Journ., 66, p. 522.

The yield of extractive matter obtained on exhausting ginger with ether and alcohol is given as follows:

Per cent. of ether extract:
Jamaica ginger (whole), 2.57 to 6.41.
" " (ground), 2.97 to 4.6.
Per cent. of alcoholic extract after ether:
Jamaica ginger (whole), 3.09 to 5.16.
" " (ground), 3.01 to 4.16.
Per cent. of alcoholic extract.
Jamaica ginger (whole), 3.94 to 5.61.
" " (ground), 3.41 to 5.67.
Cochin " (whole), 4.91 to 6.74.
" " (ground), 5.41 to 6.51.
African " (whole), 5.41 to 6.61.
" " (ground), 5.14 to 6.47.

Dieterich 1901

Extracta spissa et sicca.

Helfenberger Ann., 1901, p. 170.

One sample of extract of male fern, *D. A. IV*, gave 5.23 per cent. of "moisture" and 0.32 per cent. of ash (p. 171).

Matzdorff, M. 1901

Wertbestimmung des Rhizoma Filicis.

Apoth.-Ztg., 16, pp. 233, 256 and 273.

The various constituents of the extract of male fern are discussed with respect to their therapeutic activity. Of these filix acid is thought to be the most important. Tables showing the crude filicin and filix acid content of ethereal fluid extracts prepared by ordinary percolation and by extraction with a Soxhlet's apparatus are given.

Stoeder 1901

Bestimmung der Filixsaeure in Extractum Filicis.

Pharm. Ztg., 46, p. 541.

A method very similar in all respects to that of Fromme for the estimation of the filix acid in the extract of male fern is described.

——— 1902

Oleoresin of Insect Powder.

Southall Bros. & Barclay, Lab., Rep., 10, p. 20.

This oleoresin is said to be extracted from the powdered drug and is offered for sale, in the crude form, as an extract, or precipitated, in the form of a coarse powder.

It is said to be useful as a basis for nursery hair lotions, dusting powders and similar articles.

——— 1902

Extractum Filicis.

Caesar and Loretz, Geschaefts-Ber., Sept. 1902, p. 73.

It is stated that the crude filicin contains the amorphous acid recently shown by Kraft to be the active principle of male fern extract. The estimation of the crude filicin will, therefore, be continued by the firm.

Buttin, L. 1902

Extract de Fougère mâle.

Schweiz. Wochenschr. f. Chem. u. Pharm., 40, p. 234.

A short review of the early work on the constituents of the extract of male fern is given.

The variation in the constituents of the rhizomes due to the locality in

which they are grown, the time of the year when harvested, storing, etc., and the effect of the same upon the activity of the extract is emphasized.

Eight per cent of extract is reported as having been obtained from rhizomes harvested in the spring.

Kraft, F. 1902

. Untersuchung des Extractum Filicis.

Schweiz. Wochenschr. f. Chem. u. Pharm., 40, p. 322. [Chem. Centralb., 73, 2, p. 53; Pharm. Ztg., 48, p. 275.]

Two new substances were isolated by the author from the ethereal extract of male fern, *flavaspidin* and an amorphous acid. The amorphous acid is reported to be the active principle and to be present to the extent of 5 per cent. in a good extract.

——————— 1903

Table showing suggested standards, ranges of specific gravity, etc., for galencial preparations.

Southall Bros., & Barclay, Lab. Rep., 11, p. 23.

The standard range for the specific gravity of *Extractum Filicis liquidum* is given as 1.000 to 1.019 at 15.5°C p. 24.)

——————— 1903

Extractum Filicis.

Caesar and Loretz, Geschaefts-Ber., Sept. 1903, p. 77.

It is reported that extracts prepared from the male fern rhizomes harvested during the previous year, when assayed according to the method of Kraft, yielded 27.08 to 36.6 per cent. of crude filicin.

——————— 1903

Ginger.

Southall Bros. & Barclay, Lab. Rep., 11, p. 13.

The following table shows the proportion of oleoresin found in three varieties of commercial ginger.

	Jamaica	Cochin	African
Per cent. sol. in alcohol (90 per cent.)	4.35	4.57	9.93
Per cent. sol. in ether, Sp. gr. 0.717	4.76	6.04	11.09

——————— 1903

Capsicum.

Southall Bros. & Barclay, Lab. Rep., 11, p. 13.

A sample of *Capsicum minimum* yielded ,5.67 per cent. of material soluble in ether, Sp. gr. 0.717, and a sample of *Capsicum annum* yielded 15.34 per cent. to the same solvent.

Ballard 1903

Sur quelques condiments des colonies francaise (Anise étoilé, Cannelle, Cardamome, Curcurma, Gingembre, Girofle.

Journ. de Pharm. et de Chim., 157, pp. 248 and 296.

Ginger from the Ivory Coast is reported to have yielded 6.33 per cent. of ether extract, that from Tahiti, 3.75 per cent, p. 248.

Black pepper yielded the following percentages of extractive matter to ether: 10.15, 8.70 and 5.50.

Beythien 1903

Capsicum.

Zeitschr. Unters. Nahr. u Genussm., 5, p. 858. [Pharm. Ztg., 47, p. 549; Proc. A. Ph. A., 51, p. 747.]

The examination of a number of commercial samples of powdered capsicum showed the following:

Yield of extract to ether	(total)	12.54 to 19.70	per cent.
" " " " "	(av.)	14.94	" "
" " " " alcohol	(total)	26.55 to 35.71	" "
" " " " "	(av.)	28.94	" "

Dieterich 1903

Extracta spissa et sicca.

Helfenberger Ann., 1903, p. 240.

Three samples of extract of male fern examined showed a "moisture" content of from 5.52 to 7.38 per cent., and gave from 0.27 to 0.39 per cent. of ash (p. 241.)

Penndorff, O. 1903

Untersuchungen ueber die Beschaffenheit kaeuflicher Filix-Rhizoma und Extrakte.

Apoth.-Ztg., 18, p. 150.

The author states that the rhizomes turn brown on aging due to the breaking down of the *filix-tannic acid* into *filix-red* and sugar.

An examination of 20 samples of commercial rhizomes showed that 12 of them or over 50 per cent. contained rhizomes of *Aspidium spinulosum*, 1 sample consisted of 90 per cent. of this species.

Twenty samples of commercial extracts were examined with the following results:

4 samples — Starch present in small quantities.
1 sample — Aspidin present.
20 samples — 6.65 to 18.31 per cent. crude filicin.
20 samples — 1.06 to 7.48 per cent. filix acid.
20 samples — 0.40 to 3.00 per cent. filix acid in solution.
20 samples — 0.40 to 6.05 per cent. filix acid deposited.
7 samples — copper, more or less.

———————— 1904

Extractum Filicis.
Caesar and Loretz, Geschaefts-Ber., Sept. 1904, p. 77.

It is stated that for years the firm has placed upon the market under their name an extract of male fern containing not less than 29 per cent. of crude filicin.

Dieterich 1904

Ueber Extractum Filicis, D. A. IV.
Helfenberger Ann., 1904, p. 182.

The results obtained in the examination of 3 samples of the extract of male fern are tabulated. The results include the per cent. of "moisture" and ash, and the iodine and saponification values.

———————— 1905

Extractum Filicis.
Caesar and Loretz, Geschaefts-Ber., Sept. 1905, p. 7.

It is stated that, although the year's crop of male fern is poor, the firm guarantees a crude filicin content of 28 per cent. for their extract, (p. 71.) Fromme's method for estimating the crude filicin content is given (p. 85.)

———————— 1905

Ueber die wirksamen Bestandtheile des Farnwurzelextrakts. Pharm. Ztg., 50, p. 651.

The work of Boehm, also that of Kraft, is commented on, special reference being made to *Filmaron* isolated from the extract by the latter.

———————— 1905

The Newer Remedies.
Am. Drugg. & Pharm. Rec., 46, p. 135.

Capsolin which is recommended as a substitute for mustard papers, is said to consist of a mixture of oleoresin of capsicum, the oils of turpentine, cajuput and croton, with an ointment base. It is manufactured and marketed by Parke, Davis & Co., Detroit.

———————— 1905

The New U. S. P., Changes in Composition and Strength.
Drug Topics, 20, p. 210. [Am. Journ. Pharm., 78, p. 412.]

The new edition of the *U. S. P.* specifies acetone as the solvent for making all of the oleoresins with the exception of oleoresin of cubebs, which

is prepared with alcohol. It is stated that manufacturers have long since seen the folly of employing an expensive solvent like ether, and the adoption of acetone for this purpose is a recognition of commercial pharmaceutical advances. (p. 214.)

Dieterich 1905

Extracta spissa et sicca.

Helfenberger Ann., 1905, p. 159.

A sample of the ethereal extract of cubeb, *D. A. IV*, showed a "moisture" content of 55.91 per cent. and an ash content of 0.87 per cent. (p. 160.)

A sample of extract of male fern *D. A. IV*, gave a "moisture" content of 5.06 per cent., an ash content of 0.46 per cent. and yielded 23.22 per cent. of crude filicin (p. 161.)

Dieterich 1905

Rhizoma Zingiberis.

Helfenberger Ann., 1905, p. 131.

The following percentages of extract were obtained by exhausting ginger with different solvents, evaporating the latter and drying the residue at 100°C:

1) One part alcohol, 8 parts water — 7.86 per cent.
2) Sixty-eight per cent. alcohol — 4.88 per cent.
3) Ninety per cent. alcohol — 2.79 per cent.

Dieterich 1905

Rhizoma Filicis.

Helfenberger Ann., 1905, p. 130.

During the year, a number of lots of male fern rhizomes were examined. The air-dried rhizomes yielded 9.94 to 10.60 per cent. of ethereal extract. The rhizomes when dried at 100°C yilded as high as 11.20 per cent. to the same solvent.

Francis, J. M. 1905

The New Pharmacopœia: A Detailed Commentary on the Eighth Revision of the U. S. P.

Bull. of Pharm., 19, p. 317. [Am. Journ. Pharm., 78, p. 412.]

Under acetone, it is stated that oleoresins prepared with this solvent will separate in two layers on standing owing to the fact that this ketone possesses in a measure the combined solvent properties of both alcohol and ether.

Vanderkleed, C. E. 1905
Report of the Committee on Adulterations.
Proc. Penna. Pharm. Assoc., 28, p. 47.

Eight assays of capsicum gave 9.4 to 23.9 per cent. of oleoresin, the average being 18.13 per cent. The standard for a good drug is stated to be 15 per cent.

Vieth, H. 1905
Ueber die Beziehung zwischen chemischer Zusammensetzung und medizinischer Wirkung einiger Balsamika.
Verh. d. Ges. deutsch. Naturf. u. Aerzte, 2, p. 364. [Jahresber. d. Pharm., 66, p. 13.]

Kubebenextrakt is reported to consist of terpenes (65 per cent.), resin acids (10 per cent.), and resins (25 per cent.)

_____ 1906
Apiolin
Merck's Ann. Rep., 20, p. 34.

Apiolin is the raw ethereal oil obtained from the seed of *Petroselinum sativum* or from *Apiol viride* by extraction with a suitable solvent. It is a yellow fluid, sp. gr. 1.25 to 1.135, boiling at 280 to 300°C.

_____ 1906
Extractum Filicis.
Caesar and Loretz, Geschaefts-Ber., Sept. 1906, pp. 82 and 99.

The firm reports that the crude filicin content of the extract obtained from the current year's crop of male fern averages 27 per cent. (p. 82). Fromme's method for estimating the crude filicin is given (p. 99).

Naylor, A. H. 1906
Progress in pharmacapœias: drugs and their constituents.
Year-Book of Pharm., 43, p. 204.

It is stated that in the present state of our knowledge, neither Daccomo and Scoccianti's, Kraft's nor Stoeder's process for the quantitative estimation of filicic acid is a measure of the anthelmintic value of the extract of male fern.

Roeder, Ph. 1906
Rhizoma Filicis.
Jahresb. d. Pharm., 41, p. 46.

The author states that the rhizomes of *Aspidium filix mas* should give at most 3 per cent. of ash and should yield at least 8 per cent. of extractive matter to ether, allowing the latter to evaporate spontaneously and then heating for 2 hours at 95°C, cooling in a desiccator and weighing. Three samples of rhizomes gave 2.52 to 2.92 per cent. of ash, respectively, and 9.22 to 10.1 per cent. of ether-soluble extract.

Wollenweber, W. 1906
Ueber Filixgerbsaeure.
Arch. d. Pharm., 244, p. 466.

In connection with his work on the tannic acid in the male fern rhizomes, the author presents the results obtained in extracting the drug in a Soxhlet's apparatus with various solvents, ether, benzol, and petroleum ether. At the end of six hours, extraction was found to be practically complete in all cases. The yield obtained in each case is given as follows; ether, 10.0 per cent., benzol, 9.06 per cent., petroleum ether, 9.08 per cent.

Extraction with alcohol of varying strength yielded extractive matter in the following quantities: alcohol (90 per cent.), 20.0 per cent., alcohol (96 per cent.), 16.6 per cent.

The fixed oil content of the ethereal extract is stated to be 70 to 75 per cent.

———— 1907
Cubebs.
Evans Sons Lescher & Webb, Analyt. Notes, 1, p. 21.

The oleoresin extracted by ether from four samples of cubebs amounted to (1) 22.08, (2) 22.6, (3) 21.13 and (4) 22.8 per cent., respectively.

Blome, W. H. 1907
Cubeba.
Proc. Mich. Pharm. Assoc., 1907, p. 68. [Bull. Hygienic Lab., No. 63, p. 225.]

Five samples of cubeb are reported which assayed from 18.85 to 26.88 per cent. of oleoresin.

Van der Harst, J. C. 1907

Lupulin.

Pharm. Weekbl., 44, p. 1506. [Bull. Hygienic Lab., No. 63, p. 301.]

Two samples of lupulin were found to contain 52 and 65 per cent. of ether-soluble matter, respectively.

Patch, E. L. 1907

Report of Committee on Drug Market.

Proc. Am. Pharm. Assoc., 55, p. 314.

The samples of capsicum examined yielded from 16.2 to 26.5 per cent. of alcoholic extract (p. 324.)

Smith, O. W. 1907

Galenicals of the U. S. P. VIII.

Proc. Mo. Pharm. Assoc., 29, p. 132.

The author is of the opinion that the oleoresin of cubeb might well have been included in the class made with acetone, as the drug yields but little on subsequent extraction with alcohol. Alcohol on the other hand is open to the objection that its boiling point is so high that a considerable loss of volatile substances from the cubeb occurs when the solvent is evaporated (p. 134.)

———— 1908

Extractum Filicis.

Caesar and Loretz, Geschaefts-Ber., Sept. 1908, pp. 76 and 99.

It is stated that for years the firm has estimated the crude filicin content of the extract of male fern and marketed a standard product containing 28 per cent. of this constituent as required by the Swiss Pharmacopœia, VI, (p. 76.)

Fromme's method for estimating the crude filicin is given (p. 99.)

Dohme and Engelhardt 1908

Purity of some official and non-official drugs and chemicals.

Proc. Am. Pharm., Assoc., 56, p. 814.

A sample of lupulin yielding only 56 per cent. of ether-soluble matter is reported (p. 817.)

Patch, E. L. 1908

Report of Committee on Drug Market.

Proc. Am. Pharm. Assoc., 56, p. 765.

The different samples of capsicum examined yielded from 15 to 25.2 per cent. of alcoholic extract (p. 768.)

Spaeth, Eduard 1908

Die chemische und mikroskopische Untersuchung der Gewürze und deren Berurteilung.

Pharm. Centralh., 49, p. 581.

The paper discusses the characteristics of several commercial varieties of ginger and the composition of the drug. The quantity of material extracted by ether, alcohol, petroleum ether and methyl alcohol is given.

Vanderkleed, C. E. 1908

Report of Committee on Adulteration.

Proc. Penna. Pharm. Assoc., 31, p. 65.

Three samples of capsicum yielded from 11.59 to 18.35 per cent. of oleoresin; four samples of cubebs, 16.39 to 23.6 per cent; two samples of ginger, 5.58 to 9.55 per cent; three samples of male fern, 6.68 to 17.9 per cent., average 10.002 per cent. (p. 88.)

_____ 1909

Pharmacy Committee's Report.

Chem. & Drugg., 74, p. 288.

The Committee of Reference in Pharmacy asserts that cubebs should yield not less than 20 per cent. of oleoresin to ether, sp. gr. not over 0.720. (p. 292.)

_____ 1909

Extractum Filicis.

Caesar and Loretz, Geschaefts-Ber. Sept. 1909, pp. 67 and 84.

A crude filicin content of 28 per cent. is guaranteed by the firm for the new lot of extract of male fern (p. 67.)

Fromme's method for the estimation of the crude filicin is given (p. 84.)

_____ 1909

Apiol.

Evans Sons Lescher & Webb, Analyt. Notes, 4, p. 11.

A sample of apiol of French manufacture examined by the firm is reported as having been liquid and green in color. It yielded 40 per cent. of

its bulk to steam distillation. It is, therefore, thought that the sample was prepared by the extraction of parsley fruits with a suitable light solvent.

Bernegau, L. H. 1909
Report of the Committee on Adulteration.
Proc. Penna. Pharm. Assoc., 32, p. 119.

Ten samples of lupulin examined yielded from 34 to 65.8 per cent. of ether-soluble matter (p. 125.)

Dohme and Engelhardt 1909
Purity of some official and non-official drugs and chemicals.
Proc. A. Ph. A., Assoc., 57, p. 713.

Three samples of lupulin examined were low in ether-soluble matter yielding but 47.50, and 43 per cent., respectively (p. 716.)

Dunn, J. A. 1909
Suggested Modifications of U. S. P. and N. F. Formulas.
Proc. A. Ph. A., 57, p. 942.

It is stated that the oleoresin of male fern prepared by the *U. S. P.* method, using acetone, contains so much undesirable extractive matter that it is necessary to purify it by dissolving in ether. It is suggested that it might be worth while to consider whether the U. S. P. should not go back to the use of ether (p. 949.)

Parson, W. A. 1909
Report of the Committee on Adulteration.
Proc. Penna. Pharm. Assoc., 32, p. 119.

Three samples of lupulin yielded 66.1 and 54 per cent. of ether-soluble matter, respectively (p. 125.)

Patch, E. L. 1909
Report of Committee on Drug Market.
Proc. A. Ph. A., 57, p. 721.

The alcoholic extract from specimens of ginger examined varied from 3.7 to 6.2 per cent. (p. 739.)

Vanderkeed, C. A. 1909

Report of the Committee on Adulteration.

Proc. Penna. Pharm., Assoc., 32, p. 119.

Samples of capsicum, cubebs, ginger, and male fern examined are reported to have yielded oleoresin as follows: five samples of capsicum, 14.34 to 17.95 per cent; four samples of cubebs, 16.49 to 24.34 per cent; sixteen samples of Jamaica ginger, 3.142 to 6.91 per cent; two samples of African ginger, 8.2 and 9.036 per cent; one sample of male fern, 10.33 per cent. (p. 129.)

_____ 1910

Extractum Filicis.

Caesar and Loretz, Jahres-Ber., Sept. 1910, p. 90.

Fromme's method for the estimation of crude filicin is given.

_____ 1910

Cubebs.

Southall Bros. & Barclay, Lab. Rep., 17, p. 11.

Eight samples of cubebs, when extracted with petroleum spirits, yielded from 3.88 to 18.08 per cent. of extractive matter. The same samples on subsequent extraction with alcohol (90 per cent.) yielded from 3.4 to 5.66 per cent. of extractive matter.

_____ 1910

Capsicum.

Southall Bros. & Barclay, Lab. Rep., 17, p. 8.

Two samples of capsicum *(B. P. C.)* yielded 15.4 and 14.0 per cent., respectively, of extract to benzol.

Dohme and Engelhardt 1910

The new Hungarian Pharmacopœia.

Proc. Am. Pharm. Assoc., 58, p. 1168.

The extraction of male fern with ether, as directed in the *Ph. Hung. III*, instead of acetone as in the *U. S. P., VIII*, is thought to be desirable since the latter is liable to extract substances which might produce injurious after effects (p. 1179.)

It is further stated that the yield of ether extract as given in the Hungarian Pharmacopœia is 8 per cent. (p. 1184.)

Eldred, F. R. 1910

Some data obtained in the examination of official substances.
Proc. A. Ph. A., 58, p. 889.

Forty-eight lots of capsicum were examined. The yield of ether-soluble oleoresin, when the latter was dried for one hour on a water bath, was found to vary from 11 to 26 per cent., the average 18 per cent. (p.891.)

Gane, E. H. 1910

Pharmacopœial notes and comments.
Drug Topics, 25, p. 212.

It is stated that a good sample of cubebs should yield 20 per cent. of ether-soluble extract.

Gane and Webster 1910

Pharmacopœial notes and comments.
Drug Topics, 25, p.

Aspidium is stated to be one of the most useful of drugs when carefully collected and preserved, but that much of the rhizome is inert and is obtained from any old species of fern. It is said to be falling into disuse on this account. It is thought that the observance of more care in the collection of the drug and the preparation of the oleoresin would restore its popularity as an anthelmintic.

La Wall, C. H. 1910

Some suggested standards and changes, for the U. S. P.
Am. Journ. Pharm., 82, p. 21.

The author asserts that a test for capsicum should be included in the U. S. P. requirements for the oleoresin of ginger as many commercial samples used in making ginger ale extracts contain oleoresin of capsicum and these occasionally find their way into the pharmaceutical trade.

A method for the detection of capsicum in the oleoresin of ginger based on the neutralization of the pungent principle of the ginger with potassium hydroxide is described (p. 25.)

Vanderkleed, C. E. 1910

Report of the Committee on Adulterations.
Proc. Penna. Pharm. Assoc., 33, p. 131.

Seven samples of capsicum yielded from 15.10 to 22.27 per cent. of oleoresin; one sample of African ginger 10.12 per cent; two samples of Jamaica ginger 5.636 and 6.316 per cent., respectively (p. 147.)

1911

Extractum Filicis.

Caesar and Loretz Jahres.-Ber., Aug. 1911, pp. 76 and 105.

Regret is expressed in that the *Ph. Germ. V.* has not included an assay for oleoresin of aspidium. The crude filicin content is thought to be a satisfactory indication of the value of this preparation. A filicin content of 27 per cent. is guaranteed by the firm for the new lot of the extract prepared by them (p. 76.)

Fromme's method of estimating the crude filicin is given (p. 105.)

1911

Male fern extract.

Evans Sons Lescher & Webb, Analyt. Notes, 6, p. 48.

Five samples of male fern extract were tested. Two were found to be adulterated with castor oil (55 to 70 per cent.)

The Kraft and the Swiss pharmacopœial methods for evaluating the extracts are discussed and the results obtained in each case, along with other physical and chemical constants, are tabulated.

1911

Cubebs.

Southall Bros. & Barclay Lab. Rep., 19, p. 9.

Five samples of cubebs yielded from 4.66 to 8.78 per cent. of extract to petroleum spirit, the average being 6.95 per cent.

1911

Insect Powder.

Southall Bros. & Barclay, Lab. Rep., 19, p. 10.

Two samples of insect powder yielded 8.28 and 7.57 per cent. of oleoresin when tested by Durant's method.

One sample of Japanese insect flowers yielded 13.98 per cent. of oleoresin of an orange brown color.

1911

Oil of male fern.

Brit. & Col. Drugg., 60, p. 388.

In this article, it is stated that parcels of the extract of male fern are being condemned in London as they have been found to contain large quantities of castor oil.

Suspicion was first aroused through the low selling price of some

of the extracts. The adulterated extract was being sold for 4s per pound while reliable manufacturers would not quote prices below 5 s 6 d per pound.

1911

Ext. Filicis maris.

Chem. & Drugg., 79, p. 749 and 798.

This editorial commenting on Parry's observation, that extract of male fern is commonly adulterated with castor oil, calls attention to the tests given in the Netherlands and Swiss pharmacopœias.

Bernegau, L. H. 1911

Report of the Committee on Adulterations.

Proc. Penna. Pharm. Assoc. 34, p. 117.

Three lots of lupulin tested 58.9, 57.7 and 62.1 per cent. soluble in ether (p. 125.)

Beythien, Hemple & Others 1911

Kurze Mitteilungen aus der Praxis des Chemischen Untersuchungsamtes der Stadt Dresden.

Zeitschr. Unters. Nahr. u. Genussm., 21, p. 666.

A table is presented showing the ash content and extract content of a number of samples of ginger (p. 668.)

According to Reich the volatile ether extract content varied from 0.80 to 4.02 per cent., the non volatile from 1.66 to 6.93 per cent; the alcoholic extract from 1.33 to 4.08 per cent; the petroleum ether extract from 1.14 to 4.49 per cent; and the methyl alcohol extract from 4.40 to 12.53 per cent.

Deane, Harold 1911

Oleoresina Capsici, B. P. C.

Pharm. Journ., 87, p. 804.

The author criticises the British Pharmaceutical Codex with respect to the title *Oleoresina Capsici.* He is of the opinion that the preparation has no right to the name *oleoresin,* as it corresponds more closely to the product sold as *capsicin* or *soluble capsicin* for the use of pill makers and mineral water manufacturers.

Francis, J. M. 1911

Report of the Committee on Adulterations.

Proc. Penna. Pharm. Assoc., 34, p. 117.

Only one of eight lots of lupulin examined failed to exceed the required 60 per cent. of ether-soluble matter (p. 125.)

Gluecksmann, G. 1911

Ueber eine neue Identitaetsreaktion des Extractum Cubebarum.

Pharm. Praxis, 1911, p. 98. [Apoth.-Ztg., 27, p. 334.]

A test in which hydrochloric acid is used for producing a color reaction is described in detail.

Parry, E. J. 1911

Extract of male fern.

Pharm. Journ. 87, p. 778. [Chem. & Drugg., 79, p. 860; Am. Journ. Pharm., 84, p. 136; Apoth-Ztg., 26, p. 1046.]

The author reports on the examination of commercial extracts of male fern and finds that the greater part are undoubtedly adulterated with from 30 to 60 per cent. of castor oil. The physical and chemical constants of the commercial samples and of genuine extracts are tabulated for comparison.

Pearson, W. A. 1911

Report of the Committee on Adulterations.

Proc. Penna. Pharm. Assoc., 34, p. 126. [Bull. A. Ph. A., 6, p. 346.]

The author reports that two lots of oleoresin of aspidium were rejected because they were not green in color.

Rosendahl, H. V. 1911

Fern rhizomes, yield of extract and relative activity of.

Year-Book of Pharm., 48, p. 286. [Apoth.-Ztg., 26, p. 588; Svensk. farmac. Tidsk., 1911, p. 85.]

The yield of ethereal extract obtained from various species of fern harvested during different months of the year was found to be as follows:

	May	August	October
	Per cent.	Per cent.	Per cent.
Aspidium filix mas	—	12.5	11.0
Dryopteris spinulosa	—	17.0	—
Dryopteris dilatata	10.0	—	—
Pteris aquilina	2.0	—	—
Athyrium filix femina	0.9	—	—
Aspidium alpestris	0.7	—	—

Two grams of the extract of *Dryopteris dilatata* are stated to be therapeutically equivalent to 8 to 10 grams of the extract of *Aspidium filix mas* or four grams of the extract of *Dryopteris spinulosa.*

Vanderkleed, C. E. 1911

Report of the Committee on Adulterations.
Proc. Penna. Pharm. Assoc., 34, p. 117.

Two samples of capsicum are reported to have yielded 14.7 to 17.93 per cent., respectively, of oleoresin; one sample of subebs, 22.14 per cent; eleven samples of African ginger, 7.128 to 9.484 per cent; and eight samples of Jamaica ginger, 3.4 to 6.6 per cent. (p. 132.)

———— 1912

Extractum Filicis.
Caesar and Loretz, Jahres-Ber., Sept. 1912, p. 128.

The firm's method for estimating the crude filicin is given.

———— 1912

Capsicine.
Evans Sons Lescher & Webb, Anaylt. Notes, 7, p. 18.

Five samples of *capsicine* examined were all entirely soluble in 10 volumes of 90 per cent. alcohol.

———— 1912

Male fern extract.
Evans Sons Lescher & Webb, Analyt. Notes, 7, p. 51.

Sixteen samples of male fern extract examined in 1912 were free from castor oil and of satisfactory purity. They showed a refractive index of 1.507 to 1.509 at 15°C, and gave a filicin content of 22.9 to 26.3 per cent., when assayed according to the method given in the Swiss Pharmacopœia.

———— 1912

Capsicum.
Johnson & Johnson, Lab. Notes, 1912, p. 14.

The yield of ether extract obtained from capsicum is reported to have varied from 16 to 19 per cent.

———— 1912

Cheap extract of male fern found badly adulterated.
Merck's Report, 21, p. 29 [Apothecary, 24, p. 14.]

A sample of cheap extract of male fern examined by Merck was found to be adulterated with 25 per cent. of castor oil, and to contain only 8 per cent. of crude filicin.

———————— 1912

Male fern extract.

Southall Bros., & Barclay, Lab. Rep., 20, p. 15.

The statement of Parry that much of the male fern extract is adulterated is confirmed. The physical and chemical constants obtained in the examination of six commercial extracts are tabulated.

Dohme and Engelhardt 1912

Drug quality during the period 1906–1911.

Journ. A. Ph. A., 1, p. 99.

It is stated that there was hardly any variation in the percentage of oleoresin in the samples of cubebs examined during the last six years, (p. 101.)

Goris and Voisin 1912

The determination of the ether extract of male fern, and the unification of the methods of analysis.

Bull. Sci. Pharmacolog., 19, p. 705, [Pharm. Ztg., 58, p. 129; Journ. 90, p. 81; Year-Book of Pharm., 50, p. 337.]

It is stated that the method of the Swiss Codex gives values for crude filicin which are about 30 per cent. too high owing to the solubility of the ether solution in the solution of barium hydroxide. If the ether be driven off by heating to 50°C before filtering, the results will be comparable with those obtained by the magnesia methods.

Hooper, D. 1912

Notes on Indian drugs.

Pharm. Journ. 89, p. 391.

The examination of the rhizomes of Indian ginger, with reference to determining the relationship between maturity and oleoresin content, showed that young rhizomes develop oleoresin as they are allowed to grow. Those gathered in December yielded 6.4 per cent. of extract to alcohol (90 per cent.), while those gathered in February gave 8.3 per cent. Upon washing the extracts with water, the remaining insoluble residue amounted to 3.0 per cent. and 3.5 per cent., respectively. Some of the more mature rhizomes gave as high as 11.8 per cent. of alcoholic extract or 8.1 per cent. of washed resin.

Patch, E. L. 1912

Report of the Committee on Drug Market.

Journ. A. Ph. A., 1, p. 499.

Eight samples of Jamaica ginger gave from 3.3 to 6.0 per cent. of alcoholic extract (p. 500.)

Vanderkleed, C. E. 1912

Report of Committee on Drug Market.

Proc. Penna. Pharm. Assoc., 35, p. 165.

The assay of 4 samples of capsicum showed the oleoresin content to be from 14.41 to 16.7 per cent; five samples of cubebs yielded 1.735 to 24.49 per cent. of oleoresin; seventeen samples of Jamaica ginger, 3.444 to 6.640 per cent; ten samples of African ginger, 6.85 to 11.10 per cent (p. 179.)

———————— 1913

Miscellaneous Inquiries.

Chem. & Drugg., 82, p. 470.

Gingerin is stated to be the extract obtained upon evaporating the tincture of ginger. It is said to vary with the variety of ginger used in the preparation of the tincture.

Capsicin is stated to be commercially indefinite. It may be a strong alcoholic extract, an ethereal, a chloroformic or an acetone preparation. The accepted *capsicin* of commerce, however, is the oleoresin prepared with ether.

———————— 1913

Die Methoden zur Wertbestimmung des Filixextrakts.

Pharm. Ztg., 58, p. 129.

The methods of Goris and Voisin, and E. Schmidt for the evaluation of the extract of male fern are discussed.

———————— 1913

Extractum Filicis.

Caesar and Loretz, Jahres.-Ber., Sept. 1913, pp. 98 and 106.

Four samples of extract of male fern prepared by the firm showed a crude filicin content of 32.64, 23.7, 28.15 and 30.4 per cent., respectively, (p. 98.)

The firm guarantees the filicin content of their extract to be 27 per cent.

———————— 1913

Male fern extract.

Evans Sons Lescher & Webb, Analyt. Notes, 8, p. 44. [Year-Book of Pharm., 51, p. 244.]

Seven samples of extract of male fern examined during the year showed a filicin content of 21.3 to 25.3 per cent. and a refractive index of 1.5 to 1.51.

Three samples were impure or suspicious. They showed a refractive index of 1.495, 1,497 and 1.499, and a filicin content of 15.6, 19.6 and 19.7 per cent., respectively.

———————— 1913

Male fern extract.

Southall Bros., & Barclay, Lab. Rep., 21, p. 14.

The analytical data obtained in the examination of two commercial samples of the extract of male fern are given.

Bohrisch, P. 1913

Ueber Extractum Filicis.

Pharm. Ztg., 58, p. 601. [Chem. Abs. 8, p. 206.]

A comprehensive review of the constituents and the methods of evaluating the extract of male fern is given.

Four samples of commercial extracts in bulk were examined for density and crude filicin content. The findings for density were 0.9888, 0.9842, 0.9836 and 1.0109; for crude filicin 14.85; 15.42, 16.00 and 24.00 per cent. The same tests for five samples of the extract in capsules showed: density, 0.9824, not determined, 1.0135, 1.0255 and 0.9910; crude filicin, 15.02, 23.42, 26.77, 27.72 and 14.45 per cent.

Dohme and Engelhardt 1913

Cubebs.

Oil, Paint and Drug Rep., 83, p. 55.

The quantities of oleoresin obtained from cubebs ranged between 16 and 22 per cent.

DuMez, A. G. 1913

The physical and chemical properties of the oleoresin of Aspidium with respect to the detection of adulterations.

Philippine Journ. of Sc., 8, Sec. B., p. 523.

The methods of adulterating the oleoresin are discussed in detail The physical and chemical constants of samples prepared in the laboratory and those obtained from various commercial sources are presented with the idea of indicating to what extent they may be relied upon in detecting a deteriorated or adulterated product.

Engelhardt, H. 1913

Purity of chemicals and drugs.

Journ. A. Ph. A., 2, p. 163.

Four samples of black pepper are reported to have yielded 10.6, 12.5, 9.2 and 11 per cent., respectively, of oleoresin; six samples of capsicum, 13.1, 41.8, 15.26, 15.8, 11.3 and 11 per cent; cubebs from 18 to 25 per cent; Jamaica ginger from 2.81 to 5.24 per cent; lupulin, eight samples out of twelve, less than 60 per cent; three samples of parsley seed. 14.7, 11.4 and 13.04 per cent. (pp. 164 and 165.)

Gane, E. H. 1913

Report of Committee on Drug Market, August, 1912.
Journ. A. Ph. A., 2, p. 677.

Four lots of lupulin gave 44.94 to 65.5 per cent. of ether-soluble material, (p. 681.)

Harrison and Self. 1913

Analytical constants of extract of male fern.
Chem. & Drugg. 83, p. 182. [Year-Book of Pharm., 50, p. 494; Pharm. Journ. 91, p. 128; Pharm. Ztg., 58, p. 643.]

The analytical constants of genuine and commercial extracts of male fern are tabulated. The authors do not approve of the standards suggested by Parry.

Hill, C. A. 1913

Analytical notes on extract of male fern.
Chem. & Drugg., 83, p. 181. [Pharm. Ztg., 58, p. 643.]

The analytical constants of 23 samples of extract of male fern are discussed and tabulated. The chemical and physical constants of the oily portion are also given for comparison with those of castor oil. One commercial sample is reported to have contained 59 per cent. of the latter.

Osborne, Oliver F. 1913

A last plea for a useful Pharmacopoeia.
Journ. Am. Med. Assoc., 60, p. 1427.

Among the "useless" preparations adopted by the Committee of Revision, the author includes the oleoresins of lupulin and parsley seed. (p. 1429.)

Parry, E. J. 1913

Extract of male fern.
Chem. & Drugg., 83, p. 231.

The author confirms the results which he published in an earlier paper.

Patch, E. L. 1913

Report of the Committee on Drug Market.
Journ. A. Ph. A., 2, p. 1081.

The percentage of alcoholic extract obtained from the drugs tested is reported as follows:

Capsicum, four samples, 19 to 24 per cent; ginger, nine samples, 5.2, 5.7, 4.2, 4.0, 4.5, 4.9, 3.5, 4.8 and 4.3 per cent. pp. 1088 and 1094.

The yield of ether extract reported by Kebler is as follows:

Fifty-three samples, lupulin, 63.96 to 77..82 per cent; black pepper three lots, 10.04, 10.87 and 12.88 per cent; red pepper, eight samples, 13.0, 10.6, 14.7, 18.91, 13.12, 10.4, 13.25 and 14.7 per cent. The iodine values for the same were 132, 138, 123.4, 107, 127.3, 25.2 and 137.3. Seventeen other samples yielded from 11.22 to 20.77 per cent. The iodine value of these varied from 110 to 145.7 (pp. 1098 and 1101.)

Umney, J. C. 1913

What is capsicin?

Pharm. Journ., 91, p. 594.

Capsicin is stated to be a synonym for *Oleo-Resin of Capsicum* of the *B. P. Codex,* and, is made by extracting capsicum with 60 per cent. alcohol and subsequently evaporating off the solvent. It should not be confused with the preparations made with strong alcohol (90 per cent.), ether or acetone.

Vanderkleed, C. E. 1913

Report of the Committee on Drug Market.

Proc. Penna. Pharm. Assoc., 36, p. 77.

Thirty-seven samples of Jamaica ginger are reported to have yielded 3.10 to 5.75 per cent. of oleoresin; seventeen samples of African ginger, 6.85 to 9.92 per cent; seven samples of capsicum, 13.1 to 18.1 per cent; one sample of cubebs, 21.8 per cent.

Yagi, S. 1913

Physiologische Wertbestimmung von Filixsubstanzen und Filixextrakten.

Zeitschr, f. d. ges. exp. Med., 3, p. 64. [Therap. Monatsch., 1914, p. 443; Apoth-Ztg., 29, p. 544.]

A method in which earth worms are used for the purpose of testing the relative activity of extract of male fern and its constituents is described.

——————— 1914

Untersuchung der offizinellen vegetablischen Drogen.

Riedel's Ber. 58, p. 29.

The samples of cubebs examined are reported as having yielded 11.1 to 14.7 per cent. of extract soluble in ether 1 part and alcohol 1 part (p. 31.)

The alcohol extract obtained from capsicum varied from 31.9 to 35.3 per cent. (p. 32.)

The samples of aspidium examined gave 9.4 to 9.7 per cent. of ether-soluble extract.

———————— 1914

Ueber Gelatinkapsel-Fabrikate.

Riedel's Ber. 58, p. 45. [Apoth.-Ztg., 29, p. 310.]

Capsules from only two manufacturers contained extract of male fern of which the crude filicin content was higher than 20 per cent. The extract of male fern in capsules from four other sources showed a filicin content of from 8.57 to 16.02 per cent. (p. 48.)

———————— 1914

Extractum Filicis.

Caesar and Loretz, Jahres-Ber., Oct., pp. 23, 37, and 96.

The method of S. Yagi for the physiological standardization of the extract of male fern is stated to be too cumbersome for practical use. (p. 23.)

Extracts prepared in the laboratory showed the following crude filicin content, 25.48, 24.85, 29.7, 26.04, 26.0, 35.58, 27.35 and 33.79 per cent. (p. 37.)

It is further stated that the yield of ether extract, after evaporating on a water bath at 60°C to constant weight and drying in a desiccator for half an hour, should be about 15 to 18 per cent. (p. 96.)

———————— 1914

United States Pharmacopœia Ninth Revision. Abstracts of proposed changes with new standards and descriptions.

Journ. A. Ph. A., 3, pp. 524 and 1573. [Year-Book of Pharm., 52, p. 324.]

It is stated that the former solvent, acetone, is to be changed to ether in the following: *Oleoresina Aspidii, Oleoresina Capsici, Oleoresina Zingiberis* and *Oleoresina Piperis.* (p. 551.)

Directions are also given for the preparation of *Oleoresina Petroselini* (p. 573.)

Bohrisch, P. 1914

Ueber verschiedene verbesserungbeduerftige Artikel des Deutschen Arzneibuches V.

Apoth.-Ztg., 29, p. 901.

It is stated that a large portion of the extract of male fern made in Germany shows a crude filicin content of less than 15 per cent., while the Swiss Pharmacopœia requires a content of 26 to 28 per cent. The author, therefore, thinks it desirable that a method for the estimation of the crude filicin in this preparation be given in the German Pharmacopœia.

E'we, G. E. 1914

Report of Committee on Drug Market.

Proc. Penna. Pharm. Assoc., 37, p. 125.

The author reports as follows on the oleoresins examined:

Four samples of oleoresin of capsicum were found to be pungent in dilutions of 1 to 150,000, the arbitrary standard of H. K. Mulford Company.

Seven samples of oleoresin of ginger were pungent to the taste in dilutions of 1 to 20,000, the arbitrary standard of H. K. Mulford Company.

One lot of oleoresin of cubeb contained the waxy deposit which the U. S. P. directs should be rejected.

One lot of oleoresin of say palmetto, "U. S. P." contained 15 per cent. of water which separated on standing. It also contained a large amount of insoluble matter (p. 152.)

Linke, H. 1914

Ergebnisse, Beobachtungen und Betrachtungen bei der Untersuchung unserer Arzneimittel.

Apoth.-Ztg., 30, pp. 606 and 628.

The results obtained in the examination of extract of male fern, in bulk and in capsules, obtained from various sources are tabulated. Especially the extract marketed in capsules was found to be low in filicin content.

Patch, E. L. 1914

Report of Committee on Quality of Medicinal Products.

Journ. A. Ph. A., 3, p. 1283.

A sample of oleoresin of capsicum examined is reported as having been found to be insoluble in ether, only slightly soluble in alcohol and almost completely soluble in water (p. 1298.)

Rippetoe, J. R. 1914

The examination of some drugs with special reference to the anhydrous alcohol and ether extracts, and ash.

Am. Journ. Pharm., 86, p. 435.

Four samples of capsicum are reported as having yielded 17.02 to 24.46 per cent. of extract to alcohol, and 16.49 to 17.88 per cent to ether, (p. 437.)

Six samples of cubebs gave 8.87 to 11.04 per cent. of alcoholic extract, and 7.68 to 9.80 per cent. of ethereal extract, (p. 438.)

Two samples of Jamaica ginger yielded 4.98 to 5.5 per cent. of extractive matter to alcohol, and 2.79 to 4.97 per cent. to ether. Two samples of African ginger yielded 6.20 to 6.23 per cent. to alcohol, and 5.3 to 5.45 per cent. to ether, (p. 439.)

Three samples of lupulin yielded 32.49, 55.18 and 57.06 per cent., respectively, of ethereal extract, (p. 440.)

Scoville, W. L. 1914

Report of Committee on Quality of Medicinal Products.
Journ. A. Ph. A., 3, p. 1283.

It is stated that the samples of cubebs examined during the year gave from 18.1 to 22 per cent. of oleoresin, (p. 1287.)

Vanderkleed, C. E. 1914

Report of Committees on Drug Market.
Proc. Penna. Pharm. Assoc., 37, p. 125.

On page 160, analytical data obtained from the laboratory of H. K. Mulford Company are reported showing the following yield of oleoresin for capsicum, cubebs and ginger:

	Lowest Yield. Per cent.	Highest Yield Per cent.	Average. Per cent.
Capsicum (15 samples)	13.0	18.0	16.0
Cubebs (6 samples)	13.9	19.8	16.9
African ginger (3 samples)	8.50	9.61	9.0
Jamaica ginger (3 samples)	4.33	5.75	5.06
Male fern (4 samples)	6.85	10.12	8.23

——————— 1915

Male fern extract.
Southall Bros. & Barclay, Ann. Rep., 22 and 23, p. 17.

The filicin content of five samples of extract of male fern examined is reported as having varied from, 20.4 to 27.7 per cent., the specific gravity from 0.9885 to 1.030.

Glickman, L. H. 1915

Report of Committee on Drug Market.
Proc. Penna. Pharm. Assoc., 38, p. 138.

Ten lots of lupulin examined are reported to have yielded the following percentages of ether-soluble matter: 55.5, 55.0, 57.1, 58.6, 54.7, 55.3, 44.2, 69.2, and 68.2, (p. 149.)

Vanderkleed, C. E. 1915

Report of Committee on Drug Market.
Proc. Penna. Pharm. Assoc., 38, p. 138.

On page 155, the following data concerning the yield of oleoresin are

reported as having been obtained from the analytical laboratory of H. K. Mulford Company.

	Lowest Yield. Per cent.	Highest Yield. Per cent.	Average. Per cent.
Capsicum (6 samples)	13.85	20.84	16.65
African ginger (2 samples)	7.99	8.90	8.44
Jamaica ginger (1 sample)	3.93	3.93	3.93

Beringer, G. M. **1916**

The reasons for some of the changes in the formulas of galenicals made in the ninth revision of the United States Pharmacopœia.

Journ. A. Ph. A., vol. 5, No. 12, p. 1390.

It is stated that acetone was the menstruum directed to be used in the preparation of the oleoresins by U. S. P., eighth revision, on account of cheapness. It is further stated that, since permission has been obtained to use denatured alcohol in the manufacture of ether, the cost of the latter has been reduced to such an extent that it has again become advantageous to use it in place of acetone. Hence, its use in the new Pharmacopœia.

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STUDIES OF ZYGOSPORE FORMATION IN PHYCOMYCES NITENS KUNZE

MARY LUCILLE KEENE

The problem of sexuality in the Mucorineae offers an extremely interesting field of investigation for the physiologist. Great differences have been found to exist with regard to the production of zygospores and sporangia and considerable experimentation has been carried on to determine the conditions influencing asexual and sexual reproduction.

The cytological features are very incompletely known. Numerous studies have been made upon *Sporodinia grandis,* a homothallic form, with more or less conflicting results. They have served, however, to give us some insight into the internal changes which occur when zygospore formation takes place. With the heterothallic forms, on the other hand, there has been little done and the field open here offers many interesting possibilities.

Conjugation in the Mucorineae was first described by Ehrenberg (1820) in a form he termed *Syzygites megalocarpus (Sporodinia grandis).* De Bary (1864) described the process of conjugation and zygospore formation in both *Sporodinia grandis* and *Rhizopus nigricans.* Following this work of de Bary, various workers undertook to study and explain the conditions responsible for the production of zygospores.

Van Tieghem (1876) believed the impoverishment of the medium to be responsible for the production of the zygospores. Cornu (1876) attributed it to desiccation. Brefeld (1872) in an early paper cited cold as an agent but later (1900) he decided that zygospore formation is undoubtedly due primarily to some unknown internal cause. Bainier (1883 *a* and *b,* 1884)

concluded that the production of zygospores is influenced by the medium on which they are grown. Zopf (1888) attributed their formation to the invasion of parasites which caused a decrease in the number of sporangia produced. Klebs (1898, 1902) attributed the immediate cause of sporangial formation to transpiration, the stimulus for zygospore production being concerned with decreased transpiration. Falck (1901) claimed that zygospores would form only when the concentration of the medium is high and that the relative humidity is of slight, if any, importance.

Blakeslee (1904) confirmed Klebs' results showing that increased moisture favors the formation of zygospores. He also showed that the concentration of the medium is unimportant. Blakeslee came to the conclusion, however, that these various physical factors were but secondary influences and that pure sexual strains existed upon which zygospore production was primarily dependent. He obtained cultures of *Rhizopus nigricans* from mycelia which had developed from the suspensors of a zygospore and succeeded in isolating two pure strains which, when grown alone through numerous generations, gave rise to sporangial growth only. If they were "contrasted," that is, grown side by side, or in mixed cultures, they readily produced zygospores. Thus the heterothallic nature of *Rhizopus nigricans* was established. Later he extended his work to include *Absidia caerulea, Phycomyces nitens, Mucor mucedo,* and several undetermined *Mucors,* among the heterothallic forms.

Blakeslee's results have not been extensively tested by other investigators. Hamaker (1906) claimed that zygospores of *Rhizopus nigricans* could be produced unfailingly upon proper media. Namyslowski (1906) also believed that the nutrients were responsible for zygospore production.

The writer (1914) in an earlier paper reviewed rather completely the literature relevant to the cytology of conjugation in the Mucorineae.

The results of studies upon *Sporodinia grandis* published by the writer (1914) vary but little from those published by Dangeard (1906) in his later paper. Fusions in pairs appear to take place between the nuclei of the fusing gametes. Certain plastid-like bodies apparently concerned with the produc-

tion of an oily reserve substance are described. These bodies are small and numerous in partially mature zygospores but later become reduced to one or two large plasmatic bodies saturated with oil. A nuclear disorganization takes place. All of the nuclei do not disorganize and, even in the mature zygospore, many of the normal but slightly larger nuclei are present. The mature zygospore contains a large amount of an irregularly knotted or densely granular, red-staining substance which the writer was unable to explain. It reacted to the triple stain much as the mucorin crystals do, but appeared to arise from the disorganized nuclei.

Burgeff (1915) describes briefly the internal changes which take place at the time of conjugation and of the formation and germination of the zygospores of *Phycomyces nitens*. At the time of conjugation the nuclei become collected at the places of contact of the gametes, a large vacuole becomes evident, the wall is resorbed and the zygospore is established. The nuclei are equally distributed throughout the zygospore. No nuclear fusions are to be noted. Vacuoles containing oil appear, together with granular masses which are protein-like reserve substances. Later the oil vacuoles flow together into a few large globules. At two to four months after formation, one central oil globule is present. The nuclei lie in the outer layer of the non-granular, weakly staining, hollow, cytoplasmic sphere which surrounds the oil globule. The nuclei are homogeneous, without a membrane and are surrounded by a clear zone.

Burgeff finds that, at the time of germination of the zygospores of one variety, the nuclei are arranged in pairs in the periphery. A varying number of the nuclei appear to fuse in pairs. In another variety the conjugation of the nuclei appears to take place before germination. The oil globules become reduced and the cytoplasm becomes vacuolate. As the germ tube pushes out, the nuclei undergo mitotic division. The single chromatin grain of the nucleus forms simple, small chromosomes. The approximate number, determined by count and estimation, is twenty-four. They appear to separate into groups of twelve and become surrounded by a membrane.

The present piece of work was undertaken in the hope that some further facts concerning these internal phenomena might be offered. Furthermore, in view of the work done by Blakeslee

and of the very interesting field opened up by his explanation of the sexuality of the Mucorineae, it was thought possible that some internal differences might be found which could be correlated with the apparent inherent difference between the two strains, or to bring the problem to the point where some such correlation might be possible when the more general cytological features were known.

The previous work was carried out on *Sporodinia grandis,* a homothallic form, in which it is a very simple matter to obtain zygospores in abundance with the simplest cultural methods. The present work, however, has been done on *Phycomyces nitens,* a heterothallic form, in which certain secondary factors are relevant to zygospore production.

I am indebted for my cultures to Dr. R. A. Harper, who kindly supplied me with zygosporic plates in which the two strains had been contrasted and a line of zygospores had resulted in the characteristic fashion where the mycelia of the two strains had come together. Isolations were made from these plates and the pure plus and minus strains resolved. Mixed cultures were also obtained which produced zygospores profusely.

I am also indebted to Dr. L. O. Kunkel for pure plus and minus cultures of *Phycomyces nitens* which were, I believe, of the same stock as those furnished in plate form by Dr. Harper.

METHODS

Stock cultures of the pure plus and minus strains of *Phycomyces nitens* have been kept on various media. Rice or rye bread has been used for the most part because of the ease of manipulation. Small Erlenmeyer flasks are very satisfactory containers for culture work because there is less surface exposed for evaporation and they are less subject to contamination than the wider-mouthed culture dishes that are ordinarily employed. The cotton plugs may be covered with tin foil so that all the moisture is conserved.

At intervals the strains have been inoculated side by side on the various kinds of media and in almost every case a line of zygospores has been evident by the end of a week. Transfers of individual sporangia have been made, one from each strain, with the same marked results when grown together.

Mixed cultures have been kept growing, and here also the zygospore production has been profuse.

For cytological studies, material ranging from three days to six months in age was fixed. Various fixing reagents were employed: Flemming's solution of various strengths, chrom-acetic, Bouin's, and Gilson's. The younger stages were fixed directly on the agar and small portions of the agar were carried through the various stages of washing, dehydrating, and infiltration with paraffin. Because the young gametophores are much convoluted and stand erect from the surface of the substratum, it is difficult to obtain satisfactory sections.

The same difficulties of technic were encountered here which were met with in *Sporodinia grandis,* but in even a more serious form. The older zygospores are extremely brittle because of the heavy walls and the large amount of reserve material contained within the zygospore. The most satisfactory serial sections were obtained from material that was fixed in Flemming's solution, thoroughly washed and treated with hydrofluoric acid while in 95 per cent alcohol. That the resulting structures are normal and not the result of this treatment is evident from the fact that in material treated in the usual ways, the same structures have been found but it is almost impossible to obtain them in serial form because of their torn condition.

For infiltration with paraffin, both the xylol and the cedar oil methods have been employed with equal success. The cedar oil method is probably better for the older stages.

The younger zygospores were cut into sections of from 5 to 10 microns while the older ones were cut into thicker sections ranging from 10 to 60 microns. Owing to the size of the zygospore inclusions found at maturity, the thick sections were more satisfactory for all but the nuclear studies. For affixing the sections to the slides both the albumen-glycerin and the fish-glue fixatives were used.

The Flemming's triple stain was given the preference although anilin-safranin alone was used in many cases. The nuclei stand out quite clearly in such preparations. Gram's anilin-gentian-violet and iodine stain gave some very beautiful preparations. They closely resemble preparations made with Heidenhain's iron-alum-haematoxylin combination but are very much more quickly made.

Stained preparations of the germinating plus and minus spores were made as follows: the spores were germinated either in weak sugar solution or in agar. Bouillon is to be avoided because of precipitation in the subsequent treatment. The spores, germinated in sugar solution, were flooded onto a slide, which had been previously covered with fish-glue fixative, and allowed to stand. When almost dry, the preparation was covered with the fixing solution, either Flemming's or chrom-acetic. The latter is the more satisfactory because subsequent bleaching is not necessary. After an exposure for ten minutes, the fixing agent was drawn off by means of filter paper and the slide was washed carefully in water. Many of the spores washed off but enough remained to give very satisfactory preparations. It was found best to use alcoholic stains if possible because the less the preparations are washed in water the better they are in the end.

The spores germinated in agar were also fixed and carried through to paraffin in the usual ways. These preparations are not as good because the agar is granular and takes the stain and because in sectioning the germ tubes are cut at various angles.

The pure strains of the sporangial cultures were grown on rye bread in large flat dishes. The sporangia were collected at various stages and fixed as previously described. The studies of the sporangia in fixed material were restricted to the large sporangia which are produced in older cultures.

LIFE HISTORY AND DEVELOPMENT

Under natural conditions *Phycomyces nitens* is found growing most commonly in fresh manure. In the majority of texts and general descriptions it is described as preferring an oily substratum, but there seems to be little evidence to support this statement. It is possible to obtain a luxuriant growth on any of the ordinary media used in the laboratory for such purposes, *e. g.*, bread, rice, sugar, and vegetable agars. It requires considerable moisture and medium temperatures (18° to 28°C.) to produce the best vegetative growth.

The spores are small elliptical bodies with resistant walls. They possess from four to twelve nuclei each, typically eight.

The spores germinate readily in water or various weak sugar solutions and broths. They enlarge somewhat and one or two germ tubes push out (Pl. II, fig. 8). These grow for some distance during which time the number of nuclei present increases. Careful studies have revealed no differences between the spores of the two strains. The germ tube branches and sub-branches forming the hyphal network over and through the substratum. At intervals characteristic enlargements occur and occasionally from these, rhizoid-like branches grow out. In other cases, however, these enlargements appear merely as swellings and what they are functionally remains a question. Internally they appear much as any other portion of the mycelium; sometimes there occurs a clustering of the nuclei in this region. The small sporangia which are formed first and are produced close to the substratum appear to be typical in formation and production of spores. The aerial branches which are destined to form the large sporangia appear yellowish with a blackening toward the base. The yellow color is due to the presence of a large amount of oil, as shown by tests, which exudes in droplets when the sporangiophores are crushed. As the sporangiophores elongate they exhibit an extreme sensitiveness to light, the terminal portions bending toward the source of illumination. If grown in the dark, they elongate to a greater extent than when grown in the light.

The history of the formation of the sporangia and of the origin of the spores has been worked out by Swingle (1903). The young sporangiophores are densely crowded with cytoplasm and nuclei. The tip swells out into a small rounded structure. The contents of this are evenly distributed at first but later a zonation appears with the denser portion of the protoplasm toward the periphery and the vacuolate portion at the center. There occurs a marked streaming of the protoplasm into the sporangium at this stage and the rounded vacuoles of the central portion move out to the periphery. They become filled with stainable contents which appear bluish when the triple stain is used. The nuclei are at first evenly distributed through the outer zone, few occurring in the central region. A layer of vacuoles having stainable contents becomes arranged in a dome-shape in the denser portion. The vacuoles flatten, fuse edge to edge and form a continuous cleft which is filled with the same material that filled the vacuoles.

The spore differentiation begins at this time. The vacuoles lose their rounded form and become angular. As the edges or points of the vacuoles come in contact, they unite, forming clefts in the plasm. Furrows cut into the sporeplasm from the columella. These unite with the clefts formed by the vacuoles and cut the plasm into irregular masses. The spores vary in size and in the number of nuclei. The nuclei remain in the resting stage throughout this process; no cases of nuclear divisions were observed by Swingle.

All the cytoplasm and the nuclei are included in the spores themselves. Between the spores there occurs the intersporal slime which has originated in the vacuoles and which Swingle believes arises as a secretion from the vacuolar membrane. It is homogeneous when the spores are formed, stains a bluish brown with the triple stain, and contains no nuclei or other inclusions.

The columella wall begins to form while the spore cleavage is going on and continues to thicken until the spores are ripe. Thus it has been shown in this form, as well as by Harper (1899) for *Pilobolus* and *Sporodinia,* that the columella wall arises as a dome-shaped structure from the very first and not as a straight wall which later arches up, as has been so erroneously described in many places. The spores finally become invested with a refringent cell wall which is very resistant to stains.

Studies of the plus and minus sporangia were made in order to determine whether or not any morphological differences exist between the two strains. The sporangia of the two strains are structurally alike as far as it has been possible to determine. The young sporangiophores of both show a marked increase in the number of nuclei. The nuclei, to all appearances, are similar. The subsequent changes that take place in the formation of the columella and production of spores proceed alike in the two strains.

The character of the growth of the plus and minus strains is unlike, and when cultures of each are grown side by side it is possible to distinguish between them. The mycelium in the minus strain grows somewhat more slowly and less vigorously and the sporangia appear to be fewer and later in appearance.

Zygospore Production

Van Tieghem and Le Monnier (1873) were the first to report the zygospores of *Phycomyces nitens* and in their drawings indicate it as a homothallic form, as a result of which de Bary placed *Phycomyces* with *Sporodinia.* Van Tieghem's first cultures were obtained from cochineal. He lost these but later obtained others from horse dung and again from cochineal. He failed to obtain zygospores when cultures were made from a single sporangium. Bainier (1883*a*) described the zygospores on horse dung mixed with flax-seed flour or soaked with flour, but he also realized the uncertainty of securing them. Later he found that if, during February or March, he started fresh cultures of horse dung he almost always obtained the zygospores within a short time. Zygospores have been reported in *Phycomyces microsporus* by Van Tieghem (1876) and in *Phycomyces pirrotianus* by Morini (1896).

Until Blakeslee (1904) worked with *Phycomyces nitens,* the zygospores had not been reported in this country except in one case in which Thaxter, according to Blakeslee, described them in rabbit dung obtained from Daytona, Florida.

The general facts of the morphology of conjugation in this form have been described by de Bary (1864), to whose account Blakeslee (1904) has added a few points of interest. The majority of writers in describing the origin of the progametes say that the club-shaped progametes develop from the hyphae, increase in size, come in contract and fuse. Blakeslee, however, believes that the stimulus to development comes from contact; that they are "zygotactic" and that the progametes are normally adherent from the first. Blakeslee has devised an ingenious experiment to determine whether the origin of the progametes is dependent upon contact or upon chemical stimuli. The experiment has been repeated by the writer. A small battery jar was lined with filter paper. Suspended by means of wire in the jar, one and one-fourth inches apart, were two small cheese cloth bags containing rye bread. Blakeslee soaked these with orange juice but the author found moistening well with water to be sufficient. An inch of water was put in the bottom of the jar and paper was tied over the top. This was then sterilized in the autoclave at 8 lbs. for 15 minutes. When cool one bag was inoculated with the plus strain and the other

with the minus strain. The paper cover was replaced, tin foil was spread over the top to reduce evaporation, and the apparatus was set into the ice box where evaporation would be slow and light effects negligible. At the end of six days a line of zygospores had formed midway between the two bread bags. Thus, as Blakeslee has pointed out, any influence upon the origin or direction of growth of the gametophores was not the result of any chemical attraction due to the medium or to contact of the mycelial masses, but must have been confined to the aerial portions and is, in all probability, restricted to the hyphae affected.

Blakeslee describes the more general morphological aspects of conjugation and zygospore formation. In a later paper (1906), he describes the germination of the zygospores of *Phycomyces* as well as of several other forms. Germination is said to take place after a long period of rest and it is difficult to bring the zygospores to germination in the laboratory. In the process of germination the outer wall is ruptured and a germ tube forms which produces a rudimentary mycelium on which are borne sporangia and spores. The spores in turn give rise to the next vegetative generation. Presumably it is at the time of the formation of the spores in the germ sporangia that sex segregation occurs. According to Blakeslee, three kinds of spores may be produced in a single germ sporangium: the plus, the minus and the neutral, which upon germination give rise to the corresponding mycelia.

The effects of external conditions, as secondary influences related to the formation of zygospores in this form, have not been carefully investigated by Blakeslee. He secured zygospores on all the substrata tested, which fact disproves the idea that oily substances are essential. He obtained them sparsely on potato agar but plentifully on potato agar acidulated with orange juice. He finds that the zygospores are produced much less abundantly in the warm oven, 26°–28°C., than at room temperature.

The writer has verified these results. In securing zygospores, the following media have been used successfully: carrots, carrot agar, bean dextrose agar, condensed milk agar, potato agar, potato dextrose agar, prune agar, dextrose bouillon agar acidulated with orange juice, bread (both wheat and

rye) and rice. In every case zygospores have been secured when the medium was inoculated with spores from both strains, while at no time and on none of the above-mentioned media, have zygospores been obtained with the pure strains.

No difficulty whatever was experienced in any inoculations in securing a plentiful production of zygospores when the two strains were inoculated into the same medium. The matter of humidity seems to be a very important secondary condition and the best results are invariably obtained if the cultures are kept in a cool, humid place. As soon as the medium begins to dry out, zygospore production ceases and there occurs a marked increase in vegetative development. Considerable difficulty was experienced at first in bringing the zygospores to maturity, but later it was discovered that moisture conditions were responsible. If Petri dishes were used it was essential that a very heavy layer of agar be supplied, and the softer agars with high moisture content were more satisfactory.

This substantiates the work done by Klebs (1898, 1902) in which he pointed out the relation of transpiration to spore formation in *Sporodinia grandis,* and corroborates the results obtained by Blakeslee (1904) on *Rhizopus nigricans.* There seems to be no evidence in *Phycomyces,* at least, that zygospore formation is affected by the concentration of the medium.

Cytology of the Zygospore

When the two branches from sexually different strains of *Phycomyces nitens* come together there occur a branching and lobing which tend to interlock the hyphae (Pl. I, figs. 1 and 2). Previous to contact it is impossible to tell the gametophoric hyphae from any of the other parts of the mycelium. The nuclear conditions at this stage are the same as in the resting mycelium, as far as it has been possible to determine. There is no evidence of any excessive streaming or increase in the number of nuclei. As the branches increase in length, they become erect, the terminal portions elongate, their inner surfaces lying parallel and appressed (fig. 3). The gametophores at this time are usually characterized by a yellow color which is due to the presence of oil as shown by tests. As the progametes continue

to enlarge (fig. 4), the cytoplasm becomes somewhat vacuolate, lines of streaming may be discerned and there occurs a marked increase in the number of nuclei. Although these stages have been studied with great care, it is not possible to say with certainty that nuclear divisions take place. Certain suggestive conditions are found, as indicated in the accompanying figures (Pl. II, figs. 9, 9*a*, 9*b*), but to any one who has attempted a study of the nuclei of these forms it will be evident why a final decision cannot be rendered. The nuclei are so small that only the most conspicuous changes are apparent. It seems highly probable that nuclear divisions do occur at this time, however, as the increase in the number of nuclei in the progametes is greater than can be readily explained through nuclear migration from adjacent parts. Furthermore, these peculiar nuclear conditions are apparent at only four stages: in the progametes; in the suspensors prior to formation of the appendages; in the young sporangiophores; and in the germinating spores. The accompanying figures (9*a*, 9*b*, 10*a*, 10*b*) illustrate the appearance of the nuclei at this time. The resting nucleus is a small, dense, globular to ovoid body possessing a conspicuous, red-staining granule, probably the nucleolus. The chromatin consists of very small granules scattered through the nuclear cavity. The nuclear membrane is fairly well defined in most of the nuclei. At the time of these apparent divisions, there are present from one to three of these red-staining masses and some of the nuclei appear somewhat elongated. In what is evidently a polar view three of these red-staining bodies can be seen, one of which is usually slightly larger than the other two. In edge view, there are usually two of these bodies apparent, sometimes of equal size but often unequal. As far as it has been possible to determine, the larger of these three bodies is the nucleolus and the other two represent the chromatic masses. If they were all nucleoli they would be expected to be visible at other stages. Furthermore, the granular chromatin which is present in the resting nucleus is not found in the nuclei showing this condition. This would suggest that it had become concentrated into these small, deep-staining masses.

The tips of the gametophores remain in contact throughout, but as the gametophores increase in length they also increase in diameter and the portions back of the tip are gradually forced

apart from each other and round out. At this time, a large central vacuole becomes apparent in each and the cytoplasm at the point of contact of the two gametophores is very dense. This is evident in fresh material and is particularly well brought out in stained preparations (figs. 9 and 10). There are few, if any, small vacuoles and the nuclei are very closely placed.

The time of dissolution of the contiguous walls varies here as in *Sporodinia.* Sometimes the gametes are cut off from the suspensors before the protoplasmic masses come into contact, but in other cases, the intervening wall is resorbed before the new delimiting walls are established. This condition has been checked carefully in living material. Figure 10 shows a section through the gametophores before the formation of the cross walls. This formation, however, is a matter of a very short space of time, both being accomplished within an hour. The formation of the delimiting walls proceeds as it does in *Sporodinia.* The new wall forms first at the surface and closes in gradually in the form of a diaphragm which finally severs the intervening strand of protoplasm. The same papilla-like structures found in *Sporodinia* characterize these walls.

The intervening wall separating the two gametes is usually dissolved first at the center but may be resorbed in several places at once. (fig. 10). The protoplasm of one gamete pushes its way into the protoplasm of the other. Here again, as in *Sporodinia,* the author is not inclined to ascribe any significance to this act as due to a sexual differentiation. It appears rather to occur as the result of turgescence. If the internal pressures are not at an equilibrium, which is hardly to be expected, when the wall weakens at one spot, the gamete possessing the greater osmotic pressure is released and surges into the other. Ultimately, the separating wall disappears and the two gametes appear as one mass (fig. 11). Occasionally fragments of this wall may be discerned in the zygospore.

An interesting and somewhat perplexing condition arises in the appearance of the nuclei at this time. As the young zygospore is delimited and the protoplasm of the two gametes begins to mix, there occurs a characteristic grouping of the nuclei. There appear to be from twelve to sixteen nuclei aggregated in a group (fig. 11). It is impossible to state with certainty that in each group there are nuclei from each gamete, as there

is no possible way of distinguishing between the nuclei of the two branches. In fact the nuclei from the two gametes are apparently similar in size and content. It seems highly probable, however, that nuclei from each gamete are aggregated because nuclei in each group fuse in pairs. This is evidenced by the fact that the nuclei show an increase in size and a decrease in number prior to any process of nuclear disorganization. Prior to fusion, the nucleus is dense and granular and possesses a conspicuous nucleolus. After the fusions take place, the nuclear plasm appears vacuolate and the nucleolus is much larger. As indicated in the figures, within one zygospore (fig. 11*c*) there may be found groups of nuclei in which there occur from twelve to sixteen small nuclei and other groups in which there are five large nuclei and two to four smaller ones. There seems little doubt as to the significance of this condition, since no marked differences in the size of the nuclei are evident at any earlier stage.

There are certain large, round, red-staining bodies found within the zygospore at all stages which must not be confused with the nuclei. They are far more conspicuous than the nuclei and are larger. They take the safranin in the same way that the nucleolus does and have undoubtedly been interpreted by some workers as nuclei. They are usually contained within a clear zone and very often each possesses a small secondary body (fig. 11*d*). Their appearance suggests very strongly the crystalloids and globoids of the higher plants. These bodies are characteristic of many of the Mucorineae and have been described by various workers. The author has been able to demonstrate their occurrence at all stages in the life history of both *Sporodinia grandis* and *Phycomyces nitens*. They undoubtedly constitute a form of reserve substance, probably protein in nature. In addition to these, there also occur red-staining angular crystals (fig. 11*e*).

The new wall surrounding the zygospore is established just previous to the nuclear fusions. It is laid down inside of the old gametophore wall (fig. 11). It is more or less roughened and is probably formed in the same way as in *Sporodinia*, which has been described by Vuillemin (1904).

Following the nuclear fusions, there occur nuclear disorganizations. The cytoplasm of the zygospore becomes finely vacuo-

late. The nuclear disorganizations do not appear to be restricted to any particular region but occur throughout the whole zygospore. Some of the nuclei in each group apparently undergo disintegration. This disorganization appears to be restricted to the smaller nuclei or presumably to those which have failed to fuse. During the process of nuclear disorganization the red-staining nucleolus enlarges, stains unevenly and appears as if being dissolved. The margin takes the stain but the central portion stains faintly and may even appear brownish. As disorganization proceeds, and it does so on a large scale, the resulting masses appear to run together or coalesce forming irregular or knotted masses. Plate II, figure 12*b* and Plate III, figures 14*b*, 14*c* illustrate the various stages in the appearance of this substance. The same substance was evident in the zygospores of *Sporodinia,* but both its origin and fate were not interpreted. Here, however, there seems to be little, if any, doubt on these points. As this substance increases in volume it becomes concentrated into one or more central masses. It is without special structure. While it is alveolar in part, it is of a homogeneous texture and in no way resembles living cytoplasm. The oil that is contained within the zygospore sometimes appears as small droplets in this substance but does not mix with it. When fresh zygospores are crushed, gas bubbles may be seen to escape and disappear. Either of these conditions may explain the alveolar nature of this mass.

When fresh mature zygospores are crushed, the substance in question exudes as a semi-transparent amorphous mass. It is insoluble in water. The oil which is pressed out with it is honey-colored and rounds up into distinct globules which react typically to Sudan III and osmic acid. The oil is soluble in xylol and chloroform. The amorphous material, however, is not affected by these reagents. The alcohol in the Sudan III may cause a slight precipitation but the mass is not stained. When absolute alcohol is added, there remains a semi-transparent substance which becomes brownish upon standing. Since the stained sections indicated that this substance might have its origin in the disorganizing nuclei, an attempt was made to test it for proteins. Because of the small amount of material that was available, only rather crude microscopical

tests could be employed. The zygospores were dissected from their black coats, mounted in water and crushed under a cover-glass. The oil exuded in the form of yellow globules, while the amorphous substance was forced out more slowly and in a more or less irregular mass. Sudan III was then added; sometimes the zygospores were mounted directly in this reagent and then crushed. The oil became a brilliant red while the other substance remained clear. If the Sudan III were followed by chloroform, the oil globules immediately disappeared leaving the amorphous mass behind. The addition of ammonia water caused the latter to disappear at once. Sodium hydrate had much the same effect but acted more slowly. The xantho-proteic test was applied to other crushed zygospores and this mass took on a decided orange color. Millon's reagent failed to give the color changes. Zygospores were crushed in alkaline phenolphthalein and apparently gave an acid reaction, since the portion of the reagent through which the zygospore content was forced lost its color. The significance of this, however, is not great, because the reaction of the rest of the cytoplasm may have been acid. On the other hand, the cytoplasm and nuclei at this time are reduced to a thin parietal layer, as shown in sections (Pl. III, fig. 17), so the acid reaction was probably caused by these amorphous inclusions. Since it was impracticable to attempt to isolate this substance in question, the conclusions based upon these tests can only be of the most tentative nature but the reactions appear to be those of the nucleoproteins. These tests have served merely to separate this type of reserve material from the oil which is present and to aid in the interpretation of a rather perplexing condition existing in the mature zygospore.

At the time that the nuclear disorganizations begin, there appears a marked zonation in the zygospore (Pl. II, fig. 12). The inner coat of the zygospore, which is semi-transparent, is produced at this time. It is very thick and resistant. It is laid down within the brown, roughened wall previously formed. Thus the mature zygospore is invested with two thick coats (Pl. III, fig. 14) and parts of the original gametophore walls may still be found on the outside in many places. Toward the margin of the zygospore there appear bluish-staining areas. In the earlier conditions they appear merely as vacuoles in which

there are stainable contents. When fixed with Flemming's solution these areas are blackened by the osmic acid and require considerable bleaching before they are clear. They are responsible for the zonate appearance of the zygospore at this time (fig. 13).

The zygospore becomes more and more vacuolate, the groups of nuclei become separated, and the disorganizing nuclei may be found distributed throughout the zygospore (Pl. II, fig. 12). As the vacuolate condition increases the center of the zygospore becomes quite sponge-like in appearance. The bluish-staining bodies enlarge and begin to appear more or less reticulate (Pl. III, fig. 15). They are identical with the bodies described by the writer in *Sporodinia grandis* and are undoubtedly associated with the formation of oil. They do not seem to play as prominent a part in the maturation processes in *Phycomyces* as they do in *Sporodinia.* It may be that the protein material just described replaces to some extent, as reserve material in *Phycomyces,* the oil that is so plentiful in *Sporodinia.*

The literature referring to the possible relationship between these structures associated with oil production in the Mucorineae and the elaioplasts described by many workers, has been reviewed by the witer in a previous paper (1914). The elaioplasts or oil plastids, according to these various investigators, are bodies set aside by the protoplasm for the secretion of oil.

In *Sporodinia* the oil plastids arise as very small globular bodies. When first formed they appear as vacuoles with stained contents but careful study of them under high powers of magnification shows a coarse reticulation with a more or less homogeneous center. As they increase in size, their reticulate nature becomes more and more apparent. A coalescence between them takes place, apparently as the result of crowding, and in the mature zygospore there may occur from one to three of these plastids but usually only one large one is present

In *Phycomyces* these reticulated bodies, as already described, appear to originate at various places through the zygospore, being most apparent toward the margin. Oil is associated with them from their earliest appearance. As the zygospore matures these plastid-like bodies become vacuolate, enlarge and assume the appearance of definite cell structures. In the older zygospores studied, numerous small plastids were still to be found toward the periphery, but, as indicated in the accompanying

figures (15 and 16), a single large mass is often found. This plastid in *Phycomyces* does not resemble very closely the large plastid found in the mature zygospores of *Sporodinia,* but it undoubtedly functions in the same way. In *Sporodinia* the large plastid is finely granular and appears delicately reticulate. In *Phycomyces* its protoplasmic nature is not as evident. It is coarsely reticulate and even alveolate. It appears as if the protoplasm, when once saturated with oil, loses its granular appearance and may even deliquesce.

The large protein masses do not resemble the oil plastids either in color or in consistency in the prepared sections. In figure 16, the disorganized nuclei have not as yet become coalesced as they do somewhat later, as shown in figure 17. The amount of oil apparently becomes somewhat reduced in the older zygospores. The proportional amount of each substance varies in different zygospores.

Nuclei of the original typical form can be found throughout all the different stages described above. They stain somewhat differently, depending on the age of the zygospores. They are much reduced in number in the older zygospores but are still plentiful enough to be evident in many places.

There are several points which emphasize the fact that nuclear disorganizations take place. The one just mentioned, namely, that there is a marked reduction in the number of nuclei present in the mature zygospore, is the most important; secondly, the nuclear disorganization exactly parallels in appearance the conditions found in the suspensors, where there is no question concerning the occurrence of both nuclear and cytoplasmic disintegration. Swingle (1903) has described nuclear disorganizations in the sporangia of both *Rhizopus nigricans* and *Phycomyces nitens* as follows: *The first sign of disintegration is the appearance of a red-staining mass on one side. As the process goes on, the whole nucleus comes to appear as a slightly shrunken, homogeneous mass, often irregular in shape, and staining the same shade of red as the crystalloids. It might be argued that these red-staining bodies are crystalloids whose substance is being dissolved but I have very good evidence that such is not the case. * * * There are all stages of disintegration between the almost perfect nuclei and the most shrunken ones.*

Aside from these facts, on any conception of a nuclear-

cytoplasmic balance, it seems improbable that the immense number of nuclei which are present in the zygospore following the fusion of the two gametes, should persist in such a comparatively small structure, and nuclear disorganizations might normally be expected to occur.

There occurs an interesting change in the development which is of importance from a secondary standpoint only. Following the formation of the delimiting walls in the gametophores, there appear slight protuberances from the walls of the suspensors (Pl. I, fig. 6). These grow rather rapidly and give rise to the peculiar, dichotomously-branched appendages which grow out and surround the zygospore giving to it a very characteristic appearance (fig. 7). These appendages first appear on the same suspensor in which septation first occurred. This, however, does not appear to be due to any inherent difference between the two strains. Internally the changes are interesting.

Nuclear divisions appear to follow the cutting off of the terminal portions, or the nuclear divisions inaugurated in the progametes continue, and there results an increase in the number of nuclei in the suspensors. In a cross section of the suspensors there appear peculiar deep-staining areas opposite the places from which the appendages grow. The nuclei in these areas appear as if caught in a current and even show elongation in the direction of the current. The appendages as they elongate become filled with protoplasm and later appear vacuolate with only a thin lining layer of protoplasm in which the nuclei are distributed in about the same proportion as in the vegetative mycelium. All the protoplasm and nuclei disappear in the older stages and the appendages and suspensors appear empty (Pl. III, fig. 14). Occasionally dense, red-staining bodies may be found in the suspensors. They resemble closely the protein masses found in the partly mature zygospore and are probably of similar origin.

Zygospore germinations were secured in only one small lot of material so it has not been possible to carry the history of the internal changes to completion. It will be interesting to know what is the fate of these various substances in germination and what are the nuclear behaviors which complete the cycle.

Thus it will be seen that fundamentally, the changes which take place when sexual reproduction occurs in *Phycomyces*

nitens are essentially the same as those that occur in *Sporodinia grandis.* The conditions of nuclear fusions and subsequent disintegrations are the same. There appear, in both forms, certain plastid-like bodies which are associated with the oil which occurs as a reserve substance. In *Sporodinia* the disintegration of the nuclei results in the formation of an irregular knotted mass of material which in *Phycomyces* appears as a mass of what is probably either nucleic acid or nucleo-protein substance.

Careful cytological studies of the plus and minus strains of germinating spores, young sporangiophores, sporangia and progametes have revealed nothing in the nature of a morphological sexual differentiation. The author is satisfied that if any such means of differentiation between the two strains is to be found, it must be looked for in the germ sporangia where segregation undoubtedly occurs or more likely in physiological conditions. Perhaps microchemical tests will reveal a difference in the materials present in the two strains, especially in the progametes.

Many workers have described coenocentra in the Phycomycetes and McCormick (1912) has described the presence of a coenocentrum in the zygospores of *Rhizopus nigricans* but in *Phycomyces nitens* nothing has been encountered which answers the descriptions of a coenocentrum. While aggregations of nuclei occur, they are entirely independent of any coenocentra-like structures.

Young and partially mature zygospores have been studied in *Rhizopus nigricans,* another heterothallic form, and much the same set of conditions has been found as described in the present paper. The nuclei show the same clustered condition and subsequent nuclear disintegrations occur. The nature of the reserve substances in the mature zygospores has not been determined as yet. Work on *Zygorhynchus moelleri* was abandoned temporarily because of the very small size of the zygospores and the accompanying difficulties of technic. But, in view of the present work, there remains open an interesting problem in connection with such forms as show a morphological differentiation between the two sexual branches. It is interesting to note, as Blakeslee has pointed out, that in the heterothallic forms where we should expect to find an inequality of the gametes none occurs uniformly, while in *Zygorhynchus* and

Dicranophora, homothallic forms, the morphological differentiation is marked and constant.

Summary

1. There are no morphological differences to be found in the germinating spores of the plus and minus strains of *Phycomyces nitens.* They both germinate by means of one or two germ tubes which branch and produce the vegetative mycelium.

2. The vegetative mycelium of the minus strain grows somewhat less vigorously than that of the plus strain and the sporangia appear later.

3. The sporangia of the plus and minus strains are similar in all respects.

4. The two strains—the plus and the minus—are necessary in order to secure zygospores.

5. Zygospores were secured on various kinds of media and their production does not seem to be in any way dependent upon the concentration of the medium. Humidity, however, appears to be an important secondary factor.

6. Interlocking of lobes occurs when certain hyphal branches of the plus and minus strains come in contact. This appears to be the result of contact and not of chemical stimulation.

7. The terminal portions of these branches elongate forming the progametes. There is no difference morphologically between the two progametes.

8. The progametes show an increase in the number of nuclei as they increase in size and round out from each other. This increase in the number of nuclei is undoubtedly due to nuclear divisions.

9. Delimiting walls are formed which cut off the terminal portions, resorption of the contiguous walls occurs, the two gametes come in contact, and a mixing of the protoplasm takes place.

10. Grouping of the nuclei follows. There occur from 12 to 16 nuclei in each group.

11. Nuclear fusions in pairs follow, resulting in the reduction in the number of nuclei and in an increase in the size of the nuclei.

12. Deep-staining, crystal-like bodies are to be found at all stages. They resemble the crystalloids and globoids of the higher plants and undoubtedly constitute a form of reserve substance.

13. Zonations within the zygospore take place at the time of the formation of the second wall which invests the zygospore, and at the time of the appearance of the oil masses.

14. Disorganization of some of the nuclei follows and results in the formation of a large amount of irregular to globular masses of a deep-staining substance.

15. The oil plastids enlarge, show reduction in number and appear as definite reticulate bodies.

16. In the mature zygospore there are found two different types of reserve material.

a. A large amorphous mass of what is probably nucleoprotein substance.
b. A considerable amount of oil.

17. Nuclei of the typical form are found in much reduced numbers in zygospores six months old. The protoplasm is reduced to a thin parietal layer.

This work has been done under the direction of Professor G. M. Reed of the University of Missouri and I am greatly indebted to him for his unfailing interest and valuable suggestions.

Madison, Wisconsin.

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EXPLANATION OF FIGURES IN PLATES.

All figures were drawn with the aid of the camera lucida and with the Zeiss apochromatic objectives and compensating oculars. Figures 8*a*; 9*a*, *b*, *c*; 10*a*, *b*; 11*a*, *b*, *c*, *d*, *e*; 12*a*, *b*; 14*a*, *b*, *c*; 17*a*, *b*, were drawn with objective 1.5 mm. and ocular 12. Figure 8 was drawn with objective 1.5 mm. and ocular 8. Objective 16 mm. and ocular 12 were used with the remaining figures.

PLATE I.

Figs 1 and 2. Young gametophores of plus and minus strains showing lobing and interlocking.

Fig. 3. Young gametophores showing elongation of terminal portions. It is at this stage that the gametophores appear as erect yellow columns.

Fig. 4. Enlargement of gametophores.

Fig. 5. Formation of the delimiting walls.

Fig. 6. Showing the origin of the spine-like appendages.

Fig. 7. Mature zygospore with blackened wall and invested with appendages.

PLATE II.

Fig. 8. Germinating spore of plus strain.

Fig. 8*a*. Nuclei from germinating spore under higher magnification.

Fig. 9. Section through progametes prior to septation or resorption.

Figs. 9*a* and *b*. Nuclei from respective progametes. These may illustrate phases of nuclear divisions.

Fig. 9*c*. Crystalloids.

Fig. 10. Young conjugating branches at the time of resorption of the separating wall.

Figs. 10*a* and *b*. Nuclei from respective suspensors.

Fig. 11. Young zygospore, showing characteristic grouping of the nuclei following fusion of the protoplasmic masses.

Fig. 11*a* and *b*. Nuclei of the suspensors at time of formation of appendages.

Fig. 11*c*. Groups of nuclei showing conditions before and after nuclear fusions.

Figs. 11*d* and *e*. Crystalloids.

Fig. 12. Zonation at time of formation of second zygospore wall.

Fig. 12*a*. Nuclei at this stage.

Fig. 12*b*. Disorganizing nuclei.

Plate III.

Fig. 13. Zonation at time of formation of oil bodies.

Fig. 14. Showing disorganizing nuclei and aggregation of irregular masses resulting. Shows also the two walls of the zygospore and a small portion of the old gametophore wall.

Figs. 14*a*, *b*. Appearance of nuclei and disorganizing nuclei at this stage.

Fig. 14*c*. Irregular masses formed by coalescence of disorganizing nuclei.

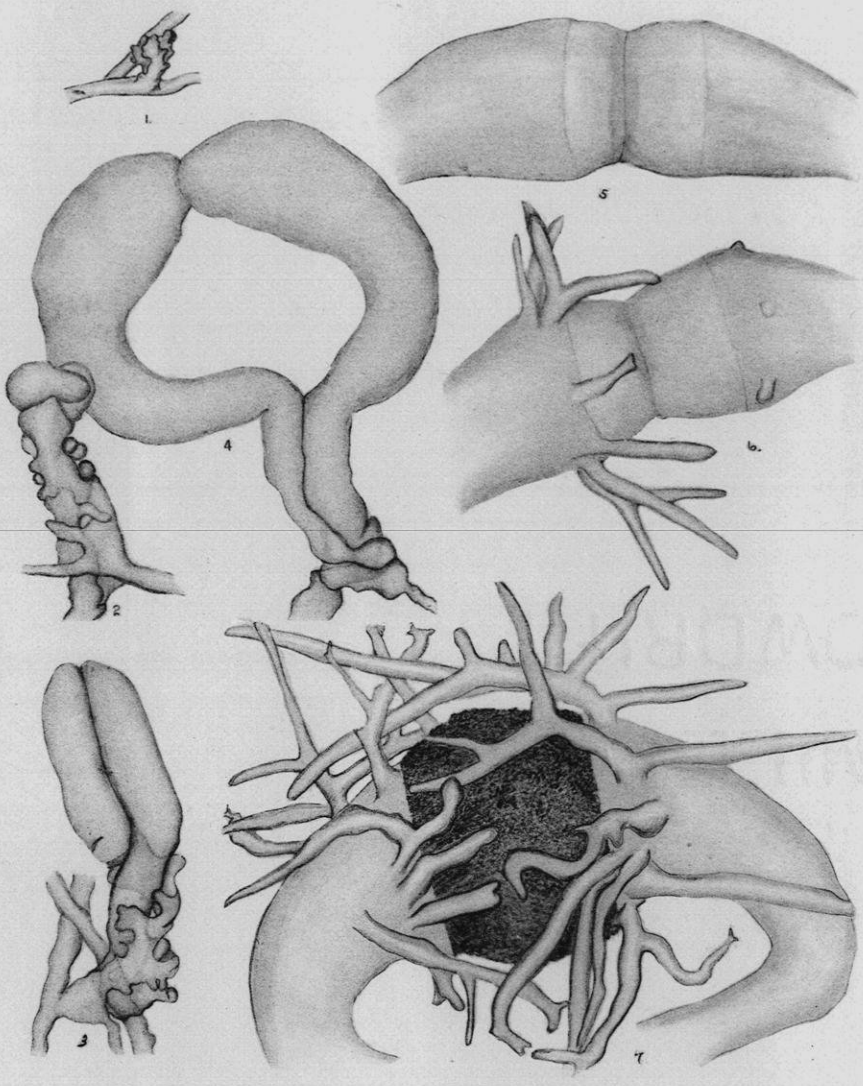
Fig. 15. Appearance of reticulated oil plastids.

Fig. 16. Large oil plastid and deep-staining area of disorganizing nuclei.

Fig. 17. Mature zygospore showing large alveolated nucleo-protein mass which results from the disorganized nuclei.

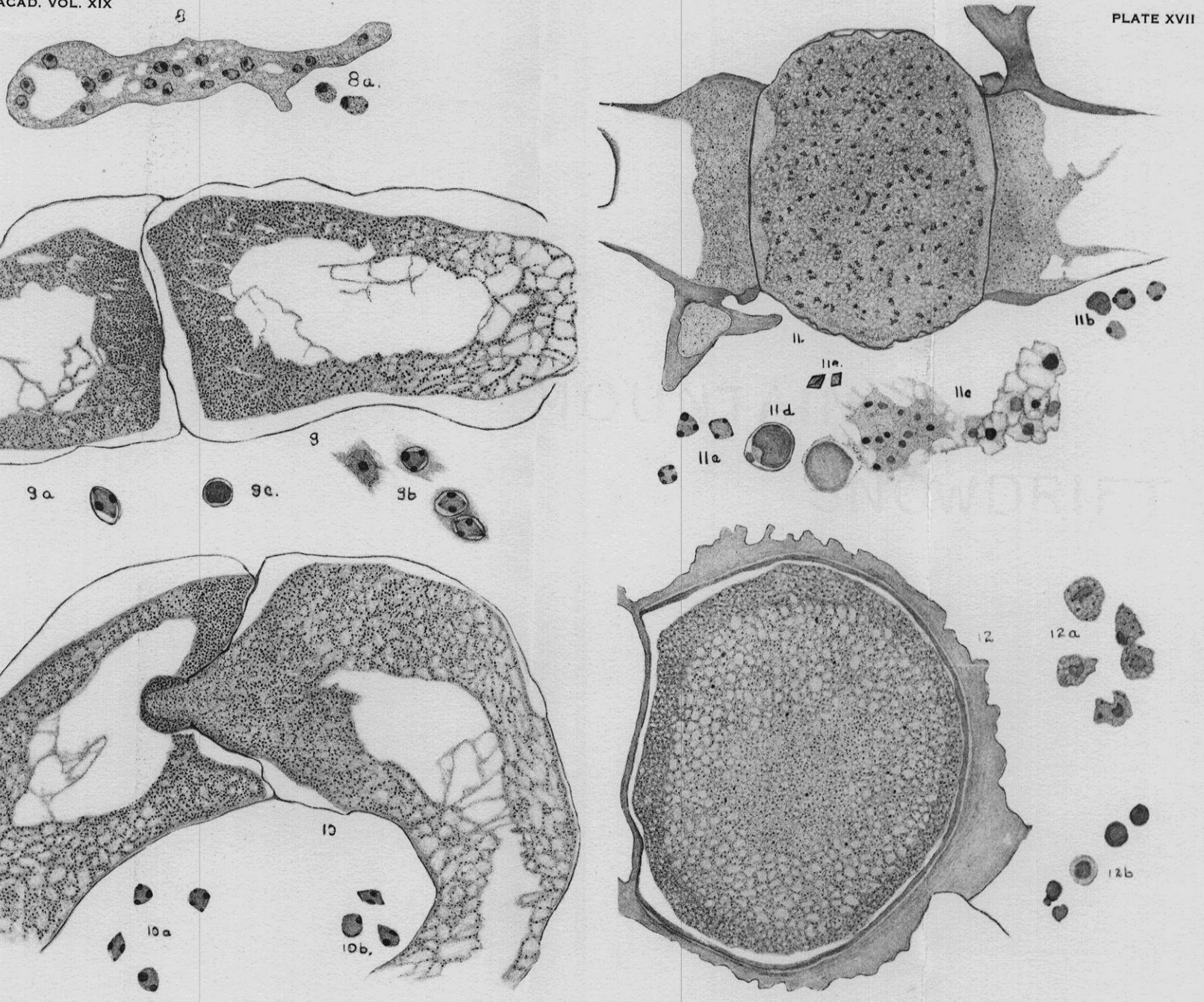
Fig. 17*a*. Appearance of a portion of the nucleo-protein mass under higher magnification.

Fig. 17*b*. Nuclei of the mature zygospore, much reduced in number.



KEENE—ZYGOSPORE FORMATION IN PHYCOMYCETES

COCKAYNE BOSTON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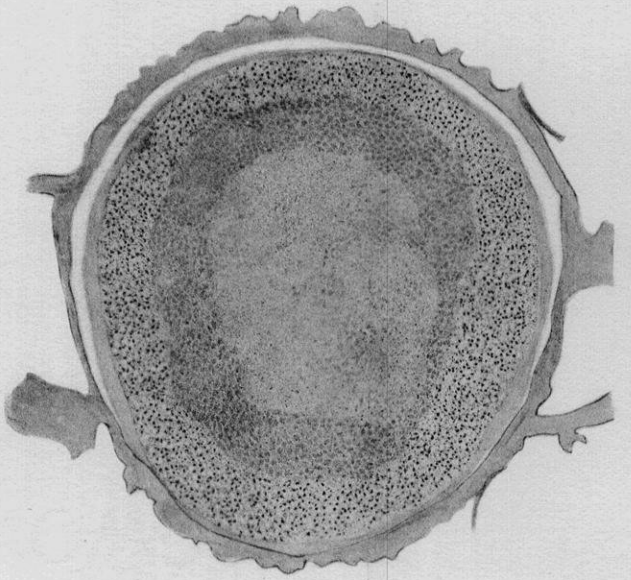

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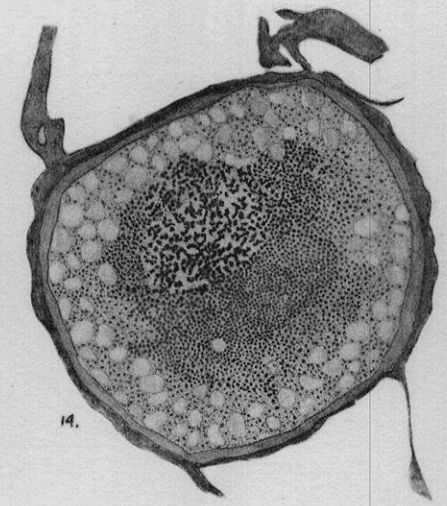

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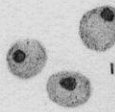

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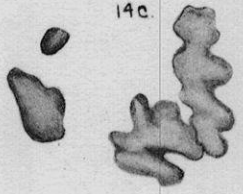

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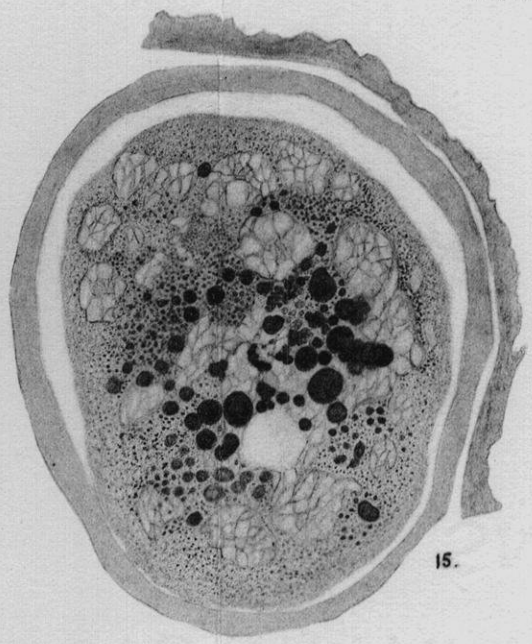

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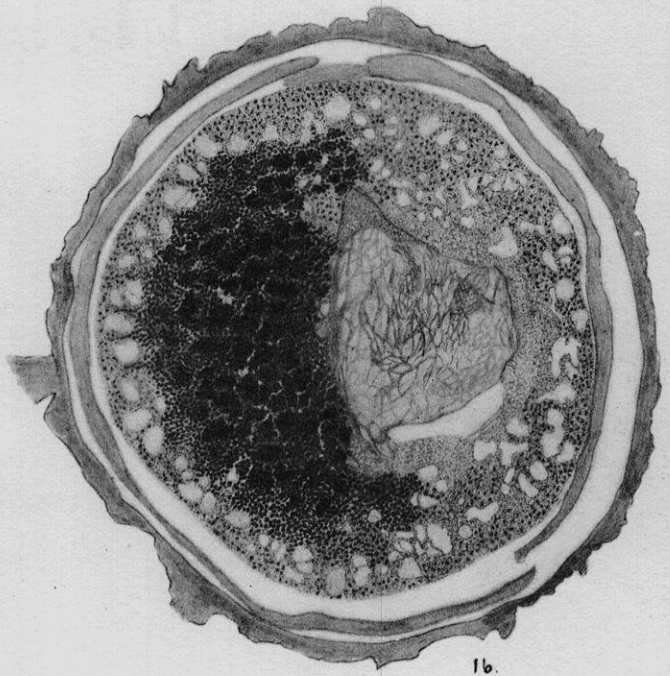

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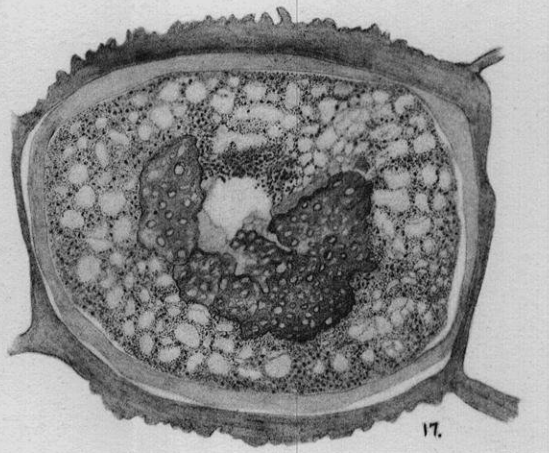

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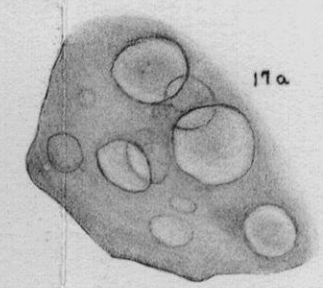

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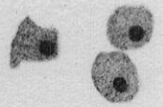

PROCEEDINGS OF THE ACADEMY, 1917 AND 1918

FORTY-SEVENTH ANNUAL MEETING, 1917.

The forty-seventh annual meeting of the Wisconsin Academy of Sciences, Arts, and Letters, in joint meeting with the Wisconsin Archeological Society, was held at Milwaukee, on Thursday and Friday, April 12 and 13, 1917, in the Trustees' Room of the Milwaukee Public Museum.

First Session, Thursday, April 12, 3 p. m.

President H. L. Ward called the meeting to order. The committee to audit the Treasurer's accounts was appointed, George Wagner and Samuel A. Barrett being named. The reports of the Secretary and Treasurer were read.

The following programme of papers was presented:

1. The Pompeius Strabo Inscription in the Palazzo dei Conservatori. M. S. Slaughter. By title.
2. The influence of French Farce on the Towneley Cycle of Mystery Plays. Louis Wann. By title.
3. Lumberjack Songs and Legends in the North West. K. Bernice Stewart. Twenty minutes.
4. An Ethnological Trip to Labrador and Hudson Bay. E. W. Hawkes. Twenty minutes. Illustrated.
5. Ethical Tales of the Eskimo. E. W. Hawkes. Twenty minutes. Illustrated.
6. The Cotton-tail in Paiute Mythology. S. A. Barrett. Fifteen minutes.
7. The Anthropological Groups of the Milwaukee Public Museum. S. A. Barrett. Ten minutes. Illustrated.
8. The New System of Taxidermy for Large Animals. Henry L. Ward. Five minutes.

9. A Museum Exhibit on Bird Protection. HENRY L. WARD. Five minutes.
10. The Eradication of Insect Pests in Collections. T. E. B. POPE. Ten minutes.

SECOND SESSION, FRIDAY, APRIL 13, 9:30 A. M.

The second session was presided over by President H. L. Ward. The following programme of papers was presented:

11. The Prehistoric Argillite Culture of New Jersey. E. W. HAWKES. Ten minutes. Illustrated.
12. The Washo Indian. S. A. BARRETT. Twenty minutes. Illustrated.
13. The Creation of the Yosemite Valley as told in Miwak Mythology. S. A. BARRETT. Fifteen minutes. Illustrated.
14. Building Laws of the Greeks and Romans. ALFRED C. CLAS. Twenty minutes.
15. The Discovery of Fluorite in the Ordovician Limestones of Wisconsin. RUFUS M. BAGG. Ten minutes.
16. The Members of the Niagara Formation in New York and their Western Extension. IRA M. EDWARDS. Ten minutes.
17. An Instance of the Uncertainty of Calculating the Thickness of Rock Formations by Measuring the Dip. IRA M. EDWARDS. Ten minutes.
18. A New Method for the Estimation of Alum and Benzoate of Soda in Foods. A. F. GILMAN. Ten minutes.
19. Some Practical Studies in Food Values. A. F. GILMAN. Twenty minutes.
20. Notes on Parasitic Fungi in Wisconsin. V. J. J. DAVIS. Ten minutes.
21. A Biochemical Study of the Plankton of Lake Mendota. HENRY A. SCHUETTE. By title.
22. Species of *Lentinus* in the Region of the Great Lakes. EDWARD T. HARPER. By title.
23. A Review of the Plover, Genus Ochthodromus Reichenbach, and its Nearest Allies. HENRY C. OBERHOLSER. By title.
24. Pigments of Flowering Plants. NELLIE A. WAKEMAN. By title.
25. The Commercial History of Ginseng of the United States. W. O. RICHTMANN. By title.
26. A Survey of the Commerce of Camphor. W. O. RICHTMANN. By title.
27. Further Studies on the Tremellineae of Wisconsin. E. M. GILBERT. By title.
28. Notes on the Fungal Flora of Lake Mendota. E. M. GILBERT. Five minutes.

29. Studies of Zygospore Formation in *Phycomyces nitens* Kunze. MARY LUCILLE KEENE. By title.

Paper 19 was discussed by Dr. J. J. Davis.

THIRD SESSION, FRIDAY, APRIL 13, 2:30 P. M.

Professor Rufus M. Bagg called the meeting to order, after which the following programme of papers was presented:

30. Proposed Changes in the Culture Area Map of North America. E. W. HAWKES. Five minues. Illustrated.
31. An Adaptation of the Dewey System to Anthropological Literature. S. A. BARRETT. Ten minutes. Illustrated.
32. The Great Basin Culture Area. S. A. BARRETT. Twenty minutes. Illustrated.
33. On the Crystalline Style of the Lamellibranchs. T. C. NELSON. Ten minutes. (Presented by GEORGE WAGNER.)
34. On a Remarkable Occurrence of Warblers. GEORGE WAGNER. Five minutes.
35. Vertebrates of Northern Michigan. A. R. CAHN. By title.
36. New American Water Mites of the Genus *Neumania*. RUTH MARSHALL. By title.
37. Studies on Myxosporidia from the Urinary Bladders of Wisconsin Fishes. J. W. MAVOR and W. STRASSER. By title.
38. Wisconsin and State Rights. LOUISE B. KELLOGG. Fifteen minutes.
39. Routes of Primitive Commerce. W. A. TITUS. By title.
40. Beloit Mound Groups. IRA M. BUELL. Fifteen minutes.
41. Indian Remains in Sheboygan County. A. GEREND. By title.
42. The Conchordal Fracture in the Flaking of Flint Implements. H. L. SKAVLEM. Twenty minutes.
43. Mounds and Sites of Green Lake. C. E. BROWN. Fifteen minutes.
44. Indian Earthworks of the Lake Chetek Region. C. E. BROWN and R. H. BECKER. By title.
45. The Milwaukee Museum School Loan Study Set on Forestry. HURON H. SMITH. Ten minutes. Illustrated.
46. Museum Exhibition of the Fungi. HURON H. SMITH. Ten minutes. Illustrated.
47. Botanical Exhibitions in the Public Museum. HURON H. SMITH. Ten minutes.
48. A Native Tree Exhibit for Wisconsin. HURON H. SMITH. Ten minutes.
49. Plans for a State Flora. HURON H. SMITH. Ten minutes.

On motion, the Secretary was instructed to cast the ballot for the following candidates for membership:

James Percy Bennett, Madison.
Mabel Mary Brown, Madison.
Ira M. Buell, Beloit.
A. R. Cahn, Madison.
Sylvester J. Carter, Milwaukee.
Ira Edwards, Milwaukee.
Ernest William Hawkes, Milwaukee.
William O. Johnson, Milwaukee.
George W. Keitt, Madison.
Charles Edward McLenegan, Milwaukee.
Maude Miller, Madison.
Thomas Edmund Burt Pope, Milwaukee.
C. Audrey Richards, Madison.
Henry A. Schuette, Madison.
Huron Herbert Smith, Milwaukee.
J. Charles Walker, Madison.
Louis Wann, Appleton.
Fred Werner, Milwaukee.
Clyde M. Woodworth, Madison.

FOURTH SESSION, FRIDAY, APRIL 13, 7:30 P. M.

The annual dinner was held in the Republican House, President H. L. Ward presiding. An informal programme and discussion was carried out. At the close of the dinner the Academy was declared adjourned, to meet in 1918 in Madison.

ARTHUR BEATTY,
Secretary.

REPORT OF THE SECRETARY FOR THE YEAR 1916.

Honorary Members	6
Life Members	11
Corresponding Members	41
Active Members	210
Total	268

CHANGES SINCE LAST REPORT.

Of the 14 applicants for membership at the last annual meeting, 13 have been enrolled, one not having completed the requirements by the payment of dues.

Active Members reported in April, 1916		216
New Members enrolled		13
		229
Deaths	2	
Resignations	3	
Dropped for non-payment of dues	15	
	20	20
		209
Transferred from Corresponding to Active Membership		1
Present Active Membership		210
New Applications for Membership		19

Membership Accounts—April 11, 1917.

Memberships paid to end of 1918 or later	3
" " " " " 1917	16
" " " " " 1916	152
" " " " " 1915	21
" " " " " 1912, 1913, or 1914	18
	210

I regret to report the loss of two active members by death—H. S. Hippensteel, Stevens Point, who died April 25, 1916; and W. J. Brinckley, Milwaukee, who died May 1, 1916.

ARTHUR BEATTY,
Secretary.

REPORT OF THE TREASURER FOR THE YEAR 1916.

Receipts.

Received from dues and initiations	$213.04
Received from sale of transactions	2.45
Received from interest on bonds	153.00
	$368.49
Balance on hand April 8, 1916	29.92
	$398.41

Disbursements.

Secretary-Treasurer's Allowance	$200.00	
Safety Deposit Box Rent	3.00	
1 Bond purchased April 2, 1917	100.00	
		$303.00
Balance on hand April 11, 1917		$95.41

ARTHUR BEATTY,
Treasurer.

MILWAUKEE, April 12, 1917.

Your auditing committee has compared the report of the treasurer with the books and vouchers, and find that the report is correct.

GEORGE WAGNER,
SAMUEL A. BARRETT.

FORTY-EIGHTH ANNUAL MEETING, 1918.

The forty-eighth annual meeting of the Wisconsin Academy of Sciences, Arts, and Letters, in joint meeting with the Wisconsin Archeological Society, was held at Madison on Thursday and Friday, April 11 and 12, 1918, in the Auditorium of the State Historical Museum.

First Session, Thursday, April 11, 3:00 p. m.

The meeting was called to order by Professor C. E. Allen, Vice-President for Sciences, as President Henry L. Ward was unable to be present on account of sickness. Dean Birge drew attention to the absence of President Ward in appropriate remarks, and moved that the Secretary send a suitable letter of condolence to President Ward. This letter was sent at once.

As this was the year for the election of officers, the chairman named the following nominating committee: Charles R. Van Hise, J. J. Davis, Charles S. Slichter.

The chairman also appointed Albert S. Flint and Charles E. Brown as members of the committee to audit the Treasurer's report.

The following programme of papers was presented:

1. Additional Wisconsin Peace Medals. Charles E. Brown. Ten minutes.
2. Votive Offerings of Indian Pottery from Colombia, S. A. George A. Collie. Twenty-five minutes.
3. The Stratigraphic Structure of Wisconsin Indian Mounds. Samuel A. Barrett. Twenty-five minutes. Illustrated. (Read in synopsis by the Secretary.)
4. Effigy Mounds in Iowa. Charles E. Brown. Ten minutes.
5. The State Collection of War Posters. Ruth O. Roberts. Fifteen minutes.

SECOND SESSION, FRIDAY, APRIL 12, 9:30 A. M.

The session was called to order by Vice President Frank G. Hubbard, who called for the report of the nominating committee. The report was as follows:

The committee on nominations beg leave to report the following nominations for officers for the ensuing term:

President, E. A. BIRGE, Madison.
Vice President, Sciences, ERATUS G. SMITH Beloit.
Vice President, Arts, A. C. CLAS, Milwaukee.
Vice President, Letters, F. L. PAXSON, Madison.
Secretary, ARTHUR BEATTY, Madison.
Treasurer, ARTHUR BEATTY, Madison.
Curator, C. E. BROWN, Madison.
Librarian, WALTER M. SMITH, Madison.

COMMITTEE ON PUBLICATION.

THE SECRETARY,
THE PRESIDENT,
C. E. ALLEN, Madison.

COMMITTEE ON LIBRARY.

THE LIBRARIAN, ex officio,
GEORGE WAGNER, Madison.
PAUL H. DERNEHL, Milwaukee.
RUFUS M. BRAGG, Appleton.
ALBERT G. GILMAN, Ripon.

COMMITTEE ON MEMBERSHIP.

THE SECRETARY,
L. J. COLE, Madison.
S. A. BARRETT, Milwaukee.
A. F. MCLEOD, Beloit.
E. M. GILBERT, Madison.

Respectfully,
CHAS. R. VAN HISE,
JOHN J. DAVIS,
CHAS. S. SLICHTER,
Committee on Nominations.

On motion of the chairman, the Secretary cast the ballot for the candidates named.

President-elect Birge was then called to the chair.

The reports of the Secretary and of the Treasurer were read, and the auditing committee vouched for the correctness of the Treasurer's accounts.

The reading of papers was then proceeded with, as follows:

6. The Work of the Wisconsin War History Commission. J. W. OLIVER. Ten minutes.
7. The Passing of a Historic Waterway. F. E. WILLIAMS. Twenty minutes.
8. The Literary Precursors of Wagner's *Meistersinger*. EDWIN C. ROEDDER. Twenty minutes.
9. A Teacher of William Wordsworth: Joseph Fawcett and *The Art of War*. ARTHUR BEATTY. Twenty minutes.
10. The Habits of the Fishes in Wisconsin. A. S. PEARSE. Twenty minutes. Illustrated.
11. Experiments on Rabbits with Immune Serum. M. F. GUYER. Thirty minutes.
12. The Relation of Age of Dam to the Production of Twins in Cattle. SARAH V. H. JONES. Ten minutes.
13. Selection for Chemical Characters in Soy Beans and Jimson-weeds. C. M. WOODWORTH. Fifteen minutes.
14. The Bottom Fauna in the Deeper Water of Lake Mendota. C. JUDAY. Ten minutes.
15. Recent Observations on the Chrysopidae of Milwaukee. ROGER C. SMITH. Eight minutes.
16. Chemistry of the Heptane Solution. EDWARD KREMERS. By title.
17. The Hydrohalogens. LEANDER SHERK. By title.
18. Terpenes as Oxygen Conveyors. E. V. LYNN. By title.

THIRD SESSION, FRIDAY, APRIL 12, 2:30 P. M.

President Birge called the afternoon session to order. On the initiative of the Treasurer the question was raised as to whether the Academy's balance should be invested in City of Madison Bonds as usual, or in securities which pay a higher rate of interest. After some discussion the Treasurer was advised to continue his usual practice.

The presentation of papers was proceeded with, as follows:

19. Housing Problems of the War. L. S. SMITH. Thirty minutes. Illustrated.

20. The Status of Chlorine in Plant Nutrition. W. E. TOTTINGHAM. Ten minutes.
21. Physiological Balance of Nutrient Solutions, with Reference to the Theory of Electrolytic Dissociation. W. E. TOTTINGHAM. Ten minutes.
22. Preliminary Notes on the Fungi Found in the Waters of Madison and Vicinity. E. M. GILBERT. Fifteen minutes.
23. Apospory in *Pteris sulcata*. W. N. STEIL. Fifteen minutes.
24. The Effect of Neutral Salts upon the Toxicity of Acidified Culture Solutions toward *Aspergillus niger*. J. P. BENNETT. Ten minutes.
25. The Relation of Certain Mineral Nutrients to the Composition of the Oat Plant. J. G. DICKSON. Ten minutes.
26. Sex Determination in a Liverwort. C. E. ALLEN. Ten minutes.
27. Notes on the Summer Birds of Door Peninsula. Wisconsin, and Adjacent Islands. HARTLEY H. T. JACKSON. By title.
28. The Relation of Vegetation to Bird Life in Texas. HARRY C. OBERHOLSER. By title.
29. Notes on Parasitic Fungi in Wisconsin, VI. J. J. DAVIS. By title.

At the close of the programme the Secretary presented the following applications for membership. On motion, the Secretary was instructed to cast the ballot in their favor:

Melvin H. Brannon, Beloit.
Frederic Doerfler, Madison.
P. H. Hawkins, Madison.
Sarah V. H. Jones, Madison.
Sterling E. Price, Madison.
W. E. Tottingham, Madison.
Charles H. Vilas, Madison.

The Secretary presented the name of Mrs. Lucius Fairchild, presented by the council for election as an Honorary member. On motion the Secretary was instructed to cast the ballot in her favor. This was done, and Mrs. Fairchild was declared an Honorary Member of the Academy.

On the completion of this item of business the Academy was declared adjourned.

ARTHUR BEATTY,
Secretary.

REPORT OF THE SECRETARY FOR THE YEAR 1917.

Honorary Members	6
Life Members	11
Corresponding Members	41
Active Members	208
Total	266

CHANGES SINCE LAST REPORT IN APRIL, 1917.

Of the 19 Applicants for membership at the last Annual Meeting, all have been enrolled.

Active Members reported in April, 1917		210
New Members enrolled since April, 1917		19
		229
Deaths	4	
Resignations	6	
Dropped for nonpayment of dues	11	
		21
Present Active Membership		208

New Applications for Membership to be acted upon at this meeting 7.

MEMBERSHIP ACCOUNTS, AS SHOWN APRIL 1, 1918.

Accounts paid to end of 1921	2
" " " " 1918	8
" " " " 1917	155
" " " " 1916	34
" " " " 1915	7
" " " " 1914	2
	208

I regret to have to report the loss of 4 Active Members by death, since the last Annual meeting. Rev. Dr. Eugene Grover Updike, of Madison, died Dec. 24, 1917; he had been a member of the Academy since 1892. Professor William Porter of Beloit

College, a member since 1894, died October, 1917. In October, 1917, William H. Ellsworth, of the Ellsworth–Thayer Mfg. Co. of Milwaukee, died, his membership dating back to 1905. Mr. George Bowman Ferry, Architect, of Milwaukee, died in January, 1918; he had been a member since 1896.

ARTHUR BEATTY,
Secretary.

April 12, 1918.

REPORT OF THE TREASURER FOR THE YEAR 1917.

Receipts.

Received from dues and initiations		$214.00
Received from sale of transactions		5.40
Received from interest on bonds		158.00
Received from 3 bonds matured April 1, 1918		300.00
		677.40
Balance on hand April 11, 1917		95.41
		762.81

Disbursements.

Secretary-Treasurer's Allowance	$200.00	
Safety-Deposit Box Rent	3.00	
Expenses of meeting, 1917	10.48	
		$213.48
Balance on hand April 12, 1918		$549.33

Present Permanent Investment consists of 25 City of Madison Bonds.

ARTHUR BEATTY.
MADISON, April 12, 1918.

Your committee, appointed to audit the Treasurer's accounts, have compared his report with the books, vouchers, and the bonds in the safety deposit box, and find that the report is correct.

ALBERT S. FLINT,
CHARLES E. BROWN.

LIST OF OFFICERS AND MEMBERS

CORRECTED TO SEPTEMBER 1, 1918.

OFFICERS.

President, EDWARD A. BIRGE, Madison.
Vice-President, Sciences, ERASTUS G. SMITH, Beloit.
Vice-President, Arts, A. C. CLAS, Milwaukee.
Vice-President, Letters, F. L. PAXSON, Madison.
Secretary, ARTHUR BEATTY, Madison.
Treasurer, ARTHUR BEATTY, Madison.
Curator, C. E. BROWN, Madison.
Librarian, WALTER M. SMITH, Madison.

COMMITTEE ON PUBLICATION.

The President, ex officio,
The Secretary, ex officio,
C. E. ALLEN, Madison.

COUNCIL.

The President, Vice-Presidents, Secretary, Treasurer, Librarian and Past Presidents retaining their residence in Wisconsin.

COMMITTEE ON LIBRARY.

The Librarian, ex officio,
GEORGE WAGNER, Madison.
PAUL H. DERNEHL, Milwaukee.
ALBERT G. GILMAN, Ripon.

COMMITTEE ON MEMBERSHIP.

The Secretary, ex officio,
L. J. COLE, Madison.
S. A. BARRETT, Milwaukee.
A. F. MCLEOD, Beloit.
E. M. GILBERT, Madison.

Past Presidents.

Honorable John W. Hoyt, M. D., LL. D.,* Washington, D. C., 1870–75.

Dr. P. R. Hoy, M. D.,* 1876–78.

President A. L. Chapin, D. D.,* 1879–81.

Professor Ronald D. Irving, Ph. D.,* 1882–84.

Professor Thomas C. Chamberlain, Ph. D., Sc. D., LL. D., Chicago, Ill., 1885–87.

Professor William F. Allen,† 1888–89.

Professor Edward A. Birge, Ph. D., Sc. D., LL. D., Madison, 1889–90.

Librarian George W. Peckham, LL. D., Milwaukee, 1891–93.*

President Charles R. Van Hise, Ph. D., LL. D., Madison, 1894–96.

Professor C. Dwight Marsh, A. M., Ph. D., Washington, D. C., 1897–99.

Professor Charles S. Slichter, M. S., Madison, 1900–1902.

Dr. John J. Davis, M. D., Racine, 1903–1905.

Professor Louis Kahlenberg, Ph. D., Madison, 1906–1909.

President Samuel Plantz, Ph. D., D. D., LL. D., Lawrence College, Appleton, 1910–1912.

Professor Dana C. Munro, A. B., A. M., Princeton, New Jersey, 1913–1915.

Director Henry L. Ward, Milwaukee, 1915–1918.

HONORARY MEMBERS.

Chamberlain, Thomas Chrowder, Hyde Park Hotel, Chicago, Ill.

A. B. (Beloit); Ph. D. (Wisconsin, Michigan); LL. D. (Michigan, Beloit, Columbia, Wisconsin); Sc. D. (Illinois). Head of Geological Department and Director of Walker Museum, University of Chicago, Consulting Geologist U. S. Geological Survey; Consulting Geologist, Wisconsin Natural History Survey; Geological Commissioner, Illinois Geological Survey; Editor, *Journal of Geology.*

Fairchild, Mrs. Lucius, 302 Monona Avenue, Madison, Wisconsin.

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*Deceased. †Deceased December 9, 1889. Professor Birge elected to fill unexpired term.

GARLAND, HAMLIN, New York, N. Y.

Vice-President, International Institute of Arts and Letters. Chairman of Cliff-Dwellers, of Chicago.

JORDAN, DAVID STARR,
President Emeritus of Stanford University, Stanford University, Cal.

M. S., Cornell University, 1872; M. D., Indiana Medical College, 1875; Ph. D., Butler College, 1878; LL. D., Cornell University, 1886, Johns Hopkins University, 1902, Illinois College, 1903; Instructor in Botany, Cornell University, 1871–72; Professor of Natural History, Lombard University, 1872–73; Principal of Appleton (Wis.) Collegiate Institute, 1873–74; Lecturer in Marine Botany at Penikese, 1873–74; Teacher of Natural History, Indianapolis High School, 1874–75; Professor of Biology, Butler College, 1875–79; Instructor in Botany, Harvard Summer School, Cumberland Gap, 1875–76 Assistant to U. S. Fish Commissioner, 1877–81; Professor of Zoology, Indiana University, 1879–85; President of Indiana University, 1885–91; President of the California Academy of Sciences, 1891–98, 1901–03, 1908; U. S. Commissioner in charge of Fur Setl. Investigations, 1896–98; of Salmon Investigations, 1904; International Commissioner of Fisheries, since 1908; President of the American Association for the Advancement of Science, 1903–09.

TRELEASE, WILLIAM, Urbana, Ill.

B. S. (Cornell); S. D. (Harvard); LL. D. (Wisconsin, Missouri, Washington University); Professor of Botany University of Wisconsin, 1883–5; Professor of Botany Washington University 1885–1913; Director Missouri Botanical Garden, 1889–1912; Professor of Botany University of Illinois, 1913–1918; Vice-President Association Internationale des Botanistes and Chairman American Board of Editors, Botaniches Centralblatt.

WHEELER, W. M., Forest Hills, Boston, Mass.

Ph. D. Professor of Economic Entomology, Harvard University.

LIFE MEMBERS

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A. B., A. M. (Williams); Ph. D. (Harvard); Sc. D. (Western University of Pennsylvania); LL. D. (Williams). Professor of Zoology and Dean of the College of Letters and Science, University of Wisconsin; Secretary of Commissioners of Fisheries, Wisconsin; Director and Superintendent, Wisconsin Geological and Natural History Survey; Member, Wisconsin State Board of Forestry; Wisconsin Conservation Commission, Vice-President, Phi Beta Kappa

DAVIS, JOHN JEFFERSON, 629 Mendota Court, Madison

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HOBBS, WILLIAM HERBERT, 820 Oxford Road, Ann Arbor, Mich.

B. S. (Worcester Polytechnic Institute); A. M., Ph. D. (Johns Hopkins). Professor of Geology, University of Michigan.

HOYT, JOHN WESLEY, Washington, D. C.

A. M. (Ohio Wesleyan); M. D. (Cincinnati); LL. D. (Missouri). Chairman of the National Committee of Four Hundred to Promote the Establishment of the University of the United States.

MARSH, CHARLES DWIGHT, 3430 Brown St., N. W., Washington, D. C.

A. B., A. M. (Amherst); Ph. D. (Chicago). Physiologist in Bureau of Plant Industry, United States Department of Agriculture.

PLANTZ, SAMUEL, 545 Union St., Appleton

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B. Met. E., B. S., M. S., Ph. D. (Wisconsin); LL. D. (Chicago, Yale, Harvard, Williams, Dartmouth). President, University of Wisconsin; Consulting Geologist, Wisconsin Geological Survey; President, Board of Commissioners, Wisconsin Geological and Natural History Survey; President, Wisconsin State Board of Forestry.

ACTIVE MEMBERS

ALLEN, BENNETT MILLS, Lawrence, Kansas
Ph. B. (De Pauw); Ph. D. (Chicago). Professor of Zoology University of Kansas.

ALLEN, CHARLES ELMER, 2014 Chamberlin Ave., Madison
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ARZBERGER, EMIL GODFREY, Washington, D. C.
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BAGG, RUFUS M. JR., 7 Brokaw Place, Appleton
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BROWN, CHARLES NEWTON,
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747 N. Main St., Springfield, Mo.
B. S. (Drury). Assistant Professor, Geology and Mineralogy, Drury College, Springfield.

HARPER, EDWARD T., Geneseo, Illinois

HARPER, MRS. ROBERT AYLMER, New York City

HARPER, ROBERT AYLMER, New York City
Professor of Botany, Columbia University.

HAWKES, ERNEST WILLIAM, Milwaukee
Anthropologist, Public Museum.

HAWKINS, PETER H., 1910 Regent St., Madison

HEDDLE, JOHN R., 1625 Monroe St., Madison

HOHLFELD, ALEXANDER RUDOLPH,
104 Breese Terrace, Madison
Ph. D. (Leipzig). Professor of German, University of Wisconsin; President, Modern Language Association of America; Member of Board of Administration, National German-American Teachers' Seminary, Milwaukee.

HOLMES, SAMUEL JACKSON, Berkeley, California
B. S., M. S. (California); Ph. D. (Chicago). Professor of Zoology, University of California.

HOTCHKISS, W. O., College Hills, Madison
Geologist, State Highway Commission.

HUBBARD, FRANK GAYLORD, 2006 Monroe St., Madison
A. B. (Williams); Ph. D. (Johns Hopkins). Professor of English, University of Wisconsin.

HUMPHREY, CLARENCE J., Madison
Pathologist, Forest Products Laboratory.

IBSEN, H. L., Madison
Assistant in Experimental Breeding, University of Wisconsin.

INGERSOLL, LEONARD R., 1933 West Lawn Ave., Madison
B. S. (Colorado College); Ph. D. (Wisconsin). Associate Professor of Physics, University of Wisconsin.

JACKSON, HARTLEY H. T., Washington, D. C.
U. S. Biological Survey.

JANA, ASHUTOSH, Haria, Bengal, India

JASTROW, JOSEPH, 237 Langdon St., Madison
A. B., A. M. (Pennsylvania); Ph. D. (Johns Hopkins). Professor of Psychology, University of Wisconsin.

JOHNSON, AARON GUY, 1910 West Lawn Ave., Madison
Plant Pathologist, University of Wisconsin.

JOHNSON, JAMES, 131 Lathrop St., Madison
Assistant Professor of Horticulture, University of Wisconsin.

JOHNSON, WILLIAM O., 1416 Lee St., Milwaukee
Assistant in Anthropology Public Museum.

JOLIVETTE, HALLIE D. M., 900 Campus Ave., Pullman, Wash.

JONES, LEWIS R., 1731 Regent St., Madison
Ph. B., Ph. D. (University of Michigan); Sc. D. (Honorary, University of Vermont). Professor of Plant Pathology, University of Wisconsin.

JONES, SARAH VAN HOOSEN, Madison
Assistant in Experimental Breeding, University of Wisconsin.

JUDAY, CHANCEY, 35 Lathrop St., Madison
A. M. (Indiana). Biologist, Wisconsin Geological and Natural History Survey.

KEITT, GEORGE WANNAMAKER, Madison
Plant Pathologist. University of Wisconsin.

KIND, JOHN LOUIS, The Irving, Sterling Court, Madison
A. B., A. M. (Nebraska); Ph. D. (Columbia). Associate Professor of German, University of Wisconsin.

KREMERS, EDWARD, 1720 Vilas St., Madison
Ph. G., B. S. (Wisconsin); Ph. D. (Gottingen); D. Sc. (Michigan). Director of Course in Pharmacy and Professor of Pharmaceutical Chemistry, University of Wisconsin.

KUHL, E. P., Minneapolis, Minn.
University of Minnesota.

KUTCHIN, MRS. HARRIET LEHMAN, Green Lake, Wis.
A. B. (Ripon); A. M. (Northwestern). Engaged in zoological research.

LANGENHAN, H. AUGUST, 1821 West Lawn Ave., Madison
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B. S. (Purdue). Instructor in Science and Mathematics, Racine High School.

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LENHER, VICTOR, 158 Summit Ave., Madison
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LEONARD, WILLIAM ELLERY, 2015 Adams St., Madison
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LOWE, JOHN N., College Station
Instructor in Zoology, A. and M. College, Texas.

MCALLISTER, FRED, Austin, Texas
Department of Botany, University of Texas.

MCCASKILL, VIRGIL E., Superior
President, State Normal School.

MCKENNA, MAURICE, 152 S. Main St., Fond du Lac
Lawyer; President Bar Association of Fond du Lac County.

MCLENEGAN, CHARLES EDWARD, Milwaukee
Librarian, Public Library.

MCLEOD, ANDREW FRIDLEY, Beloit
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MARQUETTE, WILLIAM GEORGE, New York, N. Y.
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Information of whose decease has been received since the issue of Volume XVIII

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Deceased May 1, 1916

ELLSWORTH, WILLIAM H., Milwaukee
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Deceased October 5, 1917

FERRY, GEORGE BOWMAN, Milwaukee
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Deceased January 7, 1918

HARWOOD, MARY CORINTHIA, Ripon
Professor, Ripon College.
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HIPPENSTEEL, H. S., Stevens Point
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Deceased April 25, 1916

PORTER, WILLIAM, Beloit
Professor Emeritus of Latin, Beloit College
Deceased October 14, 1917

SHERMAN, DR. LEWIS, Milwaukee
Physician and Pharmacist.
Deceased July 2, 1915

UPDIKE, EUGENE GROVER, Madison
Pastor First Congregational Church, Madison.
Deceased December 24, 1917

EXTRACTS FROM THE CHARTER OF THE ACADEMY

AN ACT to incorporate the Wisconsin Academy of Sciences, Arts, and Letters.

The people of the state of Wisconsin, represented in senate and assembly, do enact as follows:

SECTION 1. Lucius Fairchild, Nelson Dewey, John W. Hoyt, Increase A. Lapham, * * *[1] at present being members and officers of an association known as "The Wisconsin Academy of Sciences, Arts, and Letters," located at the city of Madison, together with their future associates and successors forever, are hereby created a body corporate by the name and style of the "Wisconsin Academy of Sciences, Arts, and Letters," and by that name shall have perpetual succession; shall be capable in law of contracting and being contracted with, of suing and being sued, of pleading and being impleaded in all courts of competent jurisdiction; and may do and perform such acts as are usually performed by like corporate bodies.

SECTION 2. The general objects of the Academy shall be to encourage investigation and disseminate correct views in the various departments of science, literature, and the arts. Among the specific objects of the Academy shall be embraced the following:

1. Researches and investigations in the various departments of the material, metaphysical, ethical, ethnological, and social sciences.
2. A progressive and thorough scientific survey of the state with a view of determining its mineral, agricultural, and other resources.
3. The advancement of the usual arts, through the applications of science, and by the encouragement of original invention.
4. The encouragement of the fine arts, by means of honors and prizes awarded to artists for original works of superior merit.
5. The formation of scientific, economic, and art museums.
6. The encouragement of philological and historical research, the

[1] Here follow the names of forty others. Sections 5, 6, 8 and 9 are omitted here as of no present interest. For the charter in full see *Transactions*, vol. viii, p. xi, or earlier volumes.

collection and preservation of historic records, and the formation of a general library.

7. The diffusion of knowledge by the publication of original contribution to science, literature, and the arts.

SECTION 3. Said Academy may have a common seal and alter the same at pleasure; may ordain and enforce such constitution, regulations, and by-laws as may be necessary, and alter the same at pleasure; may receive and hold real and personal property, and may use and dispose of the same at pleasure; provided, that it shall not divert any donation or bequest from the uses and objects proposed by the donor, and that none of the property acquired by it shall, in any manner, be alienated other than in the way of exchange of duplicate specimens, books, and other effects, with similar institutions and in the manner specified in the next section of this act, without the consent of the legislature.

SECTION 4. It shall be the duty of the said Academy, so far as the same may be done without detriment to its own collections, to furnish, at the discretion of its officers, duplicate typical specimens of objects in natural history to the University of Wisconsin, and to the other schools and colleges of the state.

SECTION 7. Any existing society or institution having like objects embraced by said Academy, may be constituted a department thereof, or be otherwise connected therewith, on terms mutually satisfactory to the governing bodies of the said Academy and such other society or institution.

Approved March 16, 1870.

STATUTES OF 1898.

TRANSACTIONS OF THE ACADEMY.

SECTION 341. There shall be printed by the state printer biennially in pamphlet form two thousand copies of the transactions of the Wisconsin Academy of Sciences, Arts, and Letters, uniform in style with the volumes heretofore printed for said society.

Note.—Under a ruling of the printing commissioners of the state of Wisconsin, made in response to a presentation by a committee of the Academy appointed December 29, 1897, each volume of the Transactions may be issued in two consecutive parts; so that a publication may thus be issued each year covering the papers accepted after the previous annual meeting. The Academy allows each author one hundred separate reprints of his paper from the Transactions without expense, except a small charge for printed covers when desired. Additional copies are charged for at the actual cost of printing and binding.

OF THE DISTRIBUTION OF PUBLIC DOCUMENTS.

SECTION 365. The transactions of the Wisconsin Academy of Sciences, Arts, and Letters shall be distributed as follows: One copy to each

member of the legislature, one copy to the librarian of each state institution; one hundred copies to the State Agricultural Society; one hundred copies to the State Historical Society; one hundred copies to the State University, and the remainder to said Academy.

SECTION 366. In the distribution of books or other packages, if such packages are too large or would cost too much to be sent by mail, they shall be sent by express or freight, and the accounts for such express or freight charges, properly certified to, shall be paid out of the state treasury.

STATUTES OF 1901.

CHAPTER 447.

BINDING OF EXCHANGES.

SECTION 1. Section 341 of the revised statutes of 1898 is hereby amended by adding thereto the following: The secretary of state may authorize the state printer to bind in suitable binding all periodicals and other exchanges which the Society shall hereafter receive, at a cost not exceeding one hundred and fifty dollars per annum. The secretary of state shall audit the accounts for such binding.

STATUTES OF 1917.

SECTION 35.32. That part of section 35.32 of the statutes relating to printing for the Wisconsin Academy of Sciences, Arts, and Letters is amended to read: "of each number as issued, of the transactions of the Wisconsin Academy of Sciences, Arts, and Letters, not more than two thousand copies * * * together with suitable binding at a cost not exceeding one hundred and fifty dollars per annum of all periodicals and other exchanges which said academy shall hereafter receive."

CONSTITUTION

OF THE WISCONSIN ACADEMY OF SCIENCES, ARTS, AND LETTERS.

[As amended at various regular meetings.]

ARTICLE I.—*Name and Location.*

This association shall be known as the Wisconsin Academy of Sciences, Arts, and Letters, and shall be located at the city of Madison.

ARTICLE II.—*Object.*

The object of the Academy shall be the promotion of sciences, arts, and letters in the state of Wisconsin. Among the special objects shall be the publication of the results of investigation and the formation of a library.

ARTICLE III.—*Membership.*

The Academy shall include four classes of members viz.: life members, honorary members, corresponding members, and active members, to be elected by ballot.

1. Life members shall be elected on account of special services rendered the Academy. Life membership in the Academy may also be obtained by the payment of one hundred dollars and election by the Academy. Life members shall be allowed to vote and to hold office.
2. Honorary members shall be elected by the Academy and shall be men who have rendered conspicuous services to science, arts, or letters.
3. Corresponding members shall be elected from those who have been active members of the Academy, but have removed from the state. By special vote of the Academy men of attainments in science or letters may be elected corresponding members. They shall have no vote in the meetings of the Academy.
4. Active members shall be elected by the Academy or the council and shall enter upon membership on the payment of an initiation fee of two dollars which shall include the first annual assessment of one dollar. The annual assessment shall be omitted for the president, secretary, treasurer, and librarian during their term of office.

ARTICLE IV.—*Officers.*

The officers of the Academy shall be a president, a vice-president for each of the three departments, sciences, arts and letters, a secretary, a librarian, a treasurer, and a custodian. These officers shall be chosen by ballot, on recommendation of the committee on nomination of officers,

by the Academy at an annual meeting and shall hold office for three years. Their duties shall be those usually performed by officers thus named in scientific societies. It shall be one of the duties of the president to prepare an address which shall be delivered before the Academy at the annual meeting at which his term of office expires.

Article V.—*Council.*

The council of the Academy shall be entrusted with the management of its affairs during the intervals between regular meetings, and shall consist of the president, the three vice-presidents, the secretary, the treasurer, the librarian, and the past presidents who retain their residence in Wisconsin. Three members of the council shall constitute a quorum for the transaction of business, provided the secretary and one of the presiding officers be included in the number.

Article VI.—*Committees.*

The standing committees of the Academy shall be a committee on publication, a library committee, and a committee on the nomination of members. These committees shall be elected at the annual meeting of the Academy in the same manner as the other officers of the Academy, and shall hold office for the same term.

1. The committee on publication shall consist of the president and secretary and a third member elected by the Academy. They shall determine the matter which shall be printed in the publications of the Academy. They may at their discretion refer papers of a doubtful character to specialists for their opinion as to scientific value and relevancy.
2. The library committee shall consist of five members, of which the librarian shall be *ex officio* chairman, and of which a majority shall not be from the same city.
3. The committee on nomination of members shall consist of five members, one of whom shall be the secretary of the Academy.

Article VII.—*Meetings.*

The annual meeting of the Academy shall be held at such time and place as the council may designate; but all regular meetings for the election of the board of officers shall be held at Madison. Summer field meetings shall be held at such times and places as the Academy or the council may decide. Special meetings may be called by the council.

Article VIII.—*Publications.*

The regular publication of the Academy shall be known as its Transactions, and shall include suitable papers, a record of its proceedings,

and any other matter pertaining to the Academy. This shall be printed by the state as provided in the statutes of Wisconsin. All members of the Academy shall receive gratis the current issues of its Transactions.

ARTICLE IX.—*Amendments.*

Amendments to this constitution may be made at any annual meeting by a vote of three-fourths of all the members present; *provided*, that the amendment has been proposed by five members, and that notice has been sent to all the members at least one month before the meeting.

RESOLUTIONS

REGULATIVE OF THE PROCEEDINGS OF THE ACADEMY.

THE TRANSACTIONS OF THE ACADEMY.

[*By the Academy, December* 28, 1882.]

2. The secretary of the Academy shall be charged with the special duty of overseeing and editing the publication of future volumes of the Transactions.

3. The Transactions of the Academy hereafter published shall contain: (a) a list of officers and members of the Academy; (b) the charter, by-laws and constitution of the Academy as amended to date; (c) the proceedings of the meetings; and (d) such papers as are duly certified in writing to the secretary as accepted for publication in accordance with the following regulations, and no other.

6. In deciding as to the papers to be selected for publication, the committee shall have special regard to their value as genuine, original contributions to the knowledge of the subject discussed.

9. The sub-committee on publication shall be charged with insisting upon the correction of errors in grammar, phraseology, etc., on the part of authors, and shall call the attention of authors to any other points in their papers which in their judgment appear to need revision.

[*By the Academy, June* 2, 1892.]

The secretary was given authority to allow as much as ten dollars for the illustrations of a paper when the contribution was of sufficient value to warrant it. A larger amount than this might be allowed by the committee on publication.

[*By the Academy, December* 29, 1896.]

The secretary was directed to add to the date of publication as printed on the outside of author's separates the words, "Issued in advance of general publication."

FEES OF LIFE MEMBERS.

[*By the Academy, July* 19, 1870.]

Resolved, That the fees from members for life be set apart as a permanent endowment fund to be invested in Wisconsin state bonds, or other equally safe securities, and that the proceeds of said fund, only, be used for the general purposes of the Academy.

ANNUAL DUES.

[*By the Academy, December* 29, 1892.]

Resolved, That the secretary and treasurer be instructed to strike from the list of active members of the Academy the names of all who are in arrears in the payment of annual dues, except in those cases where, in their judgment, it is desirable to retain such members for a longer time.

ARREARS OF ANNUAL DUES.

[*By the Council, December* 29, 1897.]

Resolved, That the treasurer be requested to send out the notices of annual dues as soon as possible after each annual meeting and to extend the notice to the second or third time within a period of four months where required.

SECRETARY'S ALLOWANCE.

[*By the Academy, December* 27, 1902.]

Resolved, That the Academy hereby appropriates the sum of seventy-five dollars per annum as an allowance for secretary's expenses, for which a single voucher shall be required.

SECRETARY'S ALLOWANCE.

[*By the Council, April* 5, 1912.]

Resolved, That the Academy appropriates the sum of two hundred dollars per annum for the secretary-treasurer's allowance.

ELECTION OF MRS. LUCIUS FAIRCHILD AS HONORARY MEMBER.

[*By the Council, April* 12, 1918.]

Resolved, That because of the honorable and leading part that was played by the late General Fairchild in the founding of this Academy, his widow, Mrs. Lucius Fairchild, of Madison, be voted an honorary member of the Wisconsin Academy of Sciences, Arts, and Letters.