ness and the residue (A) fractionally distilled in the Cooke-Bower fractionating column[26] at 0.1 mm. pressure. About 20% (0.35 g.) of the starting material (A) was obtained as a yellow crystalline solid and after recrystallization from ether melted at 68–69°. A mixed melting point determination, with 1-(3,4-dimethoxyphenyl)-1,2-propanedione showed no depression. The remainder of the material was not identified.

Reaction of 3-Chloro-1-(3,4-dimethoxyphenyl)-2-propanone (Series I) with a Mixture of Potassium Acetate and Glacial Acetic Acid.—The chloroketone (8.0 g.) was dissolved in glacial acetic acid (35 cc.), freshly-fused potassium acetate (21 g.) added, and the resulting solution heated for eight hours at 90–100°. The reaction mixture was diluted with water (300 cc.), neutralized with sodium carbonate, and extracted with chloroform. The extract was dried, the solvent removed and the residue fractionally distilled under reduced pressure. Each of the fractions obtained was semicrystalline. The crystalline material (40%) was separated on a porous plate and recrystallized from a mixture of ether and petroleum ether, m. p. 65–66°; no depression when mixed with an authentic sample of 2-acetoxy-1-(3,4-dimethoxyphenyl)-2-propanone (Series III).

Reaction of 1-Hydroxy-1-(3,4-dimethoxyphenyl)-2-propanone (Series II) with 5% Sulfuric Acid.—The hydroxyketone (0.4 g.) was heated at 70–80° with 5% sulfuric acid (100 cc.) for three and one-half hours and the solution extracted with chloroform. The chloroform extract was dried and the solvent removed. The residue was recrystallized from ether yielding the pure unchanged 1-hydroxy-1-(3,4-dimethoxyphenyl)-2-propanone (0.35 g.).

Reaction of 2-Hydroxy-1-(3,4-dimethoxyphenyl)-1-propanone (Series III) with Aqueous Potassium Acetate.—The hydroxyketone (0.5 g.) was refluxed for four hours with 5% aqueous potassium acetate (75 cc.). From the solution the starting material was recovered as the semicarbazone (m. p. 153–155°) in 40% yield. No 1-hydroxy-1-(3,4-dimethoxyphenyl)-2-propanone could be detected.

Reaction of 3-Chloro-1-(3,4-dimethoxyphenyl)-2-propanone (Series I) with Methanolic and Ethanolic Alkali.—The chloroketone (2.5 g.) was dissolved in methanol (15 cc.) and a solution of sodium (0.32 g.) in methanol (16 cc.) added dropwise at room temperature. The alkaline solution was acidified very slightly with hydrochloric acid, and then neutralized by addition of solid sodium bicarbonate, filtered and the filtrate taken to dryness under reduced pressure. The residue (1.75 g.) was recrystallized from

(26) Bower and Cooke, *Ind. Eng. Chem., Anal. Ed.*, **15**, 290 (1943).

petroleum ether (b. p. 30–50°) and then from aqueous ethanol; m. p. 40–41°. *Anal.* Calcd. for $(CH_3O)_2$—C_6H_3—$C_3H_4O(OCH_3)$: C, 64.3; H, 7.1; OCH_3, 41.5; mol. wt., 224. Found: C, 64.2; H, 7.0; OCH_3, 41.7; mol. wt. (Rast), 234.

Treatment of the same chloroketone with *methanolic* potassium hydroxide yielded the same compound in 80% yield.

The substitution of *ethanol* for *methanol* and sodium *ethylate* for sodium *methylate* in this experiment yielded the corresponding ethyl ether; b. p. 104° (0.04 mm.); $n^{25}D$ 1.5150; alkoxyl, calcd. for $(CH_3O)_2$—C_6H_3—$C_3H_4O(OC_2H_5)$, 39.1. Found, 39.2.

Treatment of these reaction products with semicarbazide or acidified 2,4-dinitrophenylhydrazine yielded no carbonyl derivatives.

Summary

1. Four new substances, namely, 1-bromo-, 1-hydroxy-, 1-acetoxy- and 3-acetoxy-1-(3,4-dimethoxyphenyl)-2-propanone have been synthesized.

2. The allylic and dismutation rearrangement reactions attendant upon the conversion of the two halides, 1-bromo-1-(3,4-dimethoxyphenyl)-2-propanone and 3-chloro-1-(3,4-dimethoxyphenyl)-2-propanone to the corresponding alcohols, acetates and ethyl ethers have been studied under a variety of conditions.

3. The ease with which these rearrangements occur provides valuable confirmation of the theory of Hibbert that the ethanolysis products from wood are in reality stabilized end-products formed from more reactive lignin progenitors of the type represented by oxyconiferyl alcohol (β,γ-dioxyisoeugenol) or its keto isomer, and provides strong support for his views on the structure of native lignin.

Studies on Lignin and Related Compounds. LXXV. Alkaline Nitrobenzene Oxidation of Plant Materials and Application to Taxonomic Classification[1]

By R. H. J. Creighton, R. Darnley Gibbs and Harold Hibbert

The two types of classification recognized by botanists for higher plants are (1) an *artificial* classification, or "key," compiled merely for the purpose of identification and based on any *obvious* characteristic, such as flower or leaf morphology, convenience being the sole criterion; (2) a *natural* one in which all available criteria

should be employed[2] to indicate true relationships between species, genera, etc. In general, flower morphology is the most suitable basis for classification, but many other criteria (*e. g.*, morphology of other parts, cytology, comparative anatomy and chemistry) have been employed occasionally for its support.

Chemical methods have been employed in clarifying the relationships of many closely related species. Thus, Manske[3] has pointed

(2) Gilmour, *Nature*, **139**, 1040 (1937).

(3) Manske, *Can. Chem. Process Inds.*, **23**, 199 (1939).

out that the structures of the alkaloids present in closely-related plants may serve to distinguish between species regarded as identical on the basis of morphology.

Miller[4] found the essential oils of three species of *Pycnanthemum*, *P. tullia*, *P. incanum* and *P. lanceolatum* (plants between which it is difficult to differentiate on morphological grounds) to differ markedly.

McNair's[5] studies of the essential oils from eighty-seven plants and families led him to conclude that their structure is closely related to the position of the plant in the phylogenetic scale.

Cole and Cardoso[6] isolated from members of the family *Flacourtiaceae* the five-membered ring fatty acids, gorlic acid, chaulmoogric acid, and several of the lower homologs of the latter. Of the eleven tribes or subfamilies which comprise the family *Flacourtiaceae*,[7] it is only from the *Oncobeae* (which according to Gilg is the central tribe) and the closely-related *Pangieae* that these acids have been isolated and identified. They contain an asymmetric carbon atom, and other members of the *Oncobeae* and *Pangieae* whose fatty acids have not yet been studied in detail have also yielded optically active fatty acids.

Reichert[8] has examined the starch grains from a large number of plant species and concludes that they "constitute a strictly scientific basis for the classification of plants." Both Reichert[8] and Carles[9] have carried out extensive investigations of the family *Iridaceae* which, as now classified, contains a number of genera, some of which (e. g., *Moraea* and *Homeria*) differ so widely in the reactions of their starches as to suggest misclassification. In general, their results support the earlier classification of Tournefort[10] rather than the more modern one.[11]

A close relationship exists between the proteins in certain plant extracts and the plant species,[12] in that, as the degree of relationship between species diminishes, the number of common proteins present in the plant extracts decreases proportionately. Mez and co-workers[13] on the basis of their serological investigations have constructed a "family tree" of the vegetable kingdom. The indicated phylogenetic relationships are in general accepted by taxonomists. Where differences occur Mez's results may sometimes express the truer phylogenetic relationships, though some of his serological relationships are obviously false.

The role of lignin in plant taxonomy has been confined largely to lignin color reactions, of which the Mäule test[14] has been the most valuable.

Crocker,[15] employing a wide variety of botanical groups, found that the three genera included in the *Gnetales*, namely, *Gnetum*, *Ephedra* and *Welwitschia*, all gave strongly positive Mäule tests. In general, all of the *Coniferales* gave the expected negative test, but one of the *Podocarpus* species, namely, *P. amarus*, gave a strongly positive test. Crocker's work has recently been extended by Gibbs[16] and some of his findings are included here for comparison with the present results obtained by nitrobenzene oxidation.

Analytical data have shown that a marked difference exists between the lignins isolated from the gymnosperms or "softwoods" and those isolated from the angiosperms or "hardwoods." The lignins of the former type contain 14–16% methoxyl and the latter up to 20–22%. Holmberg,[17] employing thioglycolic acid as the lignin extraction agent, investigated the extracted lignins from over fifty plant species. His results showed that, while the lignins from all of the higher plants were essentially uniform in type, the carbon analyses of lignins isolated from the gymnosperms were generally 3–5% higher than those from the angiosperms, the oxygen values of the latter lignins being correspondingly higher, even in the case of certain monocotyledons in which low methoxyl values (16.9 to 17.1%) were found. Similar results were obtained by Klason,[18] in his analyses of β-lignin sulfonic acids from various woods. He found that the lignins from gymnosperms had the formula $C_{10}H_{10}O_3$ and those from angiosperms the formula $C_{10}H_{10}O_4$. More recently further confirmation of chemical differences between the lignins from gymnosperms and angiosperms, respectively, has been secured in analyses of products obtained in the ethanolysis of wood by Hibbert and co-workers.[19] They showed that the lignin from angiosperms differs from that from gymnosperms in that in the former there are present both the 4-hydroxy-3-methoxyphenyl and the 4-hydroxy-3,5-dimethoxyphenyl nuclei, whereas in the latter the 4-hydroxy-3-methoxyphenyl nucleus only is present. Thus spruce wood, when subjected to ethanolysis, yields a mixture of 2-ethoxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (I), 1-ethoxy-1-(4-

(4) Miller. Ph.D. Thesis. University of Minnesota. 1918.

(5) McNair. *Am. J. Botany*. **19**. 168 (1932); **21**, 427 (1934); *Bull. Torrey Botan. Club*. **62**, 515 (1935).

(6) Cole and Cardoso. THIS JOURNAL. **61**, 2349 (1939).

(7) Gilg in Engler and Prantl's "Die Natürliche Pflanzenfamilien." 1925.

(8) Reichert. *Carnegie Inst. Wash. Pub.*. No. 173.

(9) Carles. *Rev. gen. botan.*. **47**, 5. 87. 144. 215. 294 (1935).

(10) "Institutiones rei herbariae." Paris. 1701.

(11) Dykes. "The Genus Iris." Cambridge University Press. 1913.

(12) Chester. *Quart. Rev. Biol.*, **12**. 19 (1937).

(13) Mez and Zeigenspeck. *Botan. Arch.*. **12**, 163 (1925).

(14) Mäule. *Fünfstück's Beitr. Wiss. Botan.*. **4**, 166 (1901).

(15) Crocker. *Bot. Gaz.*. **95**, 168 (1933).

(16) Gibbs. unpublished results.

(17) Holmberg. *Ing. Vetenskaps Akad.*. *Hand.*. No. 131 (1934).
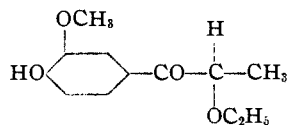
(18) Klason. *Ber.*. **63**, 1548 (1930); *Svensk Pappers Tidning*. **33**, 16 (1930); **35**, 224 (1932); **36**, 226 (1933).

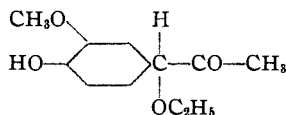(19) (a) Cramer. Hunter and Hibbert. THIS JOURNAL. **61**, 509 (1939); (b) Hunter. Cramer and Hibbert. *ibid.*. **61**, 516 (1939); (c) MacInnes. West. McCarthy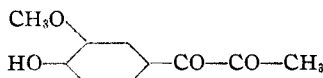 and Hibbert. *ibid.*. **62**, 2803 (1904); (d) Kulka. Hawkins and Hibbert. *ibid.*. **63**, 2371 (1941); (e) Kulka and Hibbert. *ibid.*. **65**, 1180 (1943).
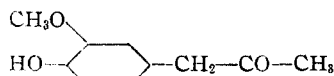
OCH₃

HO—⟨ ⟩—CO—C̶—CH₃
                |
               OC₂H₅

I. 2-Ethoxy-1-(4-hydroxy-3-
methoxypheny)-1-propanone

CH₃O

HO—⟨ ⟩—C̶—CO—CH₃
        |
       OC₂H₅

II. 1-Ethoxy-1-(4-hydroxy-3-
methoxyphenyl)-2-propanone

CH₃O

HO—⟨ ⟩—CO—CO—CH₃

III. 1-(4-Hydroxy-3-
methoxyphenyl)-1,2-
propanedione

CH₃O

HO—⟨ ⟩—CH₂—CO—CH₃

IV. 1-(4-Hydroxy-3-
methoxyphenyl)-2-propanone

CH₃O

HO—⟨ ⟩—CHO

V. Vanillin

CH₃O   H

HO—⟨ ⟩—CO—C̶—CH₃
                |
CH₃O   OC₂H₅

VI. 2-Ethoxy-1-(4-hydroxy-3,5-
dimethoxyphenyl)-1-
propanone

CH₃O

HO—⟨ ⟩—CO—CO—CH₃

CH₃O

VII. 1-(4-Hydroxy-3,5-
dimethoxyphenyl)-1,2-
propanedione

CH₃O

HO—⟨ ⟩—CH₂—CO—CH₃

CH₃O

VIII. 1-(4-Hydroxy-3,5-
dimethoxyphenyl)-2-propanone

CH₃O

HO—⟨ ⟩—CHO

CH₃O
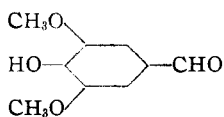
IX. Syringaldehyde
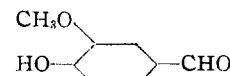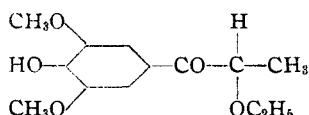
hydroxy-3-methoxyphenyl)-2-propanone (II), 1-(4-hydroxy-3-methoxyphenyl)-1,2-propanedione (III), 1-(4-hydroxy-3-methoxyphenyl)-2-propanone (IV) and v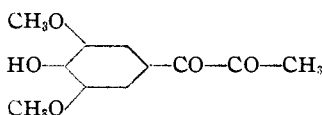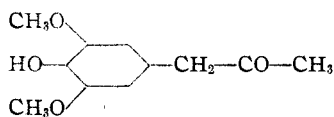anillin (V). Maple wood on similar treatment gives, in addition, the corresponding syringyl analogs (VI, VII, VIII and IX), the combined yields of pure products from angiosperms and gymnosperms amounting to 9.7 and *ca.* 3%, respectively. With certain monocotyledons, the presence of the syringyl compounds could not be definitely established.[19c]

More recently, employing the nitrobenzene oxidation technique of Freudenberg[20] spruce is found to give only vanillin upon oxidation, in a yield of 24% based on the initial Klason lignin, while maple and other dicotyledons give a combined yield of a mixture of vanillin and syringaldehyde of as high as 49%.[21] The ratio of vanillin to syringaldehyde in the isolated mixture was shown to be approximately 1:3. The monocotyledons investigated (consisting of several grasses) gave slightly lower yields of aldehydes (up to 30%), and the ratio of vanillin to syringaldehyde was approximately 1:1. A more recent investigation shows that the vanillin fraction isolated from these monocotyledons (all belonging to the grasses) contains a mixture of

vanillin and *p*-hydroxybenzaldehyde.[22]

In the present investigation the above alkaline nitrobenzene oxidation technique has been applied to over fifty plant species and the results confirm in general those obtained in the earlier work. Certain exceptions were found, however, which suggest that the method may provide a possible aid to the taxonomist in establishing the true phylogenetic relationships of certain plant species.

The procedure was essentially that previously outlined.[21] Due to the difficulty in obtaining sufficiently large quantities of material in certain cases, a reduction in the amount of starting material was found necessary. In these cases the time of reaction could be reduced from three to two hours, and the tedious step of steam distillation eliminated, when only the bisulfite soluble fraction called for investigation. The mixture of aldehydes obtained in the case of the angiosperms was separated by means of fractional sublimation of the crude mixture.[21]

Whenever possible, fresh plant material was employed, although in certain cases, notably with *Podocarpus amarus*, *P. pedunculatus* (see below), *Paulownia imperialis*, *Drimys winteri*, *Trochodendron sp.*, *Zygogynum vieillardi* and *Belliolum haplopus* (Burtt), A. C. Smith, museum specimens were used. In all cases the air-dried chips were ground to 40-mesh in a Wiley mill and solvent-extracted before use.

Podocarpus pedunculatus is not recognized by Pilger[23] but is regarded as synonymous with *P. amarus*. The close agreement found in this investigation between the two species (Table I) lends support to his views.

The results of the alkaline nitrobenzene oxidation of fifty species are shown in Tables I, II and III.

Unless otherwise specified, the air-dried wood chips were ground to 40-mesh in the Wiley mill, the resulting wood meal extracted successively with 50:50 ethanol–benzene (forty-eight hours), ethanol (twenty-four hours) and hot water (twelve hours), then air-dried, and the moisture content determined by drying a 2-g. sample overnight at 105°. The Klason lignin was determined on the oven-dried sample by the method of Ritter, Seborg and Mitchell[24] involving the use of 72% sulfuric acid.

The commonly accepted gymnosperms (Table I, Expts. 1–12) gave only vanillin, in yields of 15–24% calculated on the Klason lignin content

(20) Freudenberg, Lautsch and Engler, *Ber.*, **73**, 167 (1940).
(21) Creighton, McCarthy and Hibbert, This Journal, **63**, 3049 (1941).
(22) Creighton and Hibbert, *ibid.*, **66**, 37 (1944).
(23) Engler, "Das Pflanzenreich," 1903.
(24) Ritter, Seborg and Mitchell, *Ind. Eng. Chem., Anal. Ed.*, **4**, 202 (1932).

TABLE I
NITROBENZENE OXIDATION OF PTERIDOPHYTES AND GYMNOSPERMS

Expt.	Plant	Klason lignin, %[a]	Total aldehydes. %[b]	Vanillin. %[b]	Syringal- dehyde. %[b]	Ratio vanillin: syringal- dehyde	Mäule test[c]
1	Pteridium latiusculum (Bracken)	28.1	21.8	20.5	None		—
2	Cycas revoluta	15.6	2.7	2.5	None		—
3	Picea glauca (White Spruce)	28.6	23.7	23.5	None		—
4	Tsuga canadensis (Hemlock)	31.1	23.2	22.1	None		—
5	Pinus strobus (White Pine)	34.9	20.1	18.5	None		—
6	Thuja plicata (Western Red Cedar)	33.9	24.6	24.0	None		—
7	Sequoia sempervirens (Redwood)	31.8	24.8	23.5	None		—
8	Taxus canadensis (Ground Hemlock)	32.5	22.0	20.7	None		—
9	Agathis australis	30.8	19.9	18.7	None		—
10	Podocarpus neriifolius		Present	Present	None		Variable,
11	P. acutifolium	34.9	20.2	19.8	None		some
12	P. macrophyllus v. maki	39.9	17.4	15.1	Trace (?)		times
13	P. macrophyllus v. maki	39.0	17.0	14.9	None		faintly +
14	P. amarus	32.1	30.7	12.5	12.5	1:1.0	+ or ++
15	P. pedunculatus	30.7	31.7	13.9	14.7	1:1.0	+
16	Callitris rhomboidea	34.8	19.7	18.8	None		—
17	Tetraclinis articulata	29.5	29.2	15.5	12.0	1:0.8	++
18	Ginkgo biloba (Maidenhair Tree)	32.8	25.7	25.3	None		—
19	Ephedra trifurca	23.3	39.6	9.3	27.7	1:3.0	++ or better
20	E. sp.	25.0	36.0	8.8	26.0	1:3.0	++
21	Gnetum indicum	24.7	26.8	6.8	16.5	1:2.5	+ or ++
22	Welwitschia mirabilis	16.5	4.9	2.0	2.4	1:1.2	+

[a] Based on dry wood weight.   [b] Based on weight of initial Klason lignin.   [c] A rough attempt is made to express results quantitatively.

TABLE II
NITROBENZENE OXIDATION OF DICOTYLEDONS

No.	Plant		Klason lignin. %[a]	Total aldehydes. %[b]	Vanillin. %[b]	Syringal- dehyde. %[b]	Ratio vanillin: syring- aldehyde	Mäule test
1	Acer	(whole wood)	22.0	46.0	10.2	34.7	1:3.4	Not tested
2	rubrum	(heart wood)	23.6	39.2	9.7	28.5	1:2.9	Not tested
3	(Red maple)	(sapwood)	20.6	44.5	9.1	33.6	1:3.7	Not tested
4	A. saccharinum (Silver Maple)		18.8	51.0	12.7	35.2	1:2.8	+ or ++
5	Populus	(Aspen)	17.4	44.7	9.4	32.1	1:3.4	+ or ++
6	tremu-	(Aspen)[c]	17.0	43.3	9.1	33.4	1:3.7	Not tested
7	loides	(Aspen)[d]	20.6	41.5	8.3	31.2	1:3.7	Not tested
8	Sassafras sp.		22.1	44.5	9.9	29.5	1:3.0	+[e]
9	Quercus borealis v. maxima (N. Red Oak)		18.4	47.2	10.5	34.3	1:3.3	Not tested
10	Betula lutea (Yellow Birch)		19.6	44.9	11.0	33.7	1:3.1	++
11	Fraxinus americana (White Ash)		18.5	48.6	10.5	37.2	1:3.5	++
12	Ulmus fulva (Slippery Elm)		23.0	41.7	9.0	29.1	1:3.2	+++
13	U. americana (American Elm)		21.3	40.9	9.8	30.7	1:3.1	+++
14	Juglans cinerea (Butternut)		19.0	45.2	11.1	31.0	1:2.8	+ or ++
15	J. nigra (Black Walnut)		20.9	39.3	10.5	26.5	1:2.5	+[e]
16	Tilia americana (Basswood)		23.8	35.2	8.9	23.8	1:2.7	+ or ++
17	Corcho-	(Jute)	22.3	39.0	9.4	24.5	1:2.6	Not tested
18	rus sp.	(fiber)	12.7	32.0	7.7	23.9	1:3.1	Not tested
19	Sorbus americana		18.5	49.0	9.5	37.8	1:4.0	Not tested
20	Robinia pseudacacia		21.2	39.8	9.4	27.6	1:2.9	++
21	Paulownia imperialis		18.9	44.6	10.6	32.8	1:3.1	+
22	Liriodendron tulipifera		20.0	49.2	11.0	36.5	1:3.2	+ to +++
23	Trochodendron sp.		24.0	40.4	9.5	29.5	1:3.1	++
24	Drimys winteri		24.3	35.3	8.2	22.0	1:2.7	+
25	Belliolum haplopus		36.9	29.6	13.9	16.2	1:1.1	+ or ++
26	Zygogynum vieillardi		35.5	32.8	14.3	16.8	1:1.2	+ or ++

[a] Based on dry wood weight.   [b] Based on weight of initial Klason lignin.   [c] Extracted forty-eight hours with ethanol, then twelve hours with water.   [d] Extracted forty-eight hours with petroleum ether, then with ether.   [e] Tested by Sharma (1922).

No.	Plant species	Klason lignin. %[a]	Total aldehydes, %[b]	Vanillin. %[b]	Syring-aldehyde. %[b]	Ratio vanillin: syring-aldehyde	Mäule test
1	*Dracaena fragrans* (?)	23.8	34.9	6.9	26.2	1:3.8	++ or +++
2	*Aloe abyssinica*	18.5	37.1	8.3	27.1	1:3.3	+++
3	Bamboo (unidentified)	22.5	28.5	15.1[c]	11.5	1:0.8	+ or ++[d]
4	Rye straw	20.2	30.5	14.1[c]	15.3	1:0.9	+ or ++[d]
5	Corn cobs	13.8	21.4	17.1[c]	6.0	1:0.4	+[d]
6	Corn stalks	19.9	17.8	9.5[c]	7.8	1:0.8	+ or ++[d]
7	*Saccharum officinarum*	16.4	16.3	5.3	9.0	1:1.7	+

[a] Based on dry wood weight. [b] Based on weight of initial Klason lignin. [c] "Vanillin fraction" consists of vanillin plus p-hydroxybenzaldehyde. [d] Ethanol–benzene extracted meal was used for these tests (all other Mäule tests were on untreated plant material).

of the wood, while the dicotyledons (Table II, Expts. 1–24) yielded 35–51% of a mixture of vanillin and syringaldehyde in an approximate ratio of 1:3. The monocotyledons (Table III, Expts. 1–7) gave lower yields of mixed aldehydes (16.3 to 37.1%). In the grasses (Table III, Expts. 4 and 6) (excepting *Saccharum officinarum*) the presence of a third non-methoxyl-containing aldehyde, admixed with the vanillin, was indicated from the lower methoxyl values. As shown in the following communication, the aldehyde was p-hydroxybenzaldehyde.

A number of interesting exceptions were found to the above chemical differentiation between gymnosperms and angiosperms. Three species belonging to the *Coniferales*, namely, *Podocarpus amarus*, *P. pedunculatus* and *Tetraclinis articulata* (Table I) and also members of all the genera included in the *Gnetales* (Table I, Expts. 19–22) were found to give not only vanillin but also high yields of syringaldehyde. These species also gave a positive Mäule test, and a comparison of the results of this with the nature of the aldehydes formed on oxidation shows that a close correlation exists between the occurrence of the syringyl nucleus in the lignin of plants and the red coloration obtained in the positive Mäule test. From *Podocarpus macrophyllus v. maki*, which was found to give a very weakly positive Mäule test, the presence of the syringyl grouping could not be definitely established. In one experiment (Table I, Expt. 12) fractional crystallization of the m-nitrobenzoylhydrazone of the last sublimation fraction gave a trace (0.003 g.) of a hydrazone (m. p. 191–193°) which showed no mixed melting point depression with the m-nitrobenzoylhydrazone of syringaldehyde (m. p. 192–193°). In repeating this experiment with larger amounts of material (from a different source) (Table I, Expt. 13) and fractionating the bisulfite fraction in a semi-micro Cooke–Bower fractionating column[25] no syringaldehyde could be found. It should be emphasized, however, that the Mäule test was only *very* faintly positive with this species. The case of *Tetraclinis articulata* is particularly interesting. It has recently been

(25) Bower and Cooke. *ibid.*, **15**, 290 (1943).

separated from *Callitris* by the taxonomists and this separation is clearly supported by the chemical evidence (Table I, Expts. 16 and 17).

With *Belliolum haplopus* and *Zygogynum vieillardi* (Table II, Expts. 25, 26) the ratio of vanillin to syringaldehyde was found to be 1:1.1 and 1:1.2, respectively, proving to be, in this respect, the sole exception met with in a wide variety of dicotyledons. Both species were found to contain large amounts of Klason lignin (35 and 37%, respectively), while the yields of aldehydes based on same were only 29.6 and 32.8%. The results are of interest since these two species belong to a group of primitive angiosperms comprising the families *Magnoliaceae*, *Winteraceae* and *Trochodendraceae*, all of which are characterized by the absence of vessels. According to the most recent classification of these groups, *Belliolum* and *Zygogynum* are included, along with *Drimys winteri* (Table II, Expt. 24) in the Winteraceae while *Liriodendron* (Table II, Expt. 22) and *Trochodendron* (Table II, Expt. 23) are placed in the Magnoliaceae and Trochodendraceae, respectively. The very marked difference in the chemical behavior of *Belliolum* and *Zygogynum*, as compared with *Drimys* and also with *Trochodendron* and *Liriodendron*, suggests that further work is necessary to clarify the classification of this group of plants.

Of marked interest concerning lignin structure are the many high yields of mixed aldehydes (ratio vanillin to syringaldehyde 1:3) obtained from angiosperms amounting to 51% of the Klason lignin in the case of silver maple. If, as suggested by one of us (H. H.), native maple lignin is derived essentially from a dimeric and polymeric mixture of oxyconiferyl and oxysyringyl alcohols, and assuming the dimer units are formed by the loss of a molecule of water, the above percentage in terms of these anhydropropylphenol building units would amount to approximately 61%. This provides substantial support for the view that native maple lignin is derived *entirely* from propylphenol building units, the variations in yield between the different species being due to variations in the amounts of the polymeric forms present.

The very marked parallelism between the red color obtained on testing with the Mäule reagent and the definitely proven presence of the syringyl radical indicates the possibility of using the oxidation technique as a chemical means for checking taxonomic classification.[26]

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Summary

1. Alkaline nitrobenzene oxidation of forty-seven widely varying accepted forms of gymnosperms and angiosperms has shown that in practically all cases the former yield only vanillin, while the latter give both vanillin and syringaldehyde. This confirms the earlier findings of Hibbert and co-workers.

2. The yields of vanillin, in the case of the gymnosperms, vary between 15 and 24%, based on the Klason lignin. With the angiosperms

(26) It is the authors' intention to isolate the red coloring matter obtained by treatment of angiosperms with the Mäule reagent with the object of establishing therein the presence of the syringyl nucleus.

the combined yield of vanillin and syringaldehyde amounts to 35–51%, the ratio of the former to the latter being approximately 1:3.

3. Considering the highest combined aldehyde yield (silver maple 51%) obtained, this amounts to 61% of the Klason lignin based on the assumption that the native maple lignin is derived essentially from a mixture of anhydro polymeric forms of coniferyl and syringyl alcohols.

4. Exceptions to the above ratio (1:3) of mixed aldehydes were found in the case of certain primitive angiosperms characterized by the absence of vessels, the ratio in this case being 1:1.

5. All genera of the *Gnetales* (a group of uncertain position) gave both vanillin and syringaldehyde on oxidation, a result in harmony with their high lignin and methoxyl values and behavior with the Mäule reagent. Their relationship to the angiosperms rather than to the gymnosperms is thus indicated.

6. Only a very few members of the *Coniferales* were found to yield, on oxidation, an aldehyde mixture containing *both* vanillin and syringaldehyde and to give a definite Mäule reaction.

7. The behavior on oxidation of all the plants investigated is in complete agreement with their color reaction (or its absence) when tested with the Mäule reagent, and thus offers a new chemical procedure for taxonomic classification.

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Studies on Lignin and Related Compounds. LXXVI. Alkaline Nitrobenzene Oxidation of Corn Stalks. Isolation of p-Hydroxybenzaldehyde

BY R. H. J. CREIGHTON[1] AND HAROLD HIBBERT

Alkaline nitrobenzene oxidation of a wide variety of woods[2] has been shown to give high yields of vanillin from gymnosperms and of a mixture of vanillin and syringaldehyde from dicotyledons.

Certain monocotyledons, notably corn (cobs and stalks), bamboo and rye straw, yielded, in addition to vanillin and syringaldehyde, a non-methoxylated carbonyl derivative which could not be separated from vanillin by sublimation. The absence of methoxyl was indicated in the much lower values found for the crude *m*-nitrobenzoylhydrazones derived from the bisulfite fraction[2] isolated from the oxidation products.

The bisulfite fraction from the nitrobenzene oxidation of corn stalks has been reinvestigated and a separation of the unknown product from vanillin effected, based on solubility differences

(1) This paper represents part of a thesis presented by R. H. J. Creighton to the Faculty of Graduate Studies and Research, McGill University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, May, 1943.

(2) Creighton, Gibbs and Hibbert, THIS JOURNAL, **66**, 32 (1944).

TABLE I

METHOXYL ANALYSES OF CRUDE *m*-NITROBENZOYL HYDRAZONES FROM THE BISULFITE FRACTION ISOLATED FROM THE ALKALINE NITROBENZENE OXIDATION PRODUCTS OF MONOCOTYLEDONS

Material	OCH₃ %
Dracaena sp. (stem)	14.5
Aloe abyssinica (stem)	14.3
Bamboo (unidentified) (stem)	10.8
Zea mays L. (Corn cobs)	5.5
Zea mays L. (Corn stalks)	7.5
Secale cereale L. (Rye straw)	12.1
Saccharum officinarum L. (Sugar cane stem)	14.0
Dicotyledons (average 6 woods)	14.8
Quercus (Oak)	15.3
Populus (Aspen)	14.7
Juglans (Walnut)	15.0
Tilia (Basswood)	13.8
Robinia (pseudacacia) (Black Locust)	14.6
Liriodendron tulipifera (Yellow poplar)	15.6
Vanillin	9.8