THE OXIDATION OF LIGNIN AND RELATED COMPOUNDS

by

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CLAIMS TO ORIGINAL RESEARCH

1. The alkaline oxidation of maple and other angiosperms with nitrobenzene gives a mixture of vanillin and syringaldehyde in yields of 40-50%, the approximate ratio of the aldehydes being 1:3. This serves to establish the similarity of the lignin present in all plants as of an essentially aromatic type, the nuclei present being either of the guaiacyl or syringyl types, or both.

2. A new constituent of the aldehyde fraction, namely p-hydroxybenzaldehyde, has been isolated from corn stalks, and evidence obtained of its presence in a number of other monocotyledons.

3. Syringaldehyde has been isolated from several species of gymnosperms known to give a positive Mäule test, a reaction generally shown only by angiosperms. A close correlation between a positive Mäule test and the occurrence of the syringyl grouping in lignin has been proven.

4. Alkaline oxidation of plant species with nitrobenzene has been shown to provide a new chemical aid for the taxonomic classification of plants.

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HISTORICAL REVIEW

Early Developments in Lignin Chemistry

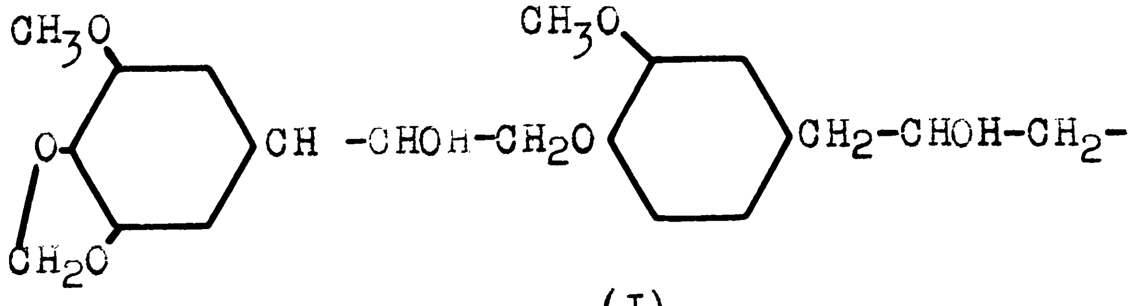
Recent reviews on the structure of lignin by von Wacek (1), Freudenberg (2), Erdtman (3) and Hibbert (4) have emphasized the essentially aromatic character of lignin and its relationship to coniferyl types.

During the period 1897 (5) to 1936 (6), Klason's original "Coniferyl-oxyconiferyl alcohol hypothesis," in which polymers of coniferyl alcohol, coniferyl aldehyde, guaiacol aldol, etc. were included, underwent various modifications. This theory which may be regarded as constituting the first phase of lignin research, was based (i) on the universal occurrence of coniferyl alcohol in young plant tissues (7), (ii) on data derived from the ethanolysis of the spruce lignin sulphonic acids and from solvent-extracted material (spruce wood) presumably containing both polymerized coniferyl and oxyconiferyl alcohol, and (iii) on the occurrence of products such as catechol, guaiacol and protocatechuic acid in the lignin alkali-fusion reaction mixture (8).

In the second phase of lignin research (1926-1932) emphasis was laid on new methods for the isolation of lignin from wood and on identification of functional groups (methoxyl, hydroxyl, carbonyl, aromatic nucleus) in the extracted lignin.

In this connection, methods were employed involving the use of concentrated acids such as sulfuric acid (9), hydrochloric acid (10) and mixtures of hydrochloric and phosphoric acids (11), as well as much milder procedures such as alcoholysis (4) by the use of alcohols, glycols, glycerol and glycerol chlorohydrin.

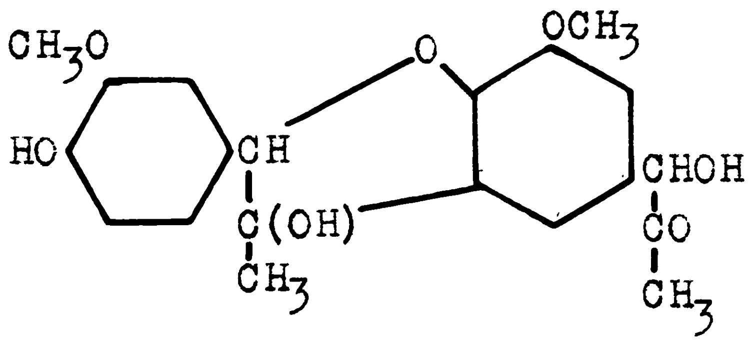
Freudenberg's earlier theory of the structure of lignin (12) was based essentially on the isolation of small amounts of catechol, protocatechuic acid and formaldehyde from spruce hydrochloric acid lignin, and on analyses of this lignin. These results indicated (a) aromatic character and the absence of free phenol groups; (b) attachment of the methoxyl groups to the aromatic nuclei; and (c) presence of aliphatic hydroxyl groups in the side chains attached to the aromatic nuclei. Freudenberg's modifications of Klason's view of lignin as a propylphenol derivative differed only in the type of side chain envisaged and in the assumption that the building units were joined through ether linkages to give "linear type" condensation polymer (I) (12, page 134). Such a polymer, however, should readily undergo degradation by hydrolytic action into simple molecular units, and, this not being the case, Freudenberg found it difficult to postulate transformation into "secondary lignins" (12, page 135).



(I)

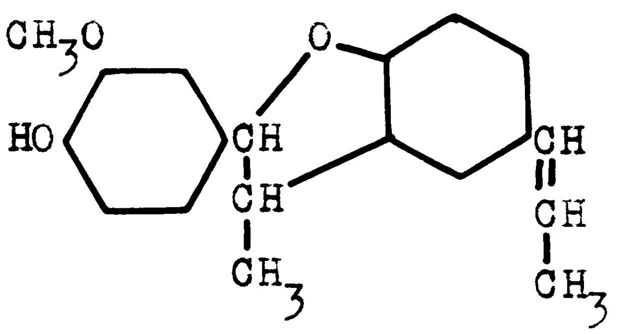
The assumed presence of the dioxymethylene group was based on the liberation of a small amount of formaldehyde (up to 1.2%) (13, 14, 15). The side chains visualized by Freudenberg were $R-CHOH-CHOH-CH_2OH$, $R-CH_2-CHOH-CH_2OH$, $R-CHOH-CH_2-CHO$ and $R-CHOH-CO-CH_3$ (12, page 135). According to his definition these compounds are "biochemically identical" (12, page 137); this expression, however, has, scientifically, no biochemical significance and can only be assumed to imply that they are in equilibrium with each other at least *in vivo*.

On the basis of the studies of alkali degradation of spruce lignin followed by methylation and oxidation (16, 17, 18), Freudenberg abandoned the ether-type linkage of assumed propylphenol units and now considers the union to be of a carbon-to-carbon type involving oxygen-ring formation between side chains and aromatic nuclei (2, page 95) (II).



(II)

(from two moles of $R-CHOH-CO-CH_3$)



(III)

It can be seen that this structure (II) is similar to the dimer resin-type polymer (e.g. dehydrodiisoeugenol, (III)) suggested by Erdtman.

One of the chief objections to this new Freudenberg conception has been the assumed presence of the dioxymethylene group either on terminal (12, page 134) or (in his later theory) centrally disposed (2, page 95, 109) aromatic nuclei, to the extent of 25 per cent of the spruce lignin structure. This theory has been seriously criticized (4), especially from the point of view of the non-isolation of piperonyl units. Furthermore the inclusion of such nuclei as centrally located units in a dehydrodiisoeugenol type of polymer implies a new and unusual form of condensation. It is also of importance to note that such a condensation polymer (II) could not be formed with syringyl derivatives where the five position (ortho to the phenol group) is blocked by a methoxyl group.

Recent Developments in Lignin Chemistry

The recent developments (constituting the third phase) which have led to a clearer understanding of the structure and origin of protolignin are those connected with studies (i) alkaline oxidation of lignin, wood and lignin sulphonic acids; (ii) high pressure hydrogenation of wood and lignin; and (iii) early experiments on the alcoholysis of wood. The results of these studies have provided experimental support for (i) the above mentioned theoretical conception of lignin originally proposed by Klason and extended by Freudenberg and (ii) the modern theory of plant respiratory catalysts as lignin progenitors developed by Hibbert (4).

1. Alkaline Oxidation of Lignin and Lignin Derivatives

(a) Early Work on the Oxidative Degradation of Lignins

Until recently, the oxidative degradation of lignin has done little to advance the modern conception of the aromatic structure of lignin. The previous work on the oxidation of lignin with ozone, (20, 21, 22) hydrogen peroxide, (23) nitric acid (24, 25, 26) and chromic acid (27) in most instances yielded only a mixture of oxalic and acetic acids, along, in some cases, with smaller amounts of formic and other simple fatty acids. Pauly and Feuerstein (28) in a patent application, treated wood-meal suspended in acetic acid with ozone and hydrolized the product claiming to obtain vanillin. No yields were given however, and the work has never been confirmed. At a later date, Freudenberg and Sohns (14) treated a methylated lignin with ozone and obtained 20 per cent oxalic acid and a trace of acetic acid as the only products.

Phillips and Goss (29) subjected methylated alkali lignin isolated from corn cobs to oxidation by both ozone and nitric acid, and obtained small amounts of anisic acid, in addition to 25 per cent oxalic acid. While ethylated lignin gave the corresponding p-ethoxy-benzoic acid, oxidation of the unmethylated lignin gave only oxalic acid.

Dorland and Hibbert (30) subjected beech lignin to ozonolysis, after which treatment it became readily soluble in bisulphite. On alkaline cleavage, these lignin sulphonic acids yielded 4 per cent vanillin and 2 per cent 4-hydroxy-3-methoxyacetophenone (VI, page 9).

In addition to oxalic acid, oxidation of lignin by nitric acid yields a mixture of substituted nitrophenols. Recently, Kalb (31) has investigated the action of nitric acid on a number of isolated lignins and lignin sulphonic acids, obtaining oxalic acid and up to 43 per cent of a mixture of substituted nitrophenols. On further treatment of the nitrophenols with a mixture of nitric acid and copper nitrate an additional quantity of oxalic acid was obtained. In this manner these authors were able to obtain 58 per cent of oxalic acid and 8 per cent of nitrophenols from a technical pine lignin isolated by the Scholler method.

Freudenberg (14) has oxidized spruce "cuproxam lignin" with hot concentrated aqueous chromic acid and obtained a 6 per cent yield of acetic acid. Since many structures containing terminal methyl groups, such as $R-CH_2-CO-CH_3$, yield better than 85 per cent of the theoretical amount of acetic acid, when oxidized with chromic acid (32), Freudenberg considered this yield to be a low percentage (20%) of the theoretical from a polymer of the type postulated by him (II).

More recently MacGregor and Hibbert (33) have applied the chromic acid oxidation technique to model substances, particularly derivatives of 3-methyl,- 4-hydroxyphenyl-propane, and find that for these compounds at least 85 per cent of the theoretical amount of acetic acid may be obtained. When spruce and maple woods were oxidized with chromic acid, only traces of acetic acid were obtained, thus indicating that the native lignin contains only a small percentage (if any) of end methyl groups. The low boiling mono-molecular oils from the ethanolysis of both spruce and maple woods were found to give yields of acetic acid amounting to 85-95 per cent of the theoretical value. With spruce and maple extracted amorphous lignins, however, a marked contrast was observed in that the spruce lignins yielded an appreciable quantity of acetic acid indicating the presence of end methyl groups in amounts corresponding to one per each 4 or 5 C_6C_3 lignin units, while with the maple lignin no acetic acid was obtained indicating the absence of terminal methyl groups.

(b) Alkali Fusion of Lignin

As previously mentioned, alkali fusion provided much of the early experimental evidence for the aromatic nature of lignin. Whem fused with alkali at temperatures of 200-300°, catechol, protocatechuic acid and oxalic acid were obtained in varying yields of up to 10, 15 and 20 per cent respectively, depending upon the reaction conditions employed (34, 35). In a systematic series of investigations, Heuser and Winsvold (8) and Heuser and Herrmann (36) found that the yields of the various isolated products varied considerably with the fusion conditions. By excluding the presence of air, the formation of oxalic acid was almost completely prevented, and correspondingly increased yields of catechol (9%) obtained. The highest values were obtained when Willstätter lignin was fused with potassium hydroxide in a nickel crucible at 280°. Under these conditions 18.7 per cent of protocatechuic acid and 5.5 per cent of catechol were obtained. It was shown that catechol was formed from protocatechuic acid by the elimination of carbon dioxide, and that this reaction was catalyzed by iron. Thus when the fusion was carried out in an iron crucible, the yield of catechol was increased to 20 per cent at the expense of the protocatechuic acid. Cellulose when treated similarly yielded no aromatic substances, leading to the conclusion that at least 25 per cent of the lignin was aromatic.

Fischer and co-workers (37) subjected lignin to what they termed "Pressure oxidation". Air was blown through a mixture of Willstätter lignin dissolved in alkali or sodium carbonate under pressures of up to 55 atmospheres. From the reaction mixture 8 per cent of aliphatic acids and 3 per cent of a mixture of benzene carboxylic acids, ranging from the monosubstituted acid (benzoic) to the hexasubstituted acid (mellitic), were obtained. Due, however, to the drastic nature of the treatment these results are of little significance in the determination of the structure of lignin.

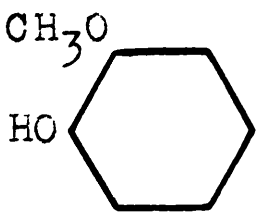
Freudenberg, (17, 18) on treatment of wood with 70 per cent alkali at 165° followed by methylation and oxidation with potassium permanganate, obtained 20-25 per cent of veratric acid, 6-12 per cent of isohemipinic acid and 2-3 per cent of dehydrodiveratric acid. When subjected to similar treatment, Erdtman's acid (III) yielded 21 per cent of veratric acid and

5 per cent isohemipinic acid. These results led Freudenberg to abandon his theories of an ether-linkage in the lignin polymer in favour of a carbon-to-carbon link (p. 3).

(c) Alkaline Cleavage of Waste Sulphite Liquors

The action of alkali on sulphite waste liquors to give vanillin was first investigated by Grafe (38) and subsequently by Kürchner, (39) Pauly and Feuerstein (40) and Hönig and Ruziczka (41), but the yields were very low, and since technical sulphite waste liquors were employed in all of these investigations, the formation of vanillin from some constituent other than lignin could not be precluded.

In 1932 Tomlinson and Hibbert (42) commensed a study of the action of alkali on isolated, carefully purified, spruce lignin sulphonic acids. When heated to 160° in 20 per cent sodium hydroxide solution, yields of vanillin (V) amounting to 6.7 per cent were obtained based on the weight of the Klason lignin present. These results, later confirmed by Hägglund and co-workers, first proved that the vanillyl nucleus is an important part of the lignin structure. In addition to vanillin, small amounts of guaiacol (IV) and 4-hydroxy-3-methoxy acetophenone (VI) have also been isolated from the "alkali cook."



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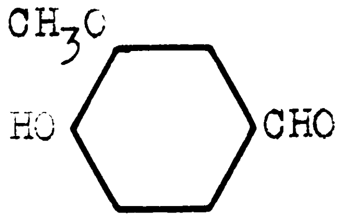

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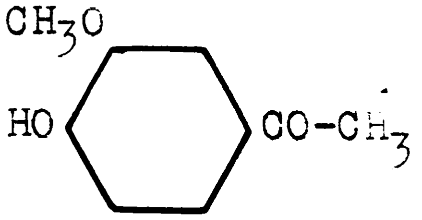

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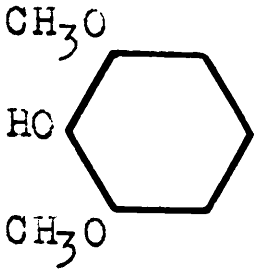

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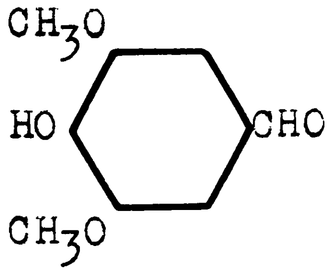

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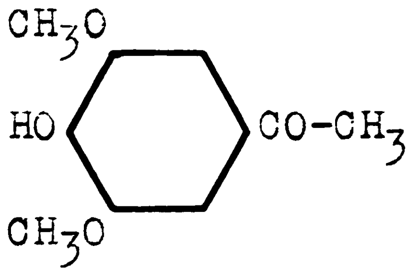


(IX)

Application of these new methods to maple lignin sulphonic acid, led to the isolation in addition to the above of pyrogallol-1,3-dimethyl ether (VII), syringaldehyde (VIII) and 4-hydroxy-1,3-dimethoxy acetophenone (IX)

(d) Nitrobenzene Oxidation of Wood and Lignin

The formation of vanillin in high yield from isoeugenol by oxidation, in aqueous alkaline solution with nitrobenzene (46a) and with m-nitrobenzene sulphonic acid (46b), has been known for some time. When waste sulphite liquor was refluxed with alkali in the presence of this reagent, the yields of vanillin were not appreciably increased. Wood meal, by the same treatment, gave 3.6 per cent vanillin, while, when subjected to boiling alkali alone, the yield was less than 1 per cent (47).

Recently Schulz (48), in a patent claim, obtained yields of from 8-10 per cent of vanillin from lignin containing materials by heating with nitrobenzene and alkali at 200° in a closed vessel.

At the same time, Freudenberg (49, 50), using aqueous alkali and a large excess of nitrobenzene, oxidized spruce wood at 160° and obtained vanillin in a 25 per cent yield based on the Klason lignin. Slightly lower yields were obtained from waste sulphite liquor and from spruce "cuproxam" lignin, which gave 18 and 22 per cent vanillin respectively. In the present investigation the author has confirmed these results, and an extension of the method to maple wood has yielded 45 per cent of the Klason lignin as a mixture of vanillin and syringaldehyde in a ratio of approximately 1:3.

(e) Alkaline Oxidation with Oxidizing Agents other than Nitrobenzene

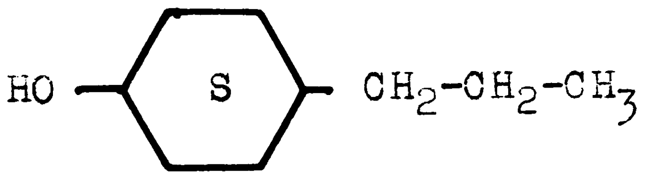
In 1936, Rang (51), in a patent, claimed to have obtained vanillin from waste sulphite liquors by oxidation in alkaline solution with air or oxygen in the presence of metallic catalysts. No yields were given. More recently, Lautsch and Piazolo (52) obtained up to 14 per cent of vanillin from lignin by boiling a suspension of the same in alkali with cobaltic hydroxide in a current of air. They found that other metallic catalysts could also be employed, but that the yields were not as good.

Pearl (53) has obtained a 22 per cent yield of vanillin from waste sulphite liquor by heating at 160° with an oxidizing mixture of copper hydroxide in alkaline solution. At reflux temperatures this yield of vanillin drops to 13 per cent. When

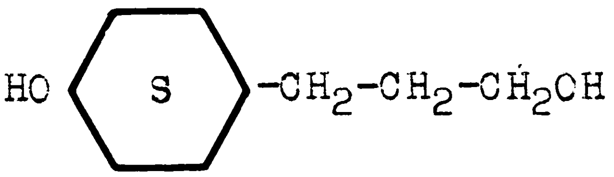
Fehling's solution at reflux temperatures was used as the oxidizing medium the yield of vanillin from waste sulphite liquor was only 10 per cent.

2. Hydrogenation of Wood and Lignin

The preliminary work concerned with the high pressure hydrogenation of lignin has been reviewed (3,4). Complete liquefaction of the wood is effected by application of this technique to spruce and maple woods (54), the protolignin being converted, in part to 4-n-propylcyclohexanol (X) and 3-(4-hydroxycyclohexyl)-propanol-1 (XI) in yields of 19.5 and 5.8 per cent respectively (based on the Klason lignin content of the wood).



(X)



(XI)

Using the carbon content of these isolated units and of the "methoxy free protolignin" in wood as a basis for calculation, the combined yield of propylcyclohexane derivatives represents a recovery of 36 per cent (54).

Examination of the yields of hydrogenation products from various amorphous fractions of maple lignin isolated by ethanolysis (55) indicates that, for a given series of such fractions, increasing solubility and increasing susceptibility to depolymerization into simple, monomolecular propylphenol units by ethanolysis are paralleled by an increasing yield of the water-insoluble propylcyclohexanol hydrogenation products. This observation is thought to indicate a prevalence of -C-O-C- bonds between the propylphenol "lignin building units" in those fractions which are readily-soluble and are easily cleaved by ethanolysis and hydrogenolysis and, conversely, an increasing incidence of -C-C-C- bonds in lignin fractions having these characteristics to a lesser extent (55).

Hatihama et al. (56) hydrogenated hydrochloric acid lignin in the presence of several less active catalysts, particularly nickel, and obtained a 50 per cent yield of ether-soluble aromatic oils containing pyrocatechol and n-propylguaiacol. Similar results have been reported by Freudenberg and co-workers (57) who, following earlier work by Babrov and Kolotova (58), extended the investigations to waste sulphite liquor. More recently, Freudenberg and Adam (59) have developed a procedure which consists of the simultaneous dry distillation and catalytic hydrogenation of isolated lignins on the surface of which various metallic catalysts had been precipitated previously. In this manner ether-soluble tarry fractions (20-50% yield) containing, among other products, phenols and phenolic ethers were obtained.

In addition to providing the foregoing proof of the presence of the propylphenol unit in lignin, hydrogenation studies have shown that the lignin building units contain terminal $-CH_2OH$ or $-CH_2-O-CH_2-$ linkages (or both types)(54, 55, 60, 61).

3. Alcoholysis of Wood

The fourth phase in the elucidation of lignin structure may be regarded as that associated with the more recent contributions of Hibbert and co-workers on the action of ethanolic hydrogen chloride on various plant materials (4).

The belief that native and extracted lignins exist only as highly polymerized, complex substances led investigators to conclude that the amorphous water-insoluble product obtained by customary extraction methods was the only form in which lignin could be isolated. Precipitation into water from concentrated alcohol, acetic acid, or other solutions has been almost an invariable step in the isolation and purification of lignin.

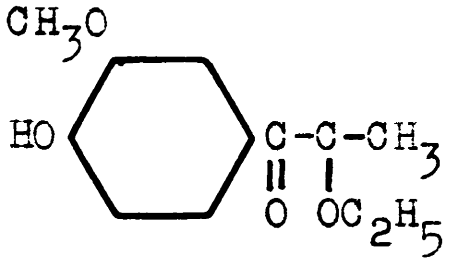
Prior to the recent developments of the ethanolysis lignin extraction process by Hibbert and co-workers, the aqueous precipitating liquors had been examined only on two occasions, namely by Friedrich and Brüda (63) (methanolysis of beech) and by Brauns and Hibbert (64) (methanolysis of spruce). The former investigators isolated a small amount of lignin which apparently had been suspended in the water as a sol, and only minute traces of methoxyl-containing material were found in true solution in the concentrated aqueous liquors. Brauns and Hibbert also found only a small quantity of methoxyl-containing material in their work.

When it was observed, however, that the sum of the weights of the amorphous water-insoluble lignin and of the Klason lignin remaining in the alcoholysis wood residue was almost always considerably less than the weight of the Klason lignin in the untreated wood, Hibbert became interested in the cause of this discrepancy. The explanation was found when Cramer, Hunter and Hibbert (65) showed that the loss in weight was due (in the case of the ethanolysis of spruce and maple woods) to the water solubility of a large portion (12% of the Klason lignin, spruce; 30%, maple) of the methoxyl-containing materials.

As a result of this important observation an extensive series of investigations was started immediately in these laboratories to determine (a) the structure of the components of the water soluble lignin fraction; (b) the factors involved in their isolation, and the bearing of these on the question as to whether the isolated ethanolysis products actually existed as such in the native lignin or were, in reality, stabilized end products derived from more complex polymers and/or more reactive simple units; (c) the significance of the simple units in relation to the structure of the amorphous, water-insoluble ethanol lignin and (d) the significance of the simple units in relation to photosynthesis, plant respiration and the formation of native lignin.

To date approximately one third (12% of Klason lignin content) of the water-soluble oils from maple wood has been shown to consist of the following products: 2-ethoxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (XII) (65) and its syringyl analogue

(XIII) (66); 1-(4-hydroxy-3-methoxyphenyl)-1,2-propanedione (XIV) (68a) and its syringyl analogue(XV) (68b); 1-(4-hydroxy-3-methoxyphenyl)-2-propanone (XVI) (69) and its syringyl analogue(XVII) (69); vanillin (V) (67) and syringaldehyde (VIII) (67).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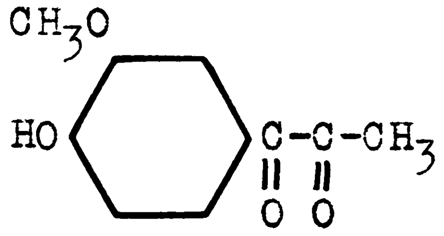
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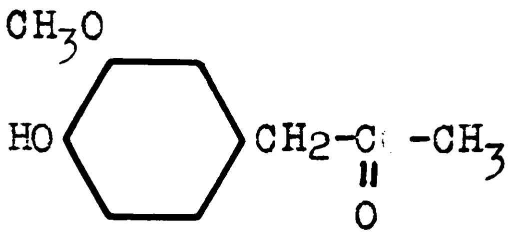
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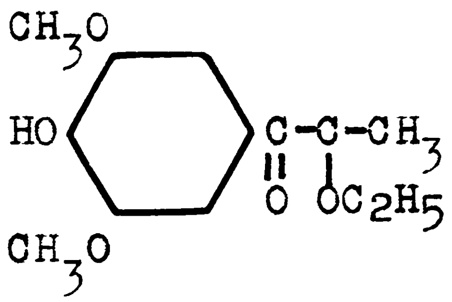
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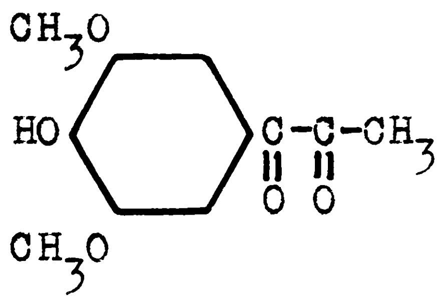
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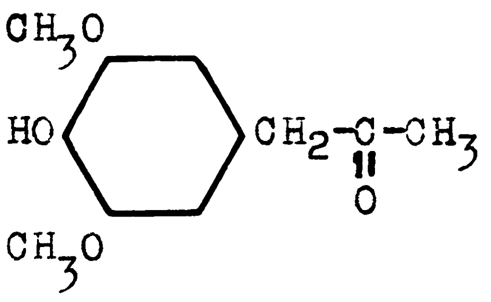
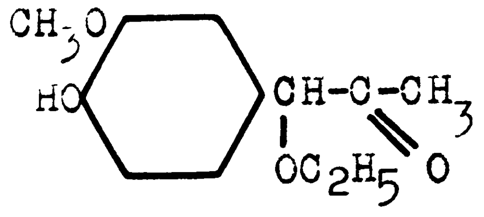

(XVII)

From spruce wood only small amounts (3% of Klason lignin) of identifiable oils have been obtained thus far. These consist of the compounds of the 4-hydroxy-3-methoxyphenyl propane series (V, XII, XIV, XVI) and an additional new isomer of (XII) 1-ethoxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone (XVIII) (70).


(XVIII)

An actual relationship between these monomeric units and extracted lignins has been established by Peniston, McCarthy and Hibbert (71). These investigators refluxed an acetylated oak lignin with anhydrous ethanolic hydrogen chloride (2%) for 15 hours. The crude, water-soluble oils thus obtained (36% of the acetyl-free lignin content of the starting material) were separated into four fractions whose characteristics were very similar to those obtained by the action of ethanolic hydrogen chloride on maple wood.

As a direct result of the isolation and identification of the propyl phenol derivatives and of 3-(4-hydroxycyclohexyl)-propanol-1 (XI) (by means of hydrogenation) interest has been centered on the potential significance of related compounds such as 3-hydroxy-1-(4hydroxy-3-methoxyphenyl)-1-propanone (XIX), 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-propanone (XX) and their corresponding syringyl derivatives:

$R\text{-}CO\text{-}CH_2\text{-}CH_2OH$ (XIX) $\qquad$ $R\text{-}CH_2\text{-}CO\text{-}CH_2OH$ (XX)

as the possible true lignin progenitors, at least, to a large extent.

4. Miscellaneous Experiments

The effect of variables in the Klason lignin determination has been carefully studied by Freudenberg and Ploetz (72, 73).

The results indicate that this determination is, to a large extent, an arbitrary one and of particular significance is the fact that reaction conditions applicable to the production of the lowest yield of lignin of highest methoxyl content in the case of soft-woods are not necessarily applicable to hard-wood. The use of hydrogen fluoride as a solvent for lignin has been suggested by Wiechert (74, 75). Preliminary investigations indicate that its use in a standardized method for the determination of lignin would eliminate many of the objections to the established sulfuric acid procedure, although the requisite expensive equipment (platinum or silver) renders its general acceptance doubtful. The difficulties encountered in applying the Klason procedure to plant products (grains, vegetables etc.) have been emphasized recently (76), in particular with respect to (a) the apparent condensation of lignin with protein material and (b) the higher values found when the plant material is pre-dried at relatively high temperature (100°C).

Benson and co-workers (77, 78), in an attempt to establish a lignin research program of industrial significance, have commenced a study of desulphonated (with sodium hydroxide) calcium lignin sulphonate. Their recent results (79) indicate that nitrated desulphonated lignin closely resembles nitrated butanol lignin.

Butanol lignin, obtained by treating wood with butanol-water and butanol-water-alkali at 160°, has been studied extensively by Bailey (80). He concludes (81) from comparative butanolysis studies on aspen and jack pine that a portion of the lignin in soft-woods (but not in hard-woods) is chemically bound to cellulose. The presence of glucosidic linkages involving phenolic hydroxyl groups of the lignin is rendered doubtful, however, by by the results of recent investigations (82) on model substances (glucosides of the ethanolysis lignin units).

The necessity for careful classification of the source of lignin under investigation is reemphasized in results obtained by Ritter (83) on the lignin content of various cross-sectional regions of birch trees. For example, the lignin content in a twenty-nine year old white birch was found to drop from 37.4 per cent at the center to 19.5 per cent at the periphery.

The similarity of the behaviour of coumarin and of acetic acid lignins toward diazomethane has led Wright (84) to suggest that lignins contain a coumarin-type of lactone linkage. von Wacek and Nittner (85) subjected beechwood tars to ozonolysis and from their analyses of the reaction products they concluded that substituted coumarones were present in the tars. The presence of two pyrone rings in each "lignin building unit" (87) is assumed from absorption spectra studies (86) on spruce native lignin (87), spruce native lignin derivatives, lignins isolated from spruce-wood by compounds containing hydroxyl and mercaptyl groups, and related compounds.

5. Biochemical Aspects of Protolignin Formation - Mechanism of Plant Syntheses of Propylphenol Derivatives

(a) Synthesis of Simple Phenols -- Various theories concerning the synthesis of phenolic compounds in plants have been based on assumed transformation of hexoses (88, 89), while a more recent theory (4) considers the phenolic substances as being formed from intermediate photosynthetic and/or plant respiratory products.

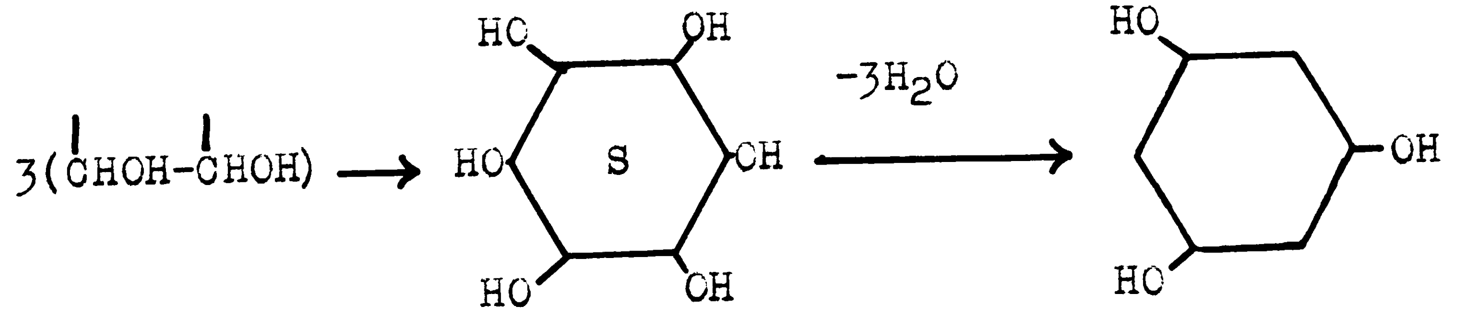
(i) Free Radical Theory (90) -- Specific types are assumed to be present in the active stages of plant growth (formic acid (XXI), glycollic aldehyde (XXII) and acetaldehyde (XXIII)) in equilibrium with the corresponding enol-radicals:

$HCOOH \rightleftharpoons \underset{|}{\overset{|}{C}}(OH)_2$ (XXI)

$CH_2CH-CHO \rightleftharpoons \overset{|}{C}HOH-\overset{|}{C}HOH$ (XXII)

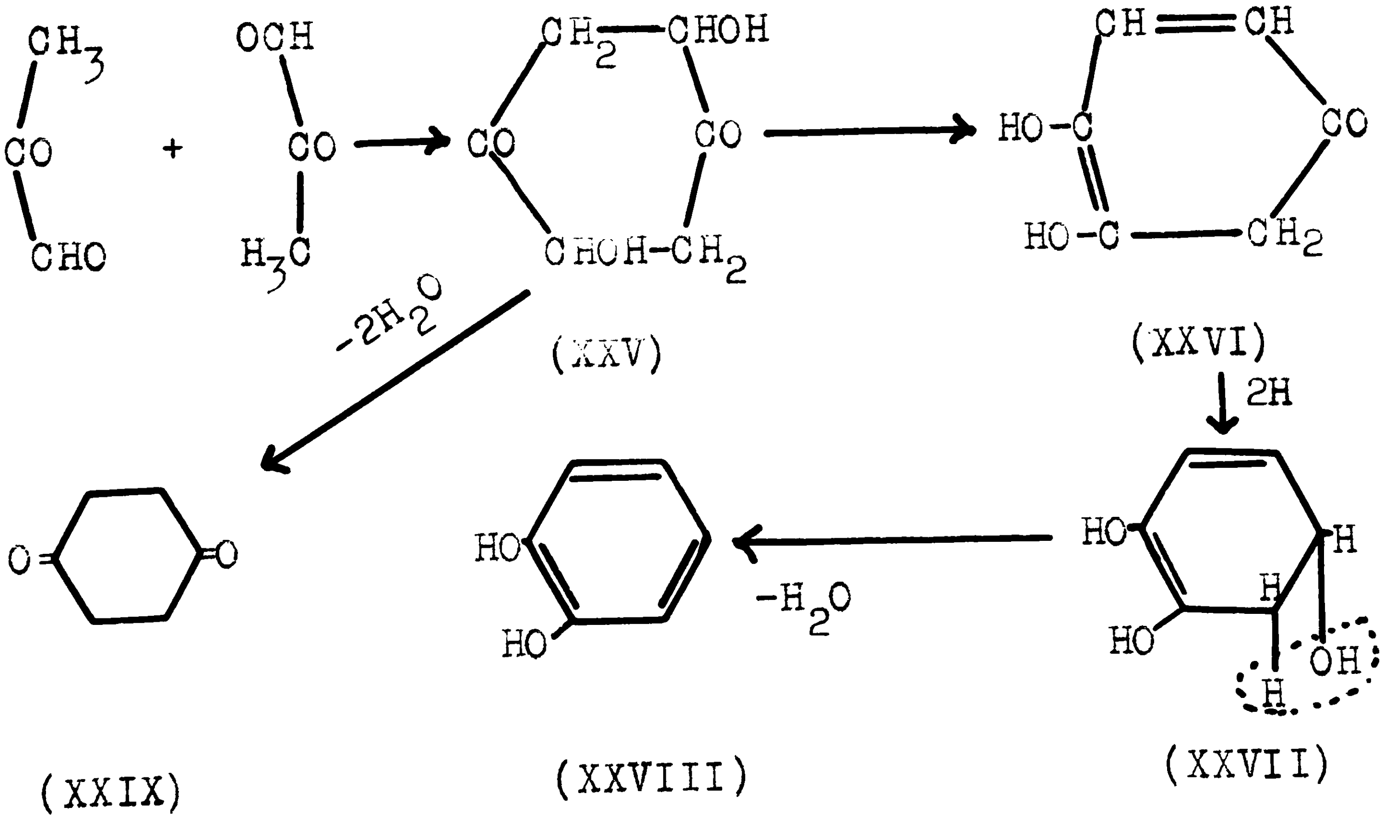
$CH_3CHO \rightleftharpoons \overset{|}{C}H_2-\overset{|}{C}HOH$ (XXIII)

Union of the free radicals may give rise to hydro-aromatic derivatives which, by loss of water, could yield phenol; for example, phloroglucinol (XXIV) from (XXII)



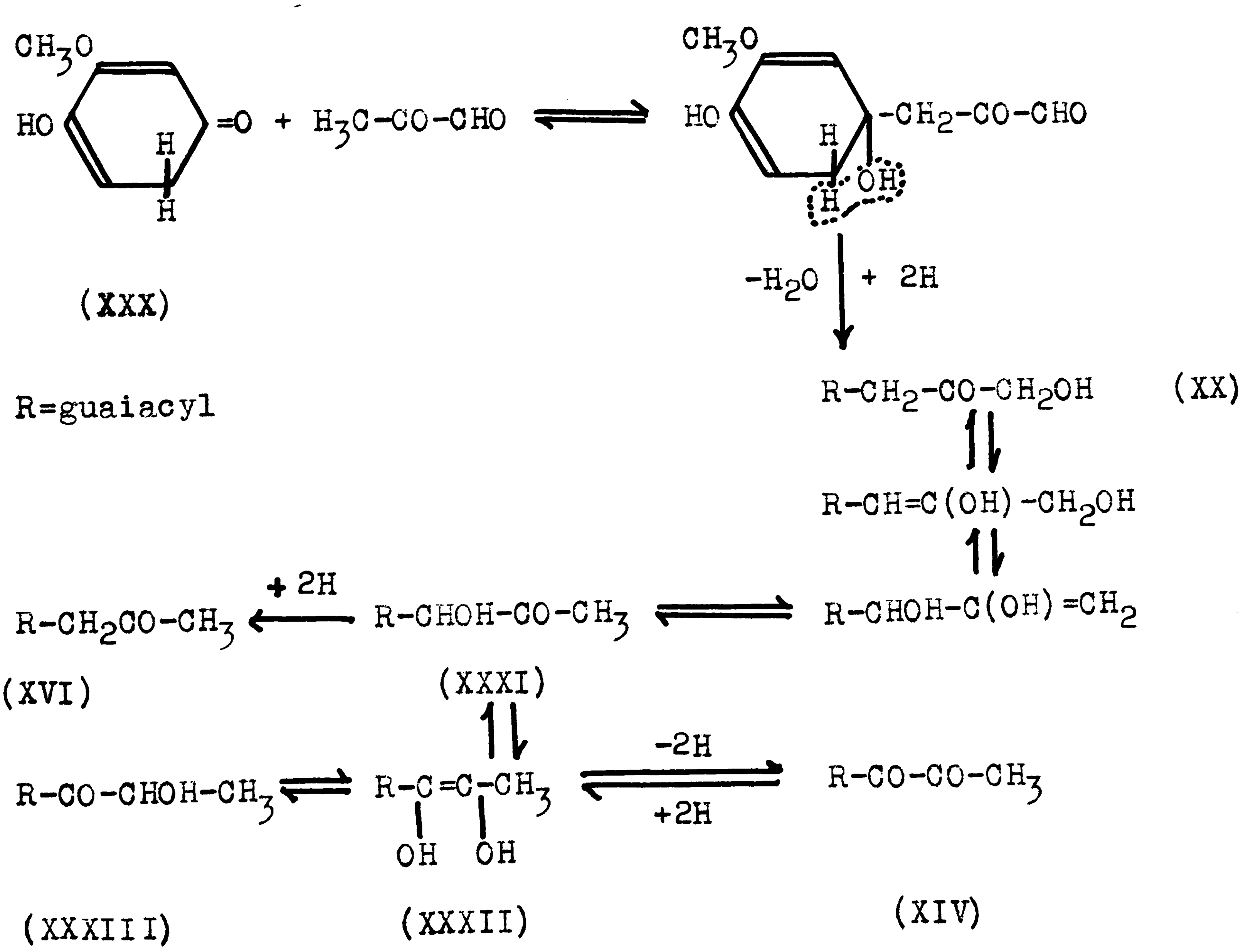
(XXII) Inositol (XXIV)

(ii) Methyl Glyoxal Theory of Phenol Formation (91) -- Methyl Glyoxal, which is a well recognized intermediate in animal cell respiratory processes (92, page 244), presumably occupies a somewhat analogous position in plant carbohydrate metabolism (93, page 264) and has actually been isolated from a number of higher plants (94). Hibbert suggests the possibility of two molecules of methyl glyoxal polymerizing to yield a cyclic dihydroxydiketone (XXV) capable of undergoing the indicated reactions to give quinone (XXIX) and 1,2-dihydroxy-4-ketocyclohexadiene (XXVI). This ketohexadiene, (XXVI), on reduction could give rise to a hydroxy-enediol, (XXVII) which could yield catechol (XXVIII) upon loss of a mole of water.



Pyrogallol (the precursor of the syringyl nucleus) could be formed by enzymatic oxidation of catechol (XXVIII) just as the latter is formed from phenol (95).

(b) Suggested Plant Synthesis of Propylphenol Derivatives -- Condensation of the "methoxylated" ketocyclohexadiene, (XXX), with a third mole of methyl glyoxal, followed by the loss of a mole of water and reduction, would yield (XX). An intramolecular change involving an allyl shift in (XX) would give the primary dismutation isomer, (XXXI), (isolated as the ethyl ether) which could then yield the enediol (XXXII), this in turn giving the benzoin derivative (XXXIII). The benzoin derivative (XXXI) on reduction could yield the desoxybenzoin (XVI). Moreover the enediol, (XXXII), is a dihydro derivative of the 1,2 diketone (XIV),



The only reaction of this series which conceivably could be open to question is concerned with the rearrangement of (XX) to (XXXI). Apparently there are no analogous reactions described in the literature, although, if it is assumed that (XX) is in equilibrium with its aldehydo form ($R-CH_2-CO-CH_2-OH \rightleftharpoons R-CH -CHOH-CHO$), the reaction undergone by its analogue benzyl glycollic aldehyde (XXXIV), in the presence of ethanol and sulfuric acid, is highly significant (96), in view of its conversion, by this means, into a mixture of phenyl acetyl carbinol and benzoyl acetone.

$$2C_6H_5-CH_2-CHOH-CHO \longrightarrow C_6H_5-CHOH-CO-CH_3 . + C_6H_5-CO-CO-CH_3$$

(XXXIV)

There is ample support in the literature for the postulated equilibrium (XXXI) $\rightleftharpoons$ (XXXIII). The simplest, and best known examples of such dismutation reactions, in the case of 1,2-hydroxyketones and aldehydes, are to be found in the field of carbohydrate chemistry, as, for example, the well known Lobry de Bruyn equilibrium transformation of glucose $\rightleftharpoons$ mannose $\rightleftharpoons$ fructose, and the glyceric aldehyde $\rightleftharpoons$ dihydroxyacetone, etc. (97). It has been pointed out (69, page 52) that in the equilibrium system $R-CHOH-CO-CH_3 \rightleftharpoons R-C(OH)=C(OH)-CH_3 \rightleftharpoons R-CO-CHOH-CH_3$ where R is a para substituted benzene radical, $R-CO-CHOH-CH_3$ is more stable than its dismutation isomer. This is in agreement with the results obtained in the ethanolysis of wood in which the products isolated are the ethyl ethers of the stabilized form $R-CO-CHOH-CH_3$, namely

2-ethoxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (XX) and its sryingyl analogue (XIII). In the light of the above literature reference (96), it is probable that these substances (XII) and (XIII) represent only stabilized end-products originating from the assumed lignin progenitors, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-propanone (XX) and its syringyl analogue respectively; (see also page 40) in other words, the first members of the proposed new aromatic system of plant respiratory catalysts (4).

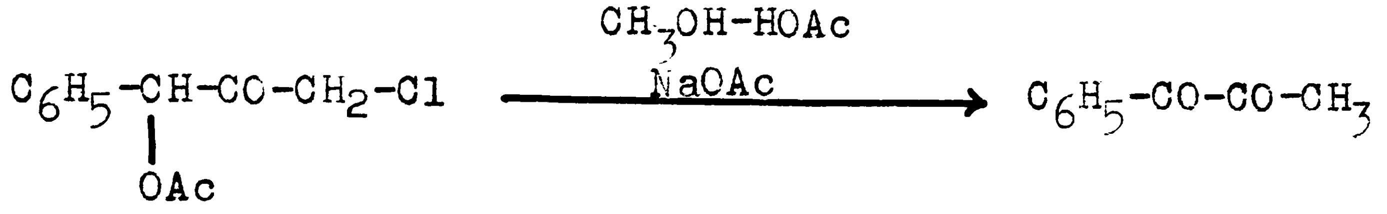
It is therefore of great importance to consider to what extent members of the second type of ethanolysis products isolated, namely, 1-(4-hydroxy-3-methoxyphenyl)-1,2-propanedione (XIV) and its syringyl analogue (XV), represent also stabilized end ethanolysis products derived from more reactive lignin progenitors. A consideration of a series of products, namely coniferyl alcohol (XXXV) ($R-CH=CH-CH_2OH$), oxyconiferyl alcohol (XXXVI) ($R-CH_2-CO-CH_2OH \rightleftharpoons R-CH=C(OH)-CH_2OH$), and dioxyconiferyl alcohol (XXXVII) ($R-CO-CHOH-CH_2CH \rightleftharpoons R-C(OH)=C(OH)-CH_2OH$), points to the possibility of the existence of a similar dismutation equilibrium involving the dioxy-ketone (XXXVII) as one constituent:

$$R-CO-CHOH-CH_2OH \rightleftharpoons R-C(CH)=C(OH)-CH_2OH \rightleftharpoons R-CHCH-CO-CH_2OH$$

Examination of enediol (XXXVII), shows it differs only from that present in the dismutation system $R-CO-CHOH-CH_3 \rightleftharpoons R-C(OH)=C(OH)-CH_3 \rightleftharpoons R-CHOH-CO-CH_3$ in that the methyl group is replaced by a terminal $-CH_2CH$, and by analogy this dioxy-coniferyl alcohol type of compound might be expected to yield 1-(4-hydroxy-3-methoxyphenyl)-1,2-propanedione in the presence of dilute acids:

$$R\text{-}C(OH)=C(OH)\text{-}CH_2OH \longrightarrow R\text{-}C(OH)_2\text{-}C(OH)=CH_2 \longrightarrow R\text{-}CO\text{-}CO\text{-}CH_3$$

Substantial experimental support for this theory is to be found in the behaviour of analogously constituted chain and cyclic derivatives. Thus Bradley and Eaton (98) observed that chloromethyl α-acetoxybenzyl ketone (XXXVIII), when heated with a solution of sodium acetate in glacial acetic acid and methanol, was converted in almost quantitative yield into benzoyl methyl ketone (XXXIX). The chlorketone (XXXVIII) when left standing at room temperature for one year decomposed spontaneously into (XXXIX):

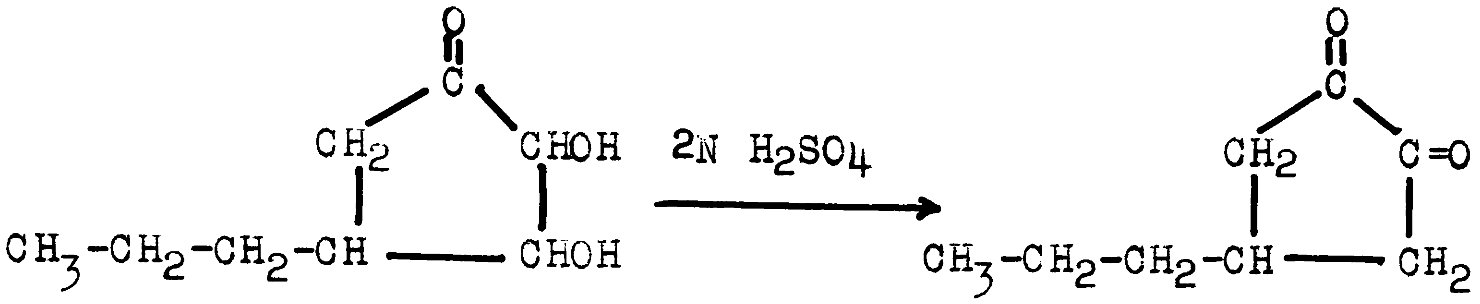


$$C_6H_5\text{-}\underset{\displaystyle OAc}{CH}\text{-}CO\text{-}CH_2\text{-}Cl \xrightarrow[NaOAc]{CH_3OH\text{-}HOAc} C_6H_5\text{-}CO\text{-}CO\text{-}CH_3$$

(XXXVIII) (XXXIX)

It seems justifiable to conclude that during this period, (XXXVIII) underwent slow hydrolysis to $C_6H_5\text{-}CHOH\text{-}CO\text{-}CH_2OH$, followed by a molecular rearrangement to the diketone as indicated above.

Further evidence for these speculations is to be found in the action of dilute sulfuric acid on the hydrogenated metabolic product terrein isolated by Raistrick and co-workers (99) by the action of several strains of Aspergillus terreus Thom on glucose as the sole organic substrate. It was found that terrein could be hydrogenated readily to yield tetrahydroterrein (XL), and this in the presence of acid underwent rearrangement to a cyclic diketone (XLI). It is evident that the molecule



(XL) (XLI)

of tetrahydroterrein contains a grouping analogous to that in dioxyconiferyl alcohol (XXXVII) so that the diketone formation is not surprising.

It therefore seems highly probable that the 1,2-diketones isolated in the ethanolysis of wood represent stabilized end products derived from one or other of the dioxyconiferyl alcohol members of that dismutation system.

Perhaps the most interesting work in this connection is that of Kohler (100) on the action of alcoholic potassium hydroxide on a variety of α-bromo ketones, essentially of the aryl-alkyl type R-CO-CHBr-CHRR' (where R is generally aromatic). Three products are formed, namely, (1) R-CO-CHOHCHRR', (2) R-CO-CO-CHRR' and (3) R-CO-CH_2-CHRR'. Thus 2-bromo-1-(4-bromphenyl)-1-propanone (XLII) and alkali yields(XLIII), (XLIV) and (XLV) in yields of 11, 20 and 20 per cent respectively.

R-CO-CHBr-CH_3
(XLII)

R-CO-CHOH-CH_3
(XLIII)

R-CO-CO-CH_3
(XLIV)

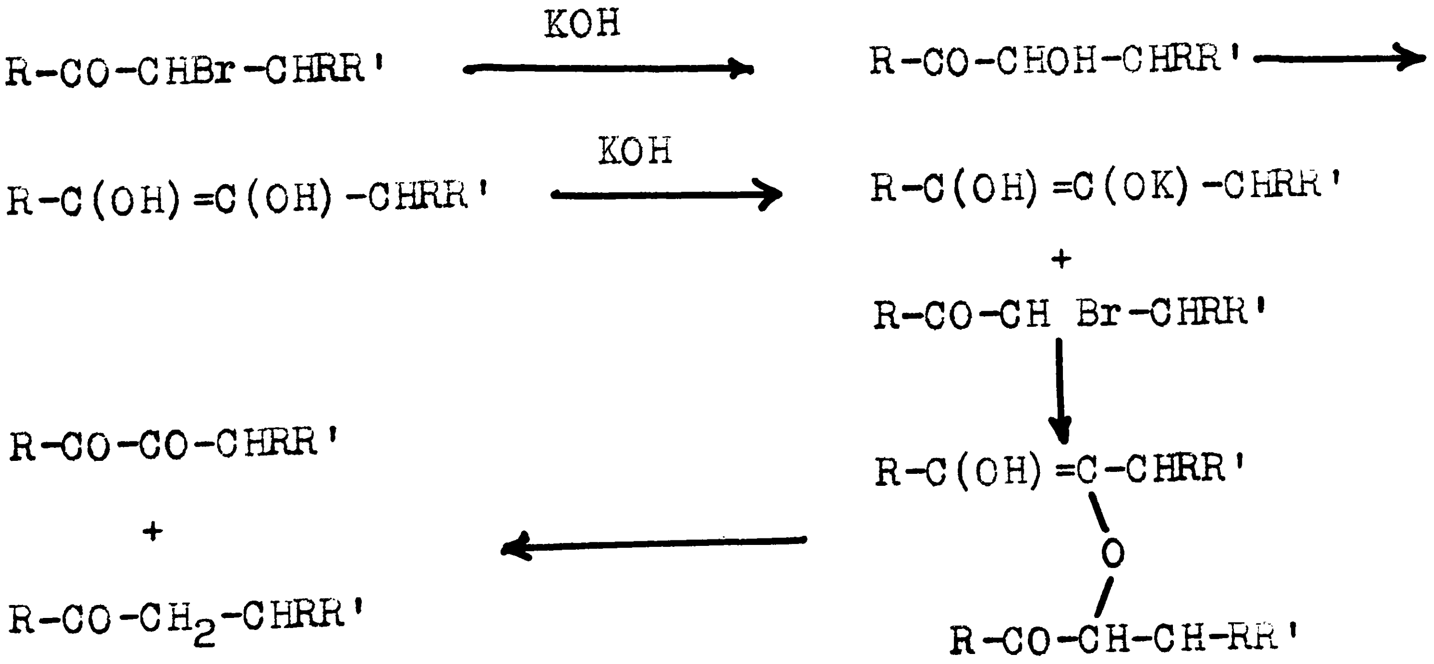
R-CO-CH_2-CH_3
(XLV)

R is 4-bromphenyl-.

This change only occurs when the strongly alkaline alcoholic solution is added slowly to the bromketone at room temperature. If added rapidly to the boiling solution a high yield of the benzoin (XLIII) is obtained, the bromine undergoing instant replacement. The diketone (XLIV) is formed in quantity only when the solution is alkaline.

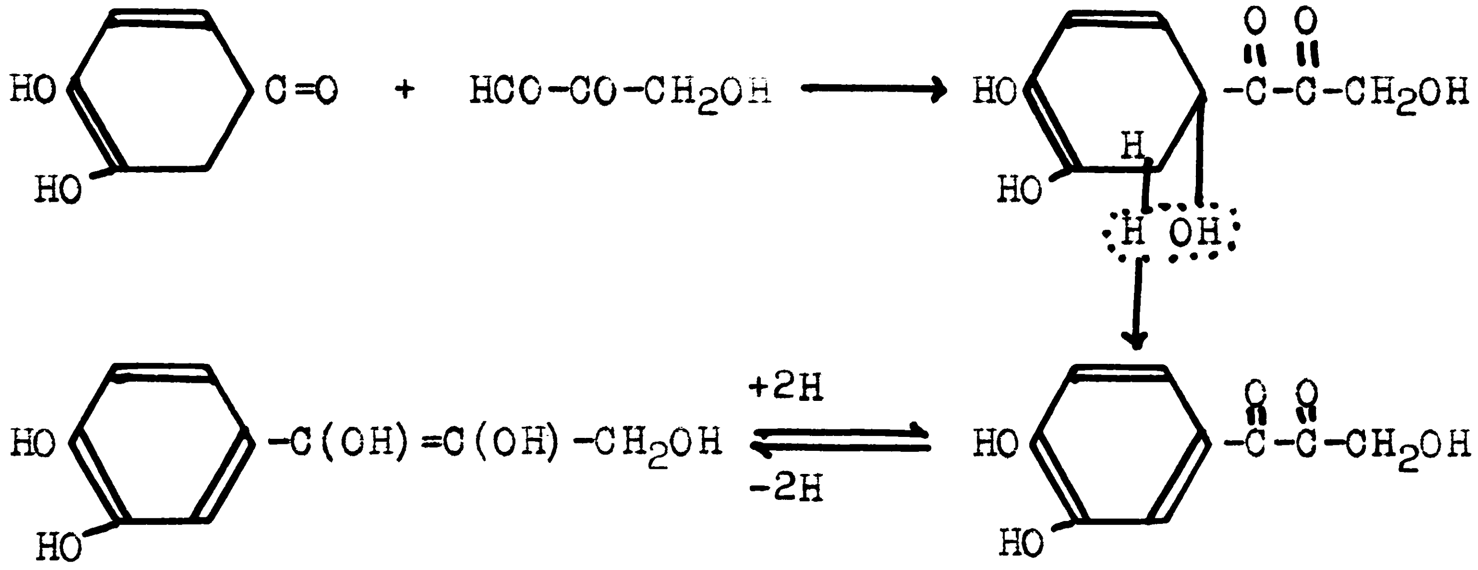
It is of interest that the benzoins R-CO-CHOH-CHRR' under the same conditions of alkaline treatment as employed for the treatment of the bromo ketone do not yield the diketones so that presumably they do not represent the intermediates, or at least the sole intermediates, in these transformations.

It is possible that reactions of the type shown below may occur:

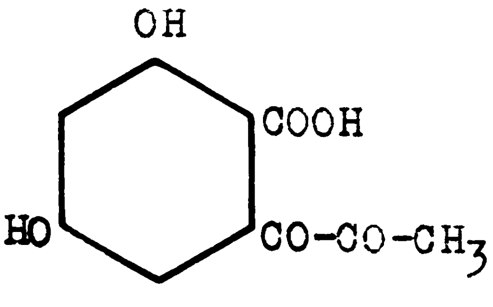


A similar type of combined intermolecular oxidation-reduction mechanism may be involved in the action of dilute acids on 1-hydroxy-1(4-hydroxy-3-methoxyphenyl)-2-propanone (XXXI) whereby the diketone (XIV) and the desoxybenzoin (XVI) are formed.

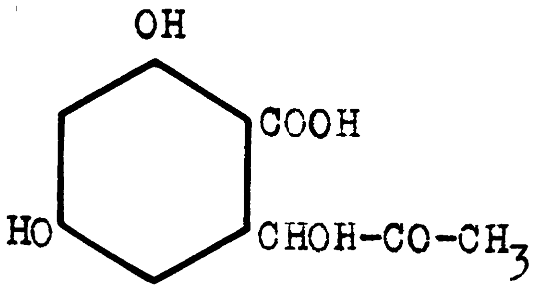
The plant origin of these dioxyconiferyl alcohol derivatives is, as yet, unknown, but it is interesting to note that their plant synthesis can be visualized as consisting of a primary condensation of two moles of methyl glyoxal, with the loss of a mole of water, followed by further condensation with a third molecule, in this case hydroxymethyl glyoxal (instead of methyl glyoxal as in the case of the end methyl group derivatives) (page 22).



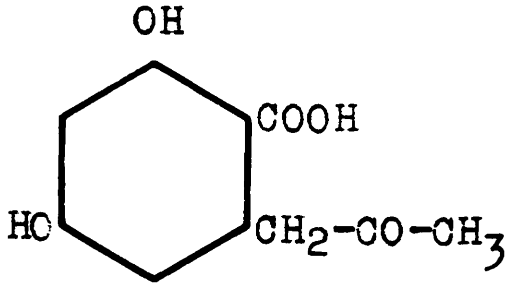
Experimental support for the view that the ethanolysis derivatives, obtained by Hibbert and co-workers (4) are true synthetic plant products is provided in the work of Oxford and Raistrick (101) on the action of Penicillium brevi-compactum and related spedies on 5% glucose solution, this being the sole organic substrate in acid medium (pH 4-5), whereby the interesting series of phenolic acids 3,5-dihydroxy-2-carboxybenzoylmethylketone (XLVI), 3,5-dihydroxy-2-carboxyphenylacetylcarbinol (XLVII) and 3,5-dihydroxy-2-carboxybenzylmethylketone (XLVIII) is formed.



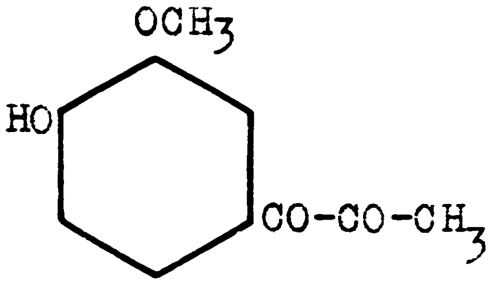
(XLVI)



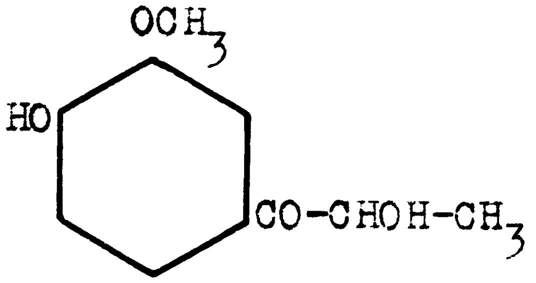
(XLVII)



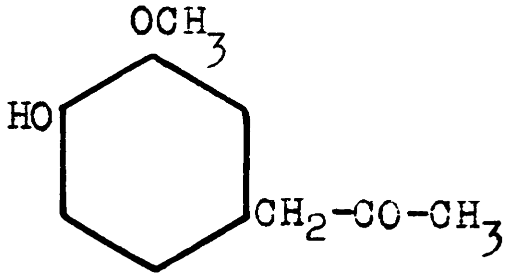
(XLVIII)



(XIV)

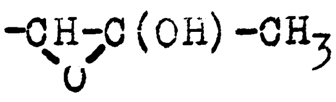
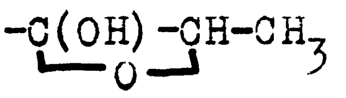


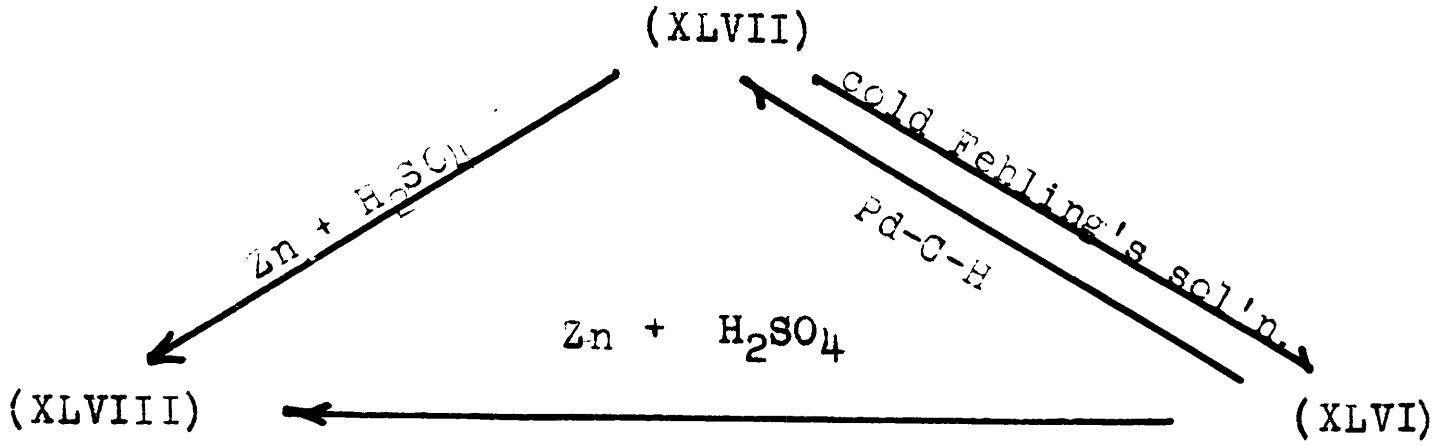
(XXXIII)



(XVI)

The products of mold metabolism, in this case, had not been subjected to the action of chemical reagents and were isolated by simple evaporation, under reduced pressure, of their aqueous solutions.

Oxford and Raistrick point out that the structure of the side chain of (XLVII) is uncertain and that it may be either one or other of the following: $-CO-CHOH-CH_3$, $-C(OH)=C(OH)-CH_3$,  $-\underbrace{CH-C}_{O}(OH)-CH_3$ or  $-\underbrace{C(OH)-CH}_{O}-CH_3$ or an equilibrium mixture of several of the possible isomerides. They found that (XLIV) was optically inactive and explained this as due to the presence of an equilibrium with its enediol form. The three compounds were found to be interconvertible, i.e.:



In most cases the principle constituent of the metabolic mixture was the diketone (XLVI). The yield of (XLVIII) was generally quite small -- about 3% of the mixture. In the case of a few species, the benzoin (XLVII) was apparently the chief constituent.

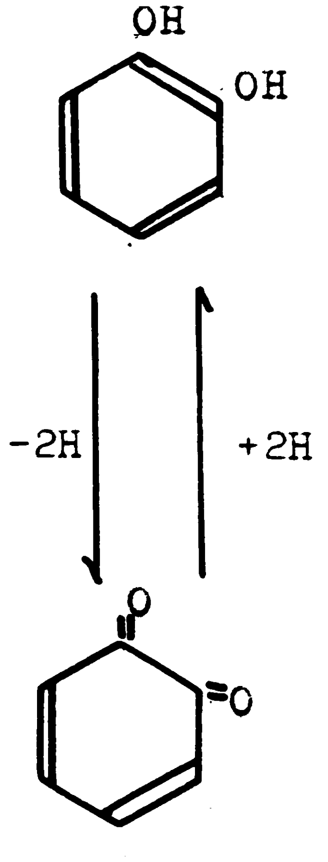
These three metabolic products (XLVI), (XLVII) and (XLVIII) show a remarkable parallelism both in regard to side chain structure and interconvertibility with certain of the aromatic derivatives isolated from the ethanolysis products of wood, namely 1-(4-hydroxy-3-methoxyphenyl)-1,2-propanedione (XIV), 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (XXXIII) and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone (XVI).

The introduction of the carboxyl group (XLVI), (XLVII) and (XLVIII), is not remarkable in the light of the ease with which carboxylation of phenols (resorcinol (102), and phloroglucinol (103)) occurs on heating with aqueous bicarbonate at quite low temperatures (50-100°).

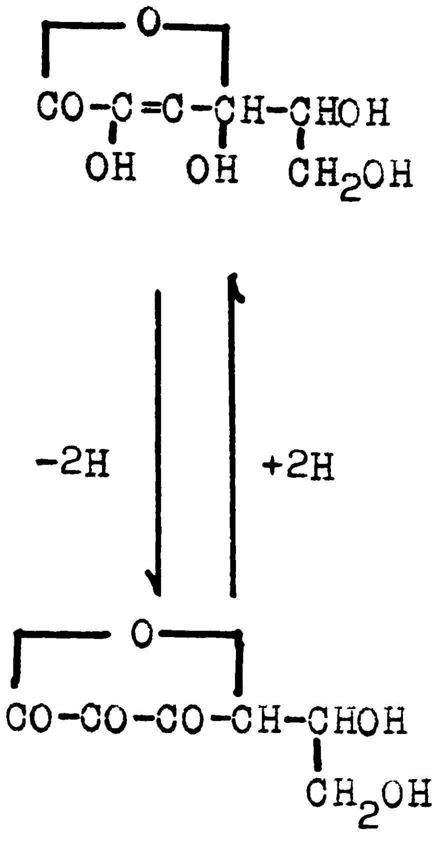
6. Possible Significance of Lignin Progenitors in Plant Oxidase Systems

The mechanism of animal respiration has engaged the attention of a large number of workers in biochemistry for some time. At present most workers in this field (92) believe that the energy necessary for the animal cell is liberated by the transformation of carbohydrates into carbon dioxide and water. This transformation consists of decarboxylation and dehydrogenation reactions. The greater part of the energy is derived from the reaction of hydrogen with oxygen. In the cell, carbohydrates undergo degradation and from the simpler fragments hydrogen is split off under the influence of enzymes known as dehydrogenases. This hydrogen is transported by what is know as a "carrier" or "hydrogen transporter". Thus hydrogen is transported from one substance to another, this resulting in a chain of reactions which serve to transport hydrogen from negative to less negative oxidation-reduction systems until finally the stage of union with oxygen is reached. Thus during the course of a series of dehydrogenation reactions energy is liberated stepwise, in small amounts, and this provides the required energy for the living organism.

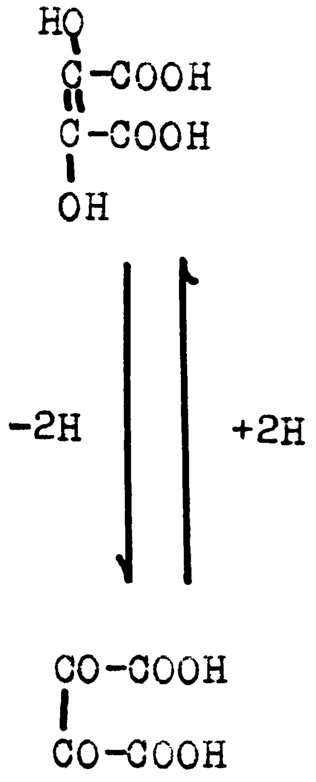
Szent-Györgyi has demonstrated the presence of a number of dehydrogenase-catalyst systems in various forms of plants; such as those of catechol (XLIX) (104), ascorbic acid (L) (105), and dioxymaleic acid (LI) (106).



(XLIX)



(L)



(LI)

Each of these can function as an oxidation-reduction system, the oxidized molecule being a 1,2-diketone and the reduced molecular an enediol.

Hibbert (4) has pointed out that the principal water-soluble ethanolysis products from spruce and maple represent enediol-1,2-diketone oxidation-reduction systems (XXXIII) and (XIV). These presumably represent as discussed above (page 24) stabilized end products, their forerunners, being the lignin progenitors present in the plant cell as a series of monomolecular, hydrogen transporting catalysts, which later undergo conversion into more complex condensation polymeric products.

The presence of coniferin in the cambial sap of practically all plants, and other considerations have led Hibbert (4) to propose a plant respiratory catalyst system similar to the C_4-dicarboxylic acid system of Szent-Györgyi (107):

Animal System
(Szent-Gyorgyi)

$$\begin{tikzcd}
\text{A} & \mathrm{HOOC{-}CH_2{-}CO{-}COOH} \\
& \text{(Oxalacetic acid)} \arrow[d, shift right=2, "+2H"'] \\
\text{B} & \mathrm{HOOC{-}CH_2{-}CHOH{-}COOH} \arrow[u, shift right=2, "-2H"'] \\
& \text{(malic acid)} \arrow[d, "-H_2O"] \\
\text{C} & \mathrm{HOOC{-}CH{=}CH{-}COOH} \\
& \text{(fumaric acid)} \arrow[d, shift right=2, "+2H"'] \\
\text{D} & \mathrm{HOOC{-}CH_2{-}CH_2{-}COOH} \arrow[u, shift right=2, "-2H"'] \\
& \text{(succinic acid)}
\end{tikzcd}$$

Plant System
(Hibbert)

$$\begin{tikzcd}
\text{A}' & \mathrm{R{-}CH_2CO{-}CH_2OH} \arrow[d, shift right=2, "+2H"'] \\
\text{B}' & \mathrm{R{-}CH_2{-}CHOH{-}CH_2OH} \arrow[u, shift right=2, "-2H"'] \arrow[d, "-H_2O"] \\
\text{C}' & \mathrm{R{-}CH{=}CH{-}CH_2OH} \\
& \text{(coniferyl alcohol)} \arrow[d, shift right=2, "+2H"'] \\
\text{D}' & \mathrm{R{-}CH_2{-}CH_2{-}CH_2OH} \arrow[u, shift right=2, "-2H"']
\end{tikzcd}$$

R- = syringyl or guaiacyl radical.

The first member (A') of the plant system, corresponding to oxalacetic acid (A), is the keto-alcohol believed to be obtained from the condensation of three moles of methyl glyoxal (page 22). It is seen that the third member of the new system is coniferyl alcohol corresponding to fumaric acid in the C_4 system. The isomeric form of (A'), $R\text{-}CO\text{-}CH_2\text{-}CH_2OH$, may also function similarly to (A'), in the new system, while (B') and its isomeric form would provide analogs of the citric and isocitric acids which are components of the Krebs animal cell oxidation system (108). The passage from Hibbert's new system to the enediol-1,2-diketone system can take place by allylic rearrangements of the oxyconiferyl alcohol or oxysyringyl alcohol isomer, as represented on (page 22).

Independent evidence in support of Hibbert's view of lignin as derived from a series of plant respiratory catalysts is to be found in certain of the experiments carried out by Raistrick and Oxford (101). Recent investigations in these laboratories (109) have proven that products containing the side chain $-CH_2-CO-CH_2OH$ attached to an aromatic ring are extraordinarily reactive substances which readily undergo, in the presence of mild reagents, a primary allyl shift followed by equilibrium dismutation changes and oxidation-reduction reactions as outlined below:

$$R\text{-}CH_2\text{-}CO\text{-}CH_2OH \rightleftharpoons R\text{-}CH{=}C(OH)\text{-}CH_2OH \rightleftharpoons R\text{-}CHOH\text{-}CO\text{-}CH_3$$

$$\begin{tikzcd} {} \arrow[r, shift left] & R\text{-}C(OH){=}C(OH)\text{-}CH_3 \arrow[l, shift left] \arrow[r, shift left] \arrow[d, shift right, "-2H"'] & R\text{-}CO\text{-}CHOH\text{-}CH_3 \arrow[l, shift left] \\ & R\text{-}CO\text{-}CO\text{-}CH_3 \arrow[u, shift right, "+2H"'] & \end{tikzcd}$$

R = phenolic nucleus.

It is highly probable that Raistrick's phenol carboxylic acids may actually function similarly as respiratory catalysts and presumably represent, at least in part, stabilized end-products originating from the more active forms. By operating exactly at neutral the point throughout, instead of under the slightly acidic conditions used by Raistrick, it might be possible to isolate, in addition to the products found by him, the more reactive analogues of the Hibbert respiratory system.

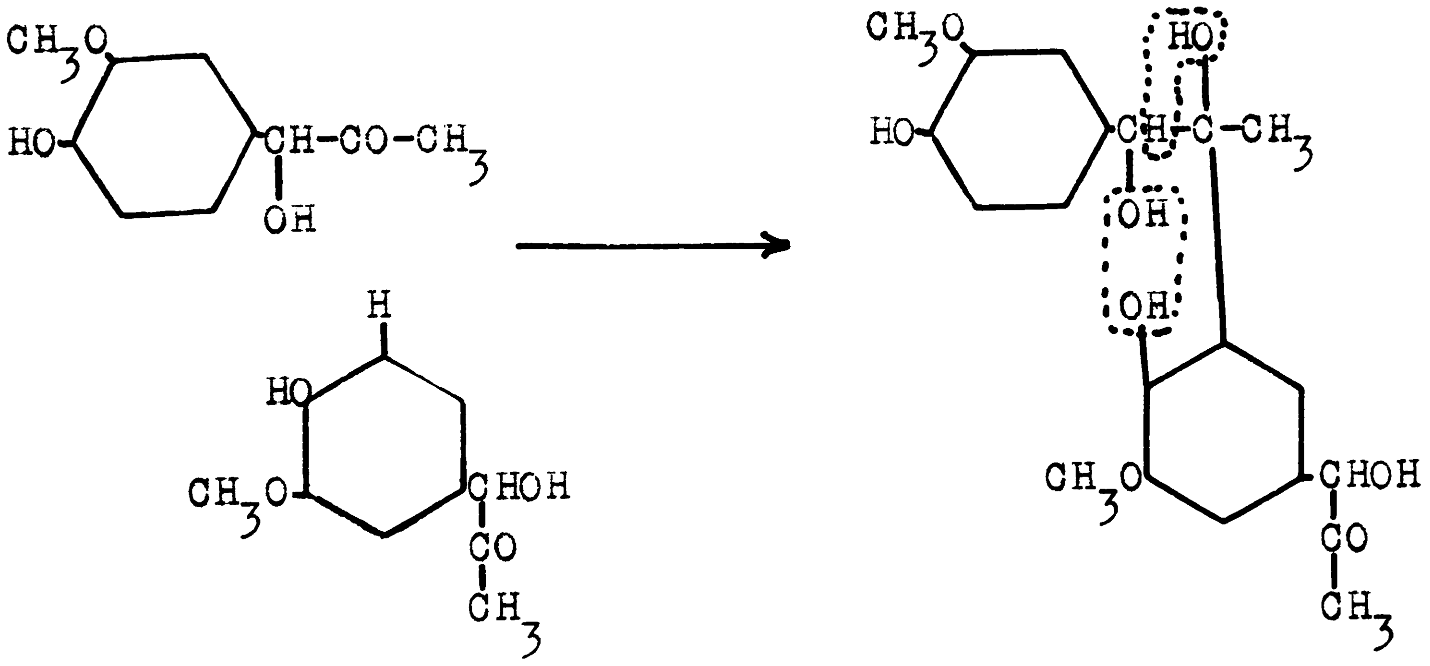
Of particular interest is the observation of Oxford and Raistrick (110) that, when the mold was deprived of substrate, the amount of the diketone (XLVI) increased, such increase, however, being accompanied by a marked simultaneous decrease in the quantity of (XLVII). Such changes are presumably due to an increased amount of oxidation of the enediol, resulting in increased water formation, which, in turn, provides the additional energy required.

7. Formation of Protolignin from Propylphenol Units

(a) Polymerization Mechanisms of 4-hydroxy-3-methoxyphenyl Units

Prominent workers in the field of lignin chemistry believe that lignin is derived from propylphenol units of the type isolated from wood by Hibbert and co-workers, or from closely related compounds, and Freudenberg, Erdtman, Holmberg and Hibbert have made significant contributions to the elucidation of the mechanism of lignin formation. Freudenberg (2) considers the union of the monomers to be of a carbon-carbon type involving oxygen-ring formation between side chains and aromatic nuclei (11) (page 3).

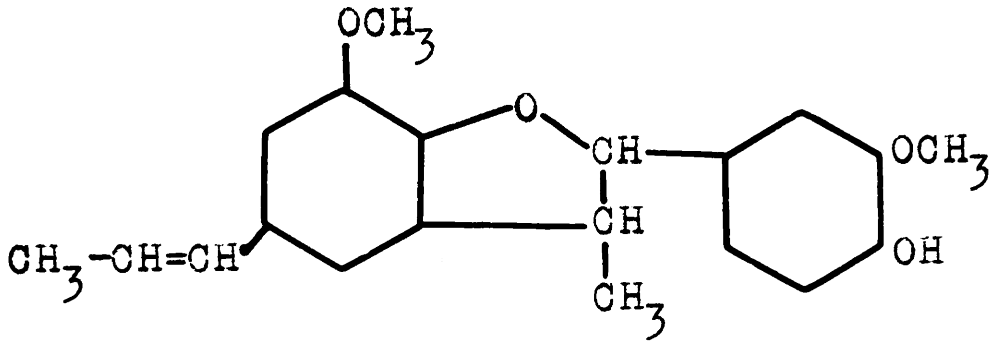
Hibbert (4) points out that the "lignin building units" containing a phenol group together with a carbonyl or an unsaturated linkage in the side chain would have a strong tendency to undergo para- and ortho-nuclear condensations both individually and collectively, under mild conditions, with formation of condensation polymers. This phenol-carbonyl type of reaction is well known, and was first suggested by Freudenberg solely on theoretical grounds, inasmuch as he has never succeeded in isolating any R-C-C-C or even R-C-C units (R = 4-hydroxy-3-methoxyphenyl):



(LII)

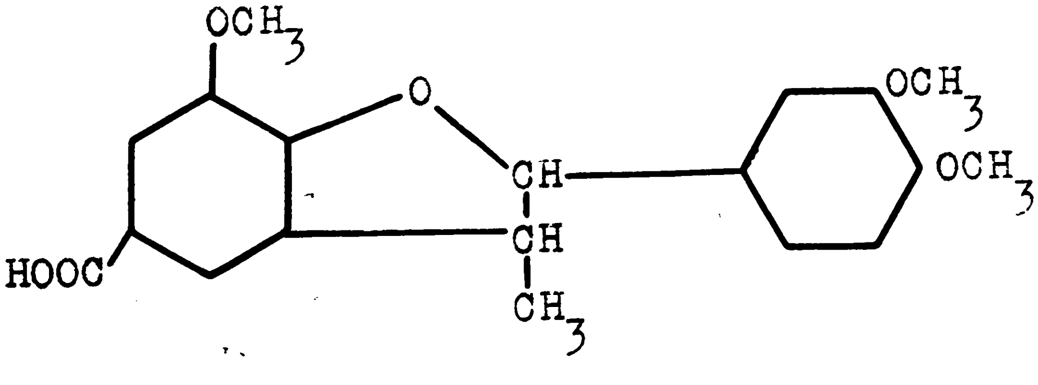
The condensation of the ortho-hydrogen atom of one unit with the carbonyl of another, followed by elimination of two molecules of water, as indicated, would result in formation of a benzofurane derivative. Condensation with a third molecule would yield a trimer and so on. It can be seen that this mechanism differs from Freudenberg's only in the order in which the first two steps are assumed to occur.

Under the action of bacterial enzymes or ferric chloride (111) isoeugenol forms a dehydrogenated dimer, dehydrodiisoeugenol shown by Erdtman (3) to have the formula (III):



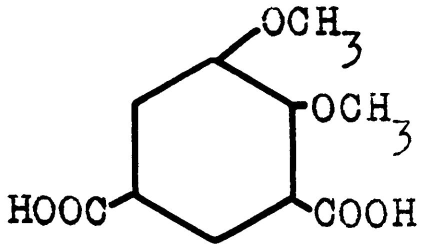
(III)

By the oxidation of the methyl ether of this dimer with cold potassium permanganate, Erdtman prepared the acid (LIII),which is regarded by Freudenberg as a model substance for the lignin polymer:

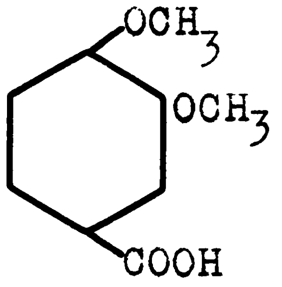


(LIII)

Freudenberg found that (LIII), after degradation with alkali, methylation, and permanganate oxidation, yielded isohemipinic acid (LIV) and veratric acid (LV) in yields of 5 and 21 per cent respectively:



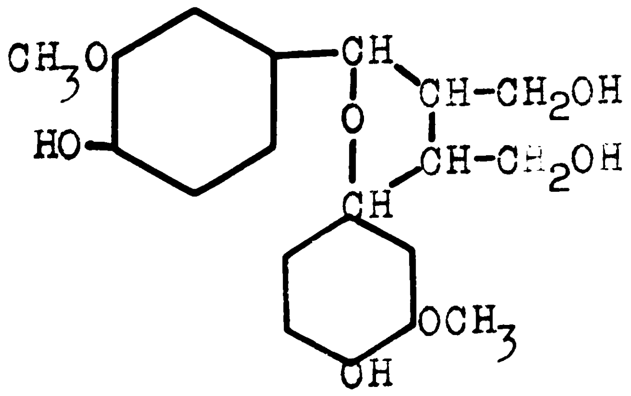
(LIV)

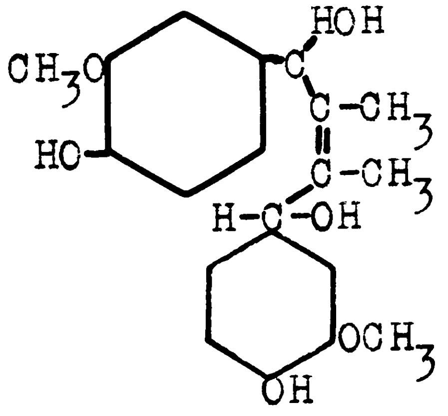


(LV)

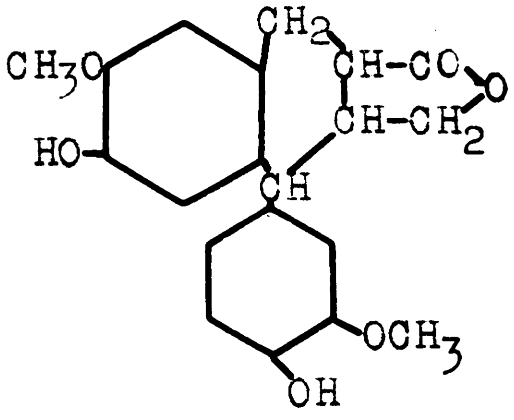
By the same treatment of spruce lignin Freudenberg obtained 2 per cent of isohemipinic acid and 12 per cent veratric acid, as well as traces of trimethyl gallic acid, and concluded (112) that some of the lignin molecules are of a type capable of yielding isohemipinic acid when submitted to alkali degradation, followed by methylation and oxidation. It is apparent that this observation supports the theory of Erdtman (3) and of Hibbert that protolignin is made up of dimers of the dehydrodiisoeugenol type (III), (see page 8).

Erdtman in his recent review (3) points out that the structure of a wide variety of plant resins, e.g. olivil (LVI), lariciresinol (LVII), conidendrin (LVIII) and others conform to a general dimer type, the monomer being a propylphenol derivative closely related to coniferyl alcohol and that the dimer formation apparently always involves the β-carbon atom of the side chain:





(LVII)



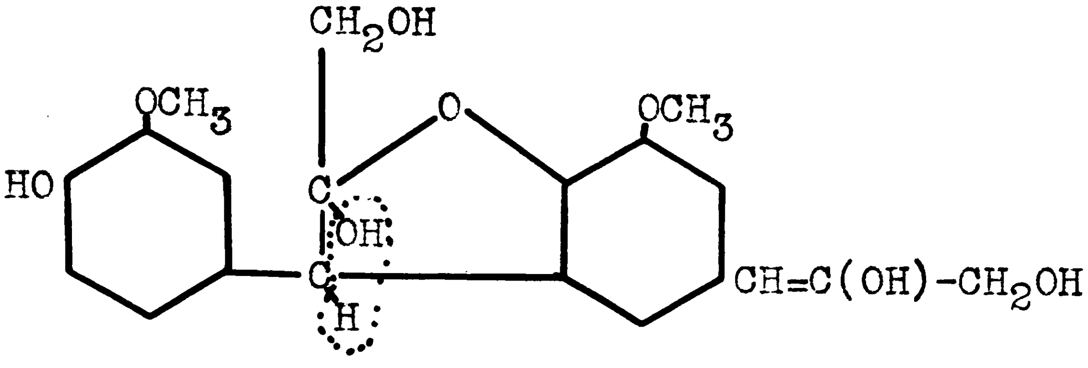
(LVIII)

The similarity between these simple natural products and dehydrodiisoeugenol, and the analogies between lignin and dehydrodiisoeugenol, have led Erdtman to the belief that the phenomenon of dehydrogenative coupling of molecules probably plays a great role in the biogenesis of plant products.

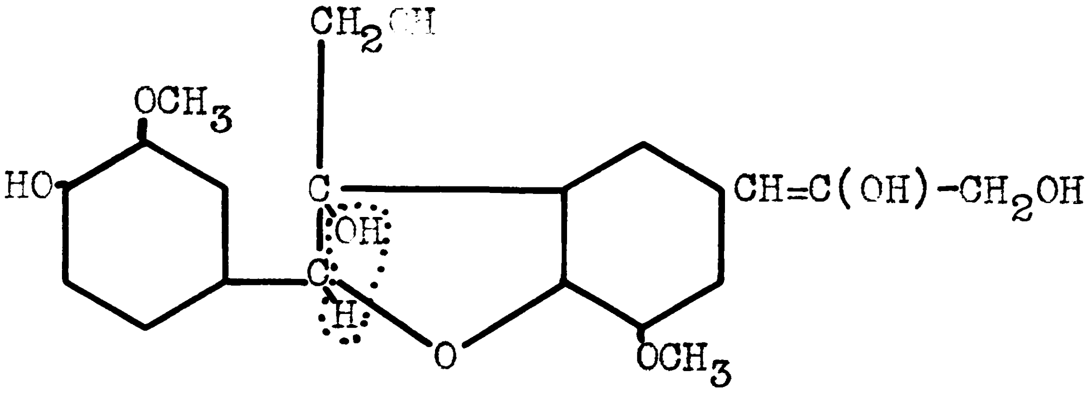
He states that the ethanolysis products 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (XXXIII) and 1-(4-hydroxy-3-methoxyphenyl)-1,2-propanedione (XIV) and their syringyl analogues are not necessarily true lignin building units, since they may represent only stabilized end products originating from dimeric types similar to dehydrodiisoeugenol. That such may well be the case has been recognized by Hibbert (4b)(see also page 24).

There is a close relationship between the enolic forms of the side chains of certain propylphenol derivatives $R-C(OH)=C(OH)-CH_3$ (XXXII); $R-CH=C(OH)-CH_3$ (XVI); $R-CH=C(OH)-CH_2OH$ (XX); and side chains of isoeugenol $R-CH=CH-CH_3$ and coniferyl alcohol $R-CH=CH-CHOH$. This fact strongly suggests that protolignin may be present in the plant as a series of polymers of the dehydrodiisoeugenol type (III) derived from monomers of the oxyconiferyl alcohol type (XXXVII). These reactive dimers during the course of ethanolysis could partially polymerize and partially undergo transformation to the stabilized ethanolysis units.

Thus application of the dehydrodiisoeugenol type of polymerization to the enolic forms of certain propylphenol units, for example oxyconiferyl alcohol (XXXVII), as pointed out by Hibbert (4b), could give rise to two general types of lignin polymers (LIX) and (LX):

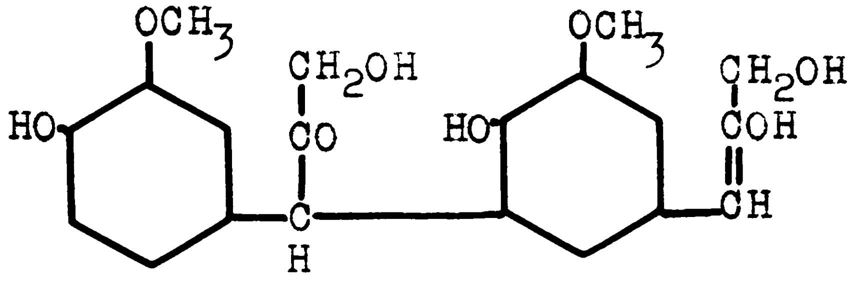


(LIX)

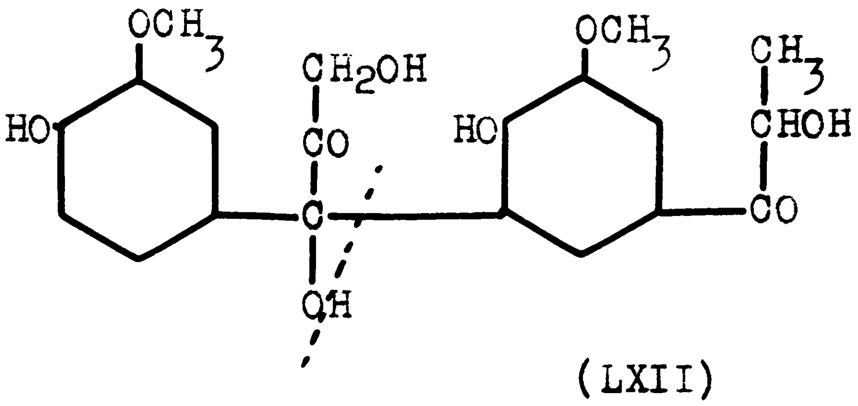


(LX)

Each of these could condense further to give a trimer, a tetramer, etc., while the elimination of a mole of water from each dimer would yield benzofurane derivatives. Ring opening of (LIX) could give rise to the (LXI) form, from which by a double allylic shift, and by cleavage, one mole of each of the diketone and of the ketol (XXXIII) could be obtained. Such a cleavage could only be possible with a reversible type of polymer and not with the benzofurane irreversible type. The same dehydrogenative-coupling type of polymerization and depolymerization can be applied also to two moles of the enediol of 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (XXXII), or to two moles of the enol of 1-(4-hydroxy-3-methoxyphenyl)-2-propanone (XVI).



(LXI)



(LXII)

(b) Polymerization Mechanism of Syringyl Units

It is apparent that the syringyl analogues, due to the blocking of the ortho positions of the phenol could not undergo the same dehydrogenative-coupling. Hibbert (4) points out that since the "lignin building units" (XXXIII), (XVI) and (XX) in their enediol forms are derivatives of styrene, they could possibly undergo the styrene type polymerization, although this is regarded as much less probable.

Inasmuch as coniferyl, oxyconiferyl, syringyl and oxysyringyl alcohols may be regarded as substituted cinnamyl alcohols, and in view of the ease with which cinnamyl derivatives, in the presence of dilute acids, not only undergo the allyl shift but also form dicinnamyl ethers (113) in high yields, it is possible that the syringyl units may exist in the woody tissue as ethers, thus accounting for their much readier extraction by ethanolysis and other methods.

In any event the type of polymerization of gymnosperms appears to be much more complex than in the case of the angiosperms, the reversible type being present in the latter to a much greater extent (4). Recent work (114) has shown that both the ether-soluble and -insoluble ethanol maple lignins undergo depolymerization on further treatment with ethanol-hydrochloric acid to give the 1,2 diketone (XIV) and the ketoalcohol (XXXIII) a result in harmony with the above theoretical speculations (page 41).

The reactions of lignin with mercaptans, particularly with thioglycollic acid, have been investigated over a period of years by Holmberg (115) and are of particular importance in any appraisal of the various suggested polymerizations mechanisms. The mercaptans, R-SH, are thioalcohols, and, in the presence of acids, react in the same way (namely through the -SH group) as alcohol with lignin in wood. This is emphasized by the fact that treatment of ethanol lignin with thioglycollic acid results in the replacement of the ethoxyl groups by the $-S-CH_2-COOH$ radical (116). By treating wood with thioglycollic acid in the presence of hydrochloric acid, Holmberg obtained products containing even more thioglycollic acid than the treated ethanol lignin.

Holmberg's experiments dealing with the reactions of model substances (presumably related to lignin) with thioglycollic acid (115) as well as related experiments carried out by Freudenberg (17), by Hägglund (117) and by Richtzenhain (118) were all designed to throw light upon the question of the actual grouping present in proto- and isolated lignins. Thus thioglycollic acid condenses with cinnamyl alcohol (119) with the loss of water to give cinnamyl thioglycollic acid.

$$C_6H_5-CH=CH-CH_2OH \quad + \quad HS-CH_2-COOH \rightarrow C_6H_5-CH=CH-CH_2-S-CH_2-COOH \quad + H_2O$$

A similar reaction occurs with butyl alcohol, α-phenylethyl alcohol, and, more significantly, with benzyl alcohol (120), its derivatives and the ethers of its derivatives, e.g. phenylmethylcarbinol (LXIII), α-ethoxyphenylethane (LXIV) benzohydrol (LXV) and benzyl ethyl ether (LXVI).

$$C_6H_5-CH(CH_3)-OH + HS-CH_2-COOH \rightarrow C_6H_5-CH(CH_3)-S-CH_2-COOH \quad + H_2O$$

(LXIII)

$$C_6H_5-CH(CH_3)-OEt + HS-CH_2-COOH \rightarrow C_6H_5-CH(CH_3)-S-CH_2-COOH + EtOH$$

(LXIV)

$(C_6H_5)_2-CHOH$
(LXV)

$C_6H_5-CH_2-OEt$
(LXVI)

(LXV) and (LXVI) react in the same way as (LXIV) and (LXV). Thioglycollic acid does not condense with such alcohols as phenylpropanol ($C_6H_5-CH_2-CH_2-CH_2OH$), and phenylethanol ($C_6H_5-CH_2-CH_2OH$) (116).

From the foregoing results, the following conclusion may be drawn: Holmberg's experiments on the reaction of thioglycollic acid with model substances have shown that this reagent condenses with derivatives of benzyl alcohol and only with a limited number of other types of alcohols. The fact that lignin condenses very readily with thioglycollic acid suggests that the former is a derivative of benzyl alcohol. Examination of the various structures proposed for lignin (II), (LX) and (LXII) shows that they are such derivatives, hence Holmberg's work lends support to the proposed theories of lignin structure.

The Oxidation of Organic Compounds with Nitrobenzene

There are but few references in the chemical literature to the use of nitrobenzene as an oxidizing agent. Use has been made of this reagent in the manufacture of fuchsin (121) and in the Skraup synthesis. Anthranilic acid is formed by intramolecular rearrangement of o-nitrotoluene when heated with alkali (122).

Ciamician and Silber (123) investigated the photochemical action of nitrobenzene on a number of organic compounds. When a mixture of an aliphatic alcohol and nitrobenzene was allowed to stand, exposed to light, for from six months to a year, there was obtained a quinoline derivative which the authors believed was formed by the action of aldehyde, produced by oxidation of the alcohol (but never isolated by the authors), on aniline (arising from the corresponding reduction of the nitrobenzene) (123). Later, however, the same authors (124) showed that there was no reaction between paraldehyde and aniline, when a mixture of the two was allowed to stand for a year exposed to the light. The quinoline was formed as a secondary reaction product as a result of the effect of the acid employed in the characterization of the alcohol-nitrobenzene reaction products. When a mixture of benzaldehyde and nitrobenzene was exposed to light at room temperature, the former was oxidized on standing to benzoic acid (123), while the nitrobenzene was reduced according to the following equation:

$$C_6H_5NO_2 \longrightarrow C_6H_5NO \longrightarrow C_6H_5NHOH \longrightarrow C_6H_5NH_2$$

Interaction with benzoic acid, and rearrangement of the above intermediate nitrobenzene reduction products, resulted in the formation and isolation of the following products: mono- and dibenzoylphenolyhydroxylamine, mono- and dibenzoyl-p-amidophenol, benzanilid, azoxybenzene and o-hydroxyazobenzene. When anisaldehyde, vanillin, piperonal and cinnamaldehyde were subjected to the same treatment as benzaldehyde, only anisaldehyde was oxidized to the corresponding acid, the others yielding an unidentifiable "mass". This resistance of the aldehyde group para to a phenolic hydroxyl group is well known. Thus whereas benzyl alcohol is converted almost quantitatively to benzoic acid when heated with nitrobenzene and alkali (125), vanillyl alcohol yields only vanillin (126). Further evidence of the effect of the para phenolic hydroxyl group upon the products of nitrobenzene oxidation is to found in the work of Ono and Imoto (127). These workers found that, when heated with nitrobenzene and alkali, p-hydroxyacetophenone yielded benzoyl formic acid (LXVII) while the corresponding o-hydroxyacetophenone yielded salicylic acid (LXVIII):

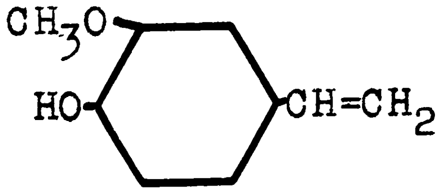
$$p\text{-}HO\text{-}C_6H_4\text{-}CO\text{-}CH_3 \longrightarrow p\text{-}HO\text{-}C_6H_4\text{-}CO\text{-}COOH$$

(LXVII)

$$o\text{-}HO\text{-}C_6H_4\text{-}CO\text{-}CH_3 \longrightarrow o\text{-}HO\text{-}C_6H_4\text{-}COOH$$

(LXVIII)

Nitrobenzene has been employed for the synthesis of vanillin and other para hydroxy aldehydes by the oxidation of the propenyl side chain in isoeugenol and related compounds. Most of the information on this reaction is to be found in the patent literature. The general procedure consists in heating a mixture of isoeugenol with alkali and nitrobenzene for several hours in a high boiling solvent or under pressure at a temperature of around 120°. It is claimed that high yields (80% or better) of vanillin may be obtained (128, 129). When isosafrole is treated in the above manner, a mixture of protocatechuic aldehyde and vanillin is obtained (129). The closely related 1-vinyl-4-hydroxy-3-methoxybenzene (LXIX) may also be oxidized to vanillin by the action of nitrobenzene and alkali (130):

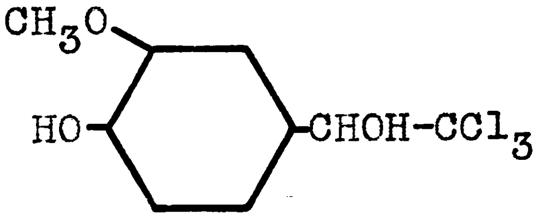


(LXIX)

Lyons and co-workers (125, 131) have investigated the oxidation, by nitrobenzene, of various side-chains attached to an aromatic nucleus. Benzyl alcohol when heated with nitrobenzene and alkali, under pressure at 140°C. for eight hours, was oxidized to benzoic acid in a yield of 93 per cent (125a). Toluene, benzyl chloride and benzyl bromide, on the otherhand, were found to be stable to the above treatment. Benzoin, when refluxed with nitrobenzene and alkali in xylene solution, was oxidized in a good yield to benzoic acid, and none of the ex-

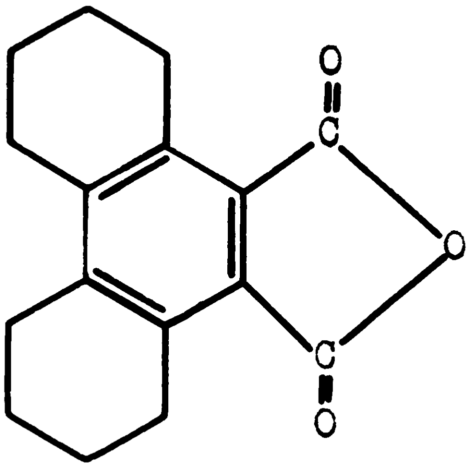
isolated from the reaction mixture (131)

Isopropyl alcohol, however, when subjected to the same treatment, was oxidized to acetone in a yield of 14 per cent. Recently Bergman (132),has obtained benzil in an almost quantitative yield from benzoin by heating the latter for three hours with nitrobenzene in the absence of all reagents. In a Belgian Patent (No. 393,344) it is claimed that the secondary alcohol, (4-hydroxy-3-methoxyphenyl) trichloromethylcarbinol (LXX) is oxidized to vanillin when treated with alkali and nitrobenzene.

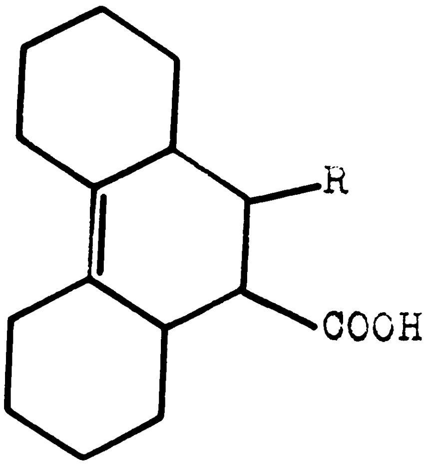


(LXX)

Bergmann (132) has recently shown that the Diels-Alder reaction, carried out in nitrobenzene solution, gives aromatic condensation in a single step. He has showed further that the action of the nitrobenzene was that of dehydrogenation, and not one involving an active intermediary stage, since the tetrahydro product, which is obtained in the usual solvents, yields the aromatic condensation product on boiling with nitrobenzene. The nitrobenzene was found to act in this manner only on systems capable of double ortho-enolization, as in the adducts with quinones and maleic anhydrides. Thus dicyclohexenyl and maleic anhydride are easily condensed in nitrobenzene with simultaneous aromatization to yield (LXXI), while with cinnamic or crotonic acid the tetrahydro products (LXXII) were obtained.



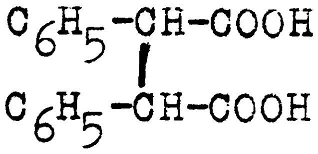
(LXXI)



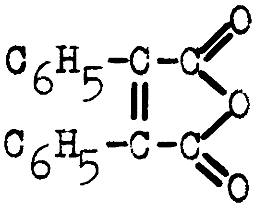
(R= CH_3; C_6H_5)

(LXXII)

As additional proof for his proposed mechanism, Bergmann showed that meso-diphenylsuccinic acid (LXXIII), when heated for twelve hours in nitrobenzene, yields diphenylmaleic anhydride (LXXIV) in about 40 per cent yield.



$$\begin{array}{l} C_6H_5\text{-}CH\text{-}COOH \\ \quad\;\;| \\ C_6H_5\text{-}CH\text{-}COOH \end{array}$$



The dienolic system involved in these reactions -C(OH)=C-C=C(OH)- may be regarded as a vinylogue of the grouping -C(OH)=C(OH)- found in the enediols of many hydroxyketones, such as dioxyconiferyl alcohol (XXXVII). As mentioned above (page 48) Bergmann has shown that benzoin is converted quantitatively to benzil by heating in nitrobenzene solution in the absence of all reagents.

Nitroso compounds have, in a few instances, been employed as oxidizing agents for organic compounds. Vanillin may be synthesized from guaiacol and formaldehyde, by heating at 100° with an alcoholic solution of p-nitrosodimethylamine and hydrogen chloride, the p-nitrosodimethylamine being reduced to p-aminodimethylamine (133). Imoto (134) has oxidized isoeugenol and isosafrole with alkyl nitrites in the presence of HCl, to give vanillin and piperonal respectively.

The Classification of Plants

Two types of classification for the higher plants are recognized by the botanist: (1) an artificial classification, compiled merely for the purpose of identification, and based on convenience as the sole criterion, and for which any obvious characteristics, such as flower or leaf morphology may be employed; (2) a natural classification, intended to show the natural relationships between species, genera, etc., and in which any or all criteria may be employed, although, for the higher plants, flower morphology has been found to be the most suitable. However, a great many other criteria (e.g. morphology of other parts, cytology, comparative anatomy and chemistry) have from time to time been used to supplement the evidence of flower morphology.

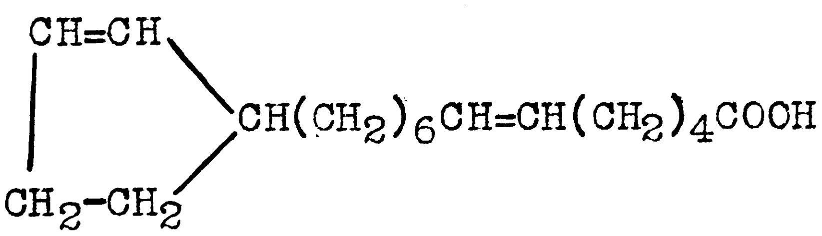
Chemical methods have been employed previously in helping to clarify the relationships of many closely related species. In recent years, Manske (135) has carried out an extensive investigation on the alkaloids of various plants, in which he has shown that the alkaloids found to be present in related species, or families, are often very similar, while at the same time they are usually sufficiently characteristic for each species that a study of the alkaloids may often help to distinguish one species from the other. The genus Senecio for example contains some 1,600 species, many of which are distinguishable one from the other only by minor differences, and then only by a specialist. Manske has examined three species of *Senecio*, namely *S. longilobus*, *S. ridellii* and *S. spartioides*, three species which have often been regarded as identical with one another, and finds that characteristic alkaloids are obtained from each (135b).

All members of the family *Papaveraceae* examined by Manske have been found, in addition to other alkaloids, to elaborate on the alkaloid protopine (135c). Although this work has not, as yet, been sufficiently extensive to allow of generalization, the classification of any member of this family, not found to contain protopine or a related alkaloid, should be open to question.

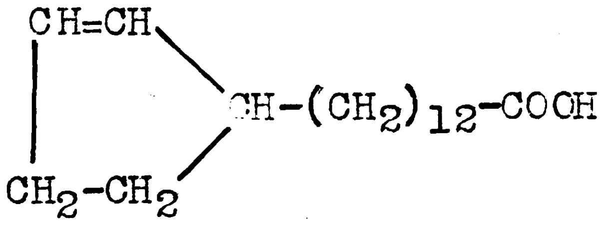
In conformity with the close botanical relationship between *Aconitum* and *Delphinium*, it has long been surmised that the alkaloids of the two genera are closely related. Recently an alkaloid isolated from *Delphinium brownii* appears to differ from the alkaloid lycoctonine , isolated from *Aconitum lycoctonum*, by just one methyl group (135d).

Many other instances of the limited occurrence of compounds or groups of compounds, specific to a species, genus or family of plants, are known in nature. Miller (136) has investigated 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, and finds that the compounds present in the essential oils of the three species are very different. On the basis of this observation he has suggested that a study of the essential oils might be of considerable aid to the taxonomist in the classification of plant species. McNair (137) has identified the essential oils from eighty-seven plants and families, and concludes that the nature of the essential oils is closely related to the position of the plant in the phylogenetic scale.

Cole and Cardosa (138) have isolated from certain members of the family *Flacourtiaceae*, the five membered ring fatty acids, gorlic acid (LXXI), chaulmoogric acid (LXXII) and several of the lower homologues of the latter.



(LXXI)



(LXXII)

The family *Flacourtiaceae* is comprised of eleven tribes or subfamilies, of which the *Oncobeae* (according to Gilg (1939)) is the central tribe. It is only from *Oncoba*, and *Carpotroche* within the *Oncobeae*, and from *Hydnocarpus* and possibly *Pangium* belonging to the closely related *Pangieae* radiating from *Oncobeae*, that these acids have been isolated and identified. Since these acids contain an asymmetric carbon atom, it is of further interest to note that it is only from members of the *Oncobeae* and the *Pangieae* that optically active fatty acids have been isolated.

Reichert (140) 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 (140) and Carles (141), have, from this point of view, carried out an extensive investigation of the family Iridaceae, which as now constituted, contains a number of genera, some of which (e.g. Moraea and Homeria) they find differ so widely in the reactions of their starches as to suggest misclassification. In general the results of these workers support the earlier classification of Tournefort (142) rather than the more modern classification.

The classification of proteins through the use of immunological tests has been used to trace the phylogenetic relationships in both plants and animals. It has been shown that a close relationship exists between the proteins present in certain plant extracts and the plant species (143) and that as the degree of relationship between species diminishes, the number of common proteins present in the plant extract diminishes proportionately. Perhaps the most extensive series of serological investigations has been carried out by Mez and co-workers (144), who, on the basis of their work constructed a "family tree" of the "vegetable kingdom". The phylogenetic relationships shown in this chart (145), are in most instances similar or identical with those generally accepted by taxonomists. Where Mez's chart shows relationships different from those generally accepted, the results based on these serological reactions may well express the truer phylogenetic relationship rather than those based on other grounds.

The role of lignin in plant taxonomy previously has been confined largely to lignin colour reactions, of which the *Mäule reaction* has been the most valuable. In 1849 Payen (146) noted that when hemp fibres were treated with chlorine water and then with ammonia, they became bright red. This is apparently the earliest record of such a reaction, which was advanced as a lignin test by Mäule in 1901 (147). At a later date, Schorger (155) noted that the wood of the angiosperms gave a strong red colour with the Mäule test, whereas that of the gymnosperms gave only an indefinite brownish grey. Schorger's observation has since been confirmed by Crocker (148), Scharma (149) and Schindler (150), and the Mäule test has been more or less accepted as a reliable means of distinguishing between the woods of angiosperms and those of gymnosperms. Crocker (151) has investigated a wide variety of botanical groups of plants by means of the Mäule test with interesting results. He finds that the three genera included in the *Gnetales*, *Gnetum*, *Ephedra* and *Welwitschia*, all give strongly positive Mäule tests.

In general, all of the coniferales examined, were found to give the expected negative test, but upon examination of several species of *Podocarpus*, he found that one of them, *P. amarus*, gave a strongly positive test. The work of Crocker has recently been greatly extended by Gibbs (152) to include 500 species belonging to 200 families. Within the genus *Podocarpus*, Gibbs has found that in addition to *P. amarus*, a large number (although not all) give weakly positive tests. *Tetraclinis articulata* was also found to give a strong positive Mäule test, while several species of Callitris, from which Tetraclinis has recently been separated, gave only negative tests. In many cases, notably among the Cycadales, the cortical fibres were found to give a positive Mäule test, while the xylem itself gave only negative results.

The observed marked difference in the chemical composition of the lignin from gymnosperms and angiosperms respectively (due to the recently discovered exclusive presence of the syringyl nucleus in the latter class) has also been employed to a restricted extent for ascertaining taxonomic differences in plant species. Holmberg (153) analyzed the thioglycollic acid lignin from forth plant species, which included both the higher and lower forms of plants. As expected the mosses were found to be almost completely free from lignin. Among the higher plant forms, he finds that the lignin from the ferns and gymnosperms (with the exception of Ephedra procera) has a methoxyl value of 15-16 per cent, while that from angiosperms and Ephedra procera has a methoxyl value of 20-22 per cent, based on the thioglycollic acid free lignin. The close agreement of the chemical analysis of the lignin from Ephedra procera with that of angiosperms is in good agreement with the above mentioned findings of Crocker and others employing the Mäule test.

MacInnes and Hibbert (154) have examined several species of the gymnosperms and angiosperms by means of the ethanolysis reaction, and find that while the former on ethanolysis yield members belonging to the 4-hydroxy-3-methoxyphenyl series, the latter yield in addition to these the corresponding syringyl analogues. The yields of the products however, were small, and in one instance (bamboo) the presence of the syringyl derivatives could not be definitely established.

Review of Experimental Results

A. Nitrobenzene Oxidation of Plant Materials

Until recently there has been little conclusive evidence of the aromatic nature of the material known as protolignin, and the earlier theories (156) according to which lignin was regarded as of carbohydrate nature were never completely disproven. Early work on the alkaline fusion of lignin yielded 20% of aromatic material, isolated as catechol and protocatechuic acid; (8,36), (page 7). This work, however, has never been repeated (157). More recently, Freudenberg, (49) by alkaline oxidation of spruce with nitrobenzene, succeeded in isolating 25% of the Klason lignin present in the wood as vanillin, along with additional small amounts of acids (chiefly vanillic acid) and unidentified phenols, amounting to 8% of the Klason lignin.

In the present investigation, the author has repeated the work of Freudenberg, and applied the method to the oxidation of maple wood. After a preliminary investigation of reaction conditions, the oxidation technique was then extended to a wide variety of other plant species.

1. Nitrobenzene-Alkali Oxidation of Spruce and Maple Woods, (Table I)

Oxidation with nitrobenzene and alkali of organic solvent extracted spruce woodmeal by the Freudenberg procedure (woodmeal

(35 g.), nitrobenzene (24 cc) and aqueous alkali (400 cc of 2N) heated for 3 hours at 160° with agitation, the mixture then acidified, extracted with benzene and the benzene extract treated with bisulphite) yielded 25% of vanillin, isolated as its m-nitrobenzoylhydrazone. In addition, a small amount (7.1%) of alkali soluble organic material also was obtained.

On repeating the above experiment with maple wood, the yield of "total aldehydes" (isolated as their mixed m-nitrobenzoylhydrazones) was 46%, based on the Klason lignin in the wood. Isolation of the crude bisulphite soluble oils, and separation of the mixture of aldehydes by means of fractional sublimation (page) yielded 10.2 and 34.7% of the Klason lignin as vanillin and syringaldehyde respectively, or a ratio of vanillin to syringaldehyde in the isolated aldehyde mixture of 1:3.4. Subsequent successive treatments, with sodium bicarbonate and alkali, of the bisulphite extracted benzene solution, yielded small amounts of "acids" and "phenols", amounting to 1.3 and 7.2% of the Klason lignin, respectively. A preliminary investigation of the latter fraction, yielded a yellow crystalline product, insoluble in cold benzene, which was identified by melting point and mixed melting point as p-hydroxyazobenzene. This product amounted to 20 per cent of the total alkali soluble fraction. The maximum yield of phenols (as distinct from aldehydes) derived from lignin amounted therefore, to 5.5% of the Klason lignin, (Table I).

The formation of hydroxyazobenzene by reduction of aromatic nitro compounds has been observed by various workers.

TABLE I

Comparison of Yields from Nitrobenzene Oxidation of Spruce and Maple Woods

		Spruce	Maple
Woodmeal, g.		35.0	38.5
Klason lignin, g.		9.2	8.5
Wood residue, g.		11.5	13.0
Total bisulphite soluble oils,	(g.)	2.50	4.12
	(% K.L.)*	27.1	48.5
"Total aldehydes", determined as m-nitrobenzoylhydrazones,	(g.)	2.28	3.92
	(% K.L.)	24.7	46.0
Vanillin,	(g.)	2.09	0.84
	(% K.L.)	22.8	10.2
Syringaldehyde,	(g.)	-	2.42
	(% K.L.)	-	34.7
Bicarbonate soluble oils,	(% K.L.)	-	1.3
Alkali soluble oils,	(% K.L.)	7.4	4.6

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*(% K.L.) = % Klason lignin

Bamburger (158) obtained o-hydroxyazobenzene as one of the products of the autoxidation-reduction of nitrosobenzene in the presence of light. o-hydroxyazobenzene, was also isolated by Ciamician and Silber (123), by the photochenical oxidation of nitrobenzene with benzaldehyde, the latter being oxidized to benzoic acid during the reaction. More recently Freudenberg (49) has isolated p-hydroxyazobenzene from the reaction mixture left from the alkaline nitrobenzene oxidation of spruce wood.

2. Influence of Reaction Variables in the Yield of Aldehydes from Maple Wood

The results of experiments by the author and by Hewson (159) on the effect of reaction variables on the yield of aldehydes from maple wood are shown in Table II. These may be summarized as follows:

(a) Time factor: No increase in yield was found on decreasing the time from three hours to one hour, while an increase to six hours resulted in a marked decrease in the yield of aldehydes from 46.0 to 42.3%.

(b) Temperature factor: Optimum yields were obtained using a temperature of about 160° (155-165°).

(c) Concentration of alkali: Increase in the alkali concentration from 2N to 3N, decreased the yield of aldehydes to 40%, and a decrease from 2N to 1N exerted the same effect.

(d) Ratio of wood to reaction mixture: A decrease in the weight of wood to volume of reaction mixture, from a ratio of

Table II

Nitrobenzene Oxidation of Maple Wood under Various Conditions

No.	Woodmeal g.	2N NaOH cc.	Nitrobenzene cc.	Time hrs.	Temp. °C.	Total Aldehydes P.I.K.L.[a]
1	38.5	400	24	3	160	46.0
2[d]	38.5	400	24	6	160	42.3
3	38.5	400	24	4	160	45.9
4	38.5	400	24	2	160	47.2
5[d]	38.5	400	24	1	160	46.1
6	38.5	400	24	2	190	31.4
7	38.5	400	24	4	135	39.4
8[d]	38.5	400[b]	24	2	160	40.0
9[d]	38.5	400[c]	24	2	160	40.0
10	19.3	400	12	3	160	48.8
11	9.6	400	6	3	145	43.0
12	10.3	200	6	3	160	45.5
13	10.3	200	6	2	160	45.0
14	4.8	100	3	2	160	45.2

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[a] P.I.K.L. is abbreviation for "Percentage Initial Klason Lignin"
[b] 3N NaOH
[c] 1N NaOH
[d] Experiments carried out by Hewson (159)

1 : 10 to a ratio 1 : 20 produced a slight increase in the yield of aldehydes from 46 to 49%, probably due to the increasing effective agitation.

(e) Decrease in total size of charge: It was found that the amount of wood used per charge could be decreased to around 5 g. without causing an appreciable change in the aldehyde yield.

3. Modified Procedure for the Oxidation of Small Amounts of Material

In order to shorten the time required for the nitrobenzene oxidation and the examination of the products, where small quantities were used (3-5 g. of wood and 3 cc nitrobenzene), the time of reaction was reduced to two hours, and since only small amounts of nitrogenous products are present in the reaction mixture, the tedious step of steam distillation of the alkaline reaction mixture was omitted.

4. Separation of Vanillin and Syringaldehyde from the Bisulphite Fraction of Maple Wood

The separation of vanillin and syringaldehyde in the aldehyde mixture isolated from the bisulphite fraction, obtained in the alkaline oxidation of maple and other angiosperms, was effected either by (a) precipitation of the sparingly soluble ammonium salt of syringaldehyde from ethanol solution, or (b) fractional sublimation of the vanillin and syringaldehyde.

(a) Precipitation of the Ammonium Salt of Syringaldehyde

The precipitation of the ammonium salt of syringaldehyde was carried out by the method of Hunter and Hibbert (160), namely, treatment of a solution of the aldehyde in anhydrous ethanol with a solution of ammonia (7%) in anhydrous ethanol. Complete precipitation (95%) of the ammonium salt of syringaldehyde was obtained from a medium in which the concentration factor represented by the product of the concentrations of the syringaldehyde and that of the ammonia (in grams per liter) was greater than 50.

Under similar conditions, the ammonium salt of vanillin was not precipitated from anhydrous ethanol solution until the similar factor (the product of the concentrations of vanillin and ammonia) exceeded 100. Since, from maple and most angiosperms, the ratio of vanillin to syringaldehyde in the mixture of the two, obtained by nitrobenzene oxidation, was 1:3, complete separation of the syringaldehyde as its ammonium salt was readily obtained. Syringaldehyde was readily recovered from its ammonium salt by acidification of a suspension of the salt in water with dilute acid, the crystalline aldehyde separating from the aqueous solution. Vanillin could not be recovered quantitatively from the ammoniacal, alcoholic filtrate, due to interaction of the aldehyde and ammonia, whereby large amounts of resins were formed. The fractional sublimation of vanillin and syringaldehyde, although slower, was therefore, generally employed as the more accurate method an analysis.

(b) Separation of Vanillin and Syringaldehyde by Fractional Sublimation

The fractional sublimation of vanillin and syringaldehyde was carried out by the metnod of Hawkins and Hibbert (161), who found that vanillin could be preferentially and quantitatively sublimed from a mixture of vanillin and syringaldehyde at 60°/1.0 mm Following complete removal of the vanillin, if the temperature was raised to 100°/0.5 mm, the syringaldehyde could then be sublimed from the remaining non-volatile impurities.

The vanillin was freed from impurities, consisting of small amounts of unknown volatile oils, by conversion into its m-nitrobenzoylhydrazone, and the yield of vanillin calculated from the weight of same. The yield of syringaldehyde was calculated from the weight of the crude sublimate (melting at 105-108°), and the aldehyde purified by recrystallization from water.

5. Taxonomic Investigation of Plant Species

It has been shown, for a limited number of plant species, both by elementary analysis (153) and by the isolation of monomeric "lignin building units" (154) that plants of the gymnosperm differ from those of the angiosperm class both in the nature and amount of lignin which they contain. The amount of lignin present in angiosperms is lower than in gymnosperms, while the methoxyl content of the lignin in the former is higher due to the presence of both the syringyl (4-hydroxy-3,5-dimethoxyphenyl) and the guaiacyl (4-hydroxy-3-methoxyphenyl) groupings.

The present investigation on the alkaline oxidation of spruce, maple and other woods with nitrobenzene showed that their behaviour towards oxidation was markedly different, both in the yields obtained and in the products formed. Thus, the yield of aldehydes from some angiosperms was twice that obtained from spruce and other gymnosperms, and consisted of a mixture of vanillin and syringaldehyde, whereas the gymnosperms gave in all cases vanillin only. It was, therefore, with a view to generalizing upon the above observations, and developing a convenient chemical aid to the taxonomic classification of botanical species, that an extensive study on a wide variety of plant species by means of nitrobenzene oxidation was undertaken.

The species studied were selected so as to provide not only as representative a list of both gymnosperms and angiosperms as possible, but also suitable material for the investigation of certain anomalies found in recent studies on plant species by use of the Mäule test (151, 152). 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), comb. nov., A.C. Smith* museum specimens were used.

Podocarpus pedunculatus received as such, is not recognized by Pilger (see ref. 151) but is regarded as synonymous with *P. amarus*. The close agreement found in this investigation between the two species (Table VIB) lends support to this latter view.

(a) Klason Lignin Content and Methoxyl Value of Various Plant Species (Tables III, IV and V.)

For the woody plants, the Klason lignin content of the gymnosperms (Table III) was found to be 29-35% of the dry wood weight, as compared with 18-23% found for the angiosperms (Tables IV and V). For the non-woody plants, *Cycas revoluta*, *Welwitschia mirabilis* (2 and 21, Table III), corn cobs and *Saccharum officinarum* (5 and 7, Table V), The Klason lignin values were considerably lower, and due to the interference in the procedure caused by the large amounts of carbohydrate and protein materials present, they can not be considered as strictly accurate evaluations of the lignin content of these plants. Thus, for *Cycas revolta* (2, Table III)

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* Received as *Bubbia haplopus* B.L. Burtt.

TABLE III

Klason Lignin and Methoxyl Analysis of Pteridophyta and Gymnosperms

No.	Plant Species	Klason lignin %	Methoxyl(a) %
Pteridophyta			
1	*Pteridium latiusculum*	28.1	4.85
Gymnosperms			
	Cycadales		
2	*Cycas revoluta*	15.6(b)	1.35
	Coniferales		
3	*Picea glauca*	28.6	4.73
4	*Tsuga canadensis*	31.1	5.41
5	*Pinus Strobus*	34.9	5.63
6	*Thuja plicata*	33.9	5.45
7	*Sequoia sempervirens*	31.8	4.99
8	*Taxus canadensis*	32.5	5.40
9	*Agathis australis*	30.8	5.19
10	*Podocarpus neriifolius*	-	5.17
11	*P. acutifolius*	34.9	5.20
12	*P. macrophyllus v. maki*	39.9	5.11
13	*P. amarus*	32.1	6.55
14	*P. pedunculatus*	30.7	6.40
15	*Callitris rhomboidea*	34.8	5.21
16	*Tetraclinis articulata*	29.5	6.20

cont'd.)

TABLE III (cont'd)

Klason Lignin and Methoxyl Analysis of Pteridophyta and Gymnosperms

No.	Plant Species	Klason lignin %	Methoxyl (a) %
	Ginkgoales		
17	*Ginkgo biloba*	32.8	5.63
	Gnetales		
18	*Ephedra trifurca*	23.3	5.61
19	*E. sp.*	25.0	6.01
20	*Gnetum indicum*	24.7	4.19
21	*Welwitschia mirabilis*	16.5	2.10

(a) Average of two values

(b) Methoxyl = 7.3%. Klason lignin calculated from methoxyl =6.8%. (See page 67).

TABLE IV

Klason Lignin and Methoxyl Analysis of Angiosperms (Dicotyledons)

No.	Plant Species	Klason Lignin %	Methoxyl(a) %
1	*Acer rubrum*	22.0	6.03
2	" (heartwood)	23.6	6.87
3	" (sapwood)	20.6	6.43
4	*A. saccharinum*	18.8	6.20
5	*Populus tremuloides*	17.4	5.17
6	" (b)	17.0	5.25
7	" (c)	20.6	5.56
8	*Sassafras sp.*	22.1	6.26
9	*Quercus borealis v. maxima*	18.4	5.59
10	*Betula lutea*	19.6	6.16
11	*Fraxinus americana*	18.5	5.53
12	*Ulmus fulva*	23.0	5.90
13	*U. americana*	21.3	5.76
14	*Juglans cinerea*	19.0	5.77
15	*J. nigra*	20.9	6.10
16	*Tilia americana*	23.8	5.73
17	*Corchorus sp.*	22.3	5.65
18	*C. sp.* (*fiber*)	12.7	4.07
19	*Sorbus americana*	18.5	6.22
20	*Robinia pseudacacia*	21.2	5.77
21	*Paulownia imperialis*	18.9	4.87
22	*Liriodendron tulipifera*	20.0	6.13

TABLE IV (cont'd).

Klason Lignin and Methoxyl Analysis of Angiosperms (Dicotyledons)

No.	Plant Species	Klason Lignin %	Methoxyl %
23	*Trochodendron sp.* -	24.0	6.69
24	*Drimys winteri*	24.3	6.63
25	*Belliolium haplopus*	36.9	7.10
26	*Zygogynum vieillardi*	35.5	7.11

(a) Average of two values.

(b) Extracted 48 hours with ethanol, then 12 hours with water.

(c) Extracted 48 hours with pet.-ether, then with ether.

Table V

Klason Lignin and Methoxyl Analysis of Angiosperms (Monocotyledons)

No.	Plant Species	Klason Lignin %	Methoxyl %[a]
1	*Dracaena fragrans* (?)	23.8	6.80
2	*Aloe abyssinica*	18.5	5.25
3	Bamboo (unidentified)	22.5	4.24
4	Rye Straw	20.2	3.67
5	Corn Cobs	13.8	2.77
6	Corn-Stalks	19.9	3.50
7	*Saccharum officinarum*	16.4	3.12

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[a] Average of two values

the Klason lignin, amounting to 15%, isolated by treatment with 72% sulfuric acid, was found to contain only 7.6% methoxyl. Since only vanillin was obtained on oxidation of the plant with nitrobenzene, indicating that only the 4-hydroxy-3-methoxyphenyl nucleus is present in the lignin, the theoretical methoxyl value for the Klason lignin (assuming a C_6C_3 lignin building unit) should be around 17%. On this basis, the Klason lignin content of Cycas revoluta would be only 6.8%. With certain of the monocotyledons (Table V), notably the grasses, the condensation of proteins with the lignin under the influence of 72% sulfuric acid has been shown to give erroneous results in the Klason lignin analysis (76). A calculation of the true Klason lignin content, based on methoxyl analysis, is more difficult for these monocotyledons since, in addition to vanillin and syringaldehyde, it has been shown, in at least one instance (corn-stalks) there is also present an appreciable amount of p-hydroxybenzaldehyde (page 93).

The methoxyl values of the gymnosperms are slightly lower than for the angiosperms, the average methoxyl values being 5 and 6% respectively, (Tables III, IV and V). A few exceptions were found among the gymnosperms, notably Podocarpus amarus, P. pedunculatus, Tetraclinis articulata and two species of Ephedra, all of which have methoxyl values of around 6% (Table III). All of these plants were shown by the nitrobenzene oxidation technique to contain the syringyl grouping to a greater or lesser extent (Table VIB). Podocarpus macrophylla v. maki, (Table III) on the other hand, which was found by Gibbs (152) to give a weakly positive Mäule test (usually given only by plants found to yield syringaldehyde on oxidation) had a methoxyl content of only 5.1%. Nitrobenzene oxidation of P. macrophylla v. maki, failed to show definitely the presence of the syringyl grouping, although in one instance, traces of syringaldehyde were isolated in the form of its m-nitrobenzoylhydrazone.

(b) Alkaline Oxidation of Various Plant Species with Nitrobenzene

The results of the alkaline oxidation of 50 botanical species with nitrobenzene are shown in Tables VI-VIII. The gymnosperms (Table VI) generally gave only vanillin, in yields of 20-25% calculated on the Klason lignin content of the wood, while the angiosperms (dicotyledons, Table VIIB) yielded 40-50% of a mixture of vanillin and syringaldehyde in an approximate ratio of 1:3 respectively.

A number of interesting exceptions however, were found. Three species belonging to the Coniferales, namely Podocarpus amarus, P. pedunculatus and Tetraclinis articulata, and also members of all the genera included in the Gnetales, were found to give high yields of syringaldehyde as well as vanillin (Table VIB). These results are in good agreement with the work of Crocker (151) and the recent, more extensive work of Gibbs (152) on the behaviour of these species towards the Mäule test (this consists of successive treatments of a thin cross section of the plant material with dilute potassium permanganate, hydrochloric acid and finally, after washing well with water, with dilute ammonium hydroxide (page 55)).

TABLE VIA

Nitrobenzene Oxidation of Pteridophyta and Gymnosperms

Reaction Conditions

No.	Plant Species	Wood[a] g.	Klason lignin g.	Nitro-benzene cc	2N NaOH MaOH cc	Time hrs.
Pteridophyta						
1	Pteridium latiusculum	3.70	1.05	3	100	2
Gymnosperms						
Cycadales						
2	Cycas revoluta	6.90	1.07	3	100	2
Coniferales						
3	Picea glauca	35.0	9.10	24	400	3
4	Tsuga canadensis	3.40	1.06	3	100	2
5	Pinus Strobus	3.30	1.15	3	100	2

(cont'd)

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[a] Calculated dry weight.

TABLE VIA (cont'd).

Nitrobenzene Oxidation of Pteridophyta and Gymnosperms

Reaction Conditions

No.	Plant Species	Wood[a] g.	Klason lignin g.	Nitro-benzene cc	2N NaOH MaOH cc	Time hrs.
6	*Thuja plicata*	2.93	1.00	3	100	2
7	*Sequoia sempervirens*	3.20	1.02	3	100	2
8	*Taxus canadensis*	3.10	1.01	3	100	2
9	*Agathis australis*	3.20	1.00	3	100	2
10	*Podocarpus neriifolius*	1.80	-	1.5	50	2
11	*P. acutifolius*	2.96	1.03	3	100	2
12	*P. macrophyllus v. maki*	4.75	1.89	3	100	2
13	"	14.7	5.86	17	350	2
14	*P. amarus*	4.66	1.50	3	100	2
15	*P. pedunculatus*	3.20	1.19	3	100	2
16	*Callitris rhomboidea*	2.84	0.99	3	100	2
17	*Tetraclinis articulata*	3.15	0.93	3	100	2

(cont'd.)

TABLE VIA (cont'd.)

Nitrobenzene Oxidation of Pteridophyta and Gymnosperms

Reaction Conditions

No.	Plant Species	Wood g.	Klason lignin g.	Nitro-benzene cc	2N NaOH MaOH cc	Time hrs.
	Ginkgoales					
18	*Ginkgo biloba*	10.2	3.34	9	200	3
	Gnetales					
19	*Ephedra trifurca*	17.3	4.03	12	400	3
20	*E. sp.*	9.40	2.35	6	200	3
21	*Gnetum indicum*	9.20	2.28	6	200	3
22	*Welwitschia mirabilis*	14.2	3.34	6	200	2

TABLE VIB

Nitrobenzene Oxidation of Pteridophyta and/Gymnosperms

Summary of Yields

No.	Plant Species	Total aldehydes P.I.K.L.	Vanillin P.I.K.L.	Syring-alde-hyde P.I.K.L.	Ratio Vanillin: Syring-aldehyde
Pteridophyta					
1	*Pteridium latiusculum*	21.8	20.5	-	-
Gymnosperms					
Cycadales					
2	*Cycas revoluta*	2.7	2.5	-	-
Coniferales					
3	*Picea glauca*	23.7	23.5	-	-
4	*Tsuga canadensis*	23.2	22.1	-	-
5	*Pinus Strobus*	20.1	18.5	-	-
6	*Thuja plicata*	24.6	24.0	-	-
7	*Sequoia sempervirens*	24.8	23.5	-	-
8	*Taxus canadensis*	22.0	20.7	-	-
9	*Agathis australis*	19.9	18.7	-	-
10	*Podocarpus neriifolius*	Present	Present	-	-
11	*P. acutifolius*	20.2	19.8	-	-
12	*P. macrophyllus v. maki*	17.4	15.1	Trace	-
13	"	16.8	15.5	-	-
14	*P. amarus*	30.7	12.5	12.5	1:1.0
15	*P. pedunculatus*	31.7	13.9	14.7	1:1.0
16	*Callitris rhomboidea*	19.7	18.8	-	-

(cont'd)

TABLE VIB (cont'd.)

Nitrobenzene Oxidation of Pteridophyta and Gymnosperms

Summary of Yields

No.	Plant Species	Total aldehydes P.I.K.L.	Vanillin P.I.K.L.	Syring-aldehyde P.I.K.L.	Ratio Vanillin: Syring-aldehyde
17	*Tetraclinis articulata*	29.2	15.5	12.08	1:0.8
	Ginkgoales				
18	*Ginkgo biloba*	25.7	25.3	-	-
	Gnetales				
19	*Ephedra trifurcae*	39.6	9.3	27.7	1:3.0
20	*E. sp.*	36.0	8.8	26.0	1:3.0
21	*Gnetum indicum*	26.8	6.8	16.5	1:2.5
22	*Welwitschia mirabilis*	4.9	2.0	2.4	1:1.2

TABLE VIIA

Nitrobenzene Oxidation of Angiosperms (Dicotyledons)

Reaction Conditions

No.	Plant Species	Wt. used dry wood g.	Klason lignin g.	Nitro-benzene cc	2N NaOH cc	Time hrs.
1	*Acer rubrum*	38.5	8.48	24	400	3
2	" (heartwood)	10.4	2.46	6	200	3
3	" (sapwood)	10.3	2.12	6	200	3
4	*A. saccharinum*	5.25	1.00	3	100	2
5	*Populus tremuloides*	11.2	1.95	6	200	3
6	" (a)	22.0	3.74	12	400	3
7	" (b)	5.00	1.06	3	100	2
8	*Sassafras sp.*	19.0	4.20	12	400	3
9	*Quercus borealis v. maxima*	5.50	1.01	3	100	2
10	*Betula lutea*	4.80	0.95	3	100	2
11	*Fraxinus americana*	4.70	0.87	3	100	2

(a) Extracted 48 hours with ethanol, then 12 hours with water.

(b) Extracted 48 hours with pet.-ether, then with ether.

Nitrobenzene Oxidation of Angiosperms (Dicotyledons)

Reaction Conditions

No.	Plant Species	Wt. used dry wood g.	Klason lignin g.	Nitro-benzene cc	2N NaOH cc	Time hrs.
12	*Ulmus fulva*	4.50	1.03	3	100	2
13	*U. americana*	4.70	1.00	3	100	2
14	*Juglans cinerea*	5.26	1.00	3	100	2
15	*J. nigra*	4.80	1.00	3	100	2
16	*Tilia americana*	4.20	1.00	3	100	2
17	*Corchorus sp.*	10.3	2.30	6	200	3
18	*C. sp.* (*fiber*)	33.5	4.26	12	400	3
19	*Sorbus americana*	5.40	1.00	3	100	2
20	*Robinia pseudacacia*	4.70	1.00	3	100	2
21	*Paulownia imperialis*	5.30	1.00	3	100	2
22	*Liriodendron tulipifera*	4.95	0.99	3	100	2

(cont'd.)

TABLE VIIA (cont'd).

Nitrobenzene Oxidation of Angiosperms (Dicotyledons)

Reaction Conditions

No.	Plant Species	Wt. used dry wood g.	Klason lignin g.	Nitro-benzene cc	2N NaOH cc	Time hrs.
23	*Trochodendron sp.*	4.15	1.00	3	100	2
24	*Drimys winteri*	4.15	1.00	3	100	2
25	*Belliolium haplopus*	3.20	1.19	3	100	2
26	*Zygogynum vieillardi*	3.20	1.14	3	100	2

TABLE VIIB

Nitrobenzene Oxidation of Angiosperms (Dicotyledons)

Summary of Yields

No.	Plant Species		Total[(a)] aldehydes P.I. K. L.	Vanillin P.I.K.L.	Syringaldehyde P.I.K. L.	Ratio Vanillin / Syringaldehyde
1	*Acer rubrum*		46.0	10.2	34.7	1:3.4
2	"	(heartwood)	39.2	9.7	28.5	1:2.9
3	"	(sapwood)	44.5	9.1	33.6	1:3.7
4	*A. saccharinum*		51.0	12.7	35.2	1:2.8
5	*Populus tremuloides*		44.7	9.4	32.1	1:3.4
6	"	(b)	43.3	9.1	33.4	1:3.7
7	"	(c)	41.5	8.3	31.2	1:3.7
8	*Sassafras sp.*		44.5	9.9	29.5	1:3.0
9	*Quercus borealis v. maxima*		47.2	10.5	34.3	1:3.3
10	*Betula lutea*		44.9	11.0	33.7	1:3.1
11	*Fraxinus americana*		48.6	10.5	37.2	1:3.5
12	*Ulmus fulva*		41.7	9.0	29.1	1;3.2
13	*U. americana*		40.9	9.8	30.7	1:3.1
14	*Juglans cinerea*		45.2	11.1	31.0	1:2.8
15	*J. nigra*		39.3	10.5	26.5	1:2.5
16	*Tilia americana*		35.2	8.9	23.8	1:2.7
17	*Corchorus sp.*		39.0	9.4	24.5	1:2.6
18	*C. sp.* (fiber)		32.0	7.7	23.9	1:3.1
19	*Sorbus americana*		49.0	9.5	37.8	1:4.0
20	*Robinia pseudacacia*		39.8	9.4	27.6	1:2.9

TABLE VIIB (cont'd)

Nitrobenzene Oxidation of Angiosperms (Dicotyledons)

Summary of Yields

No.	Plant Species	Total aldehydes P. I. K. L.	Vanillin P.I.K.L.	Syringaldehyde P.I.K.L.	Ratio Vanillin / Syringaldehyde
21	Paulownia imperialis	44.5	10.6	32.8	1:3.1
22	Liriodendron tulipifera	49.2	11.0	36.5	1:3.2
23	Trochodendron sp.	40.4	9.5	29.5	1:3.1
24	Drimys winteri	35.3	8.2	22.0	1:2.7
25	Belliolium haplopus	29.6	13.9	16.2	1:1.1
26	Zygogynum vieillardi	32.8	14.3	16.8	1:1.2

(a) P. I. K. L. is abbreviation for "Percentage Initial Klason Lignin".

(b) Extracted 48 hours with ethanol, then 12 hours with water.

(c) Extracted 48 hours with pet.-ether, then with ether.

Table VIIIA

Nitrobenzene Oxidation of Angiosperms (Monocotyledons)

Reaction Conditions

No.	Plant Species	Wt. used[a] g.	Klason Lignin g.	Nitrobenzene cc.	2N NaOH cc.	Time hrs.
1	*Dracaena fragrans* (?)	4.30	1.07	3	100	2
2	*Aloe abyssinica*	5.35	1.00	3	100	2
3	Bamboo (unidentified)	9.6	2.14	6	200	3
4	Rye Straw	19.2	3.88	12	400	3
5	Corn Cobs	20.5	2.83	12	400	3
6	Corn-Stalks	5.00	1.00	3	100	2
7	*Saccharum officinarum*	3.80	0.62	1.5	100	2

---

[a] Calculated dry weight of wood

Table VIIIB

Nitrobenzene Oxidation of Angiosperms (Monocotyledons)

Summary of Yields

Plant Species	Total Aldehydes P.I.K.L.[a]	Vanillin P.I.K.L.[a]	Syringaldehyde P.I.K.L.[a]	Ratio Vanillin: Syringaldehyde
Dracaena fragrans (?)	34.9	6.9	26.2	1 : 3.8
Aloe abyssinica	37.1	8.3	27.1	1 : 3.3
Bamboo (unidentified)	28.5	15.1[b]	11.5	1 : 0.8
Rye Straw	30.5	14.1[b]	15.3	1 : 0.9
Corn Cobs	21.4	17.1[b]	6.0	1 : 0.4
Corn-Stalks	17.8	9.5[b]	7.8	1 : 0.4
Saccharum officinarum	16.3	5.3	9.0	1 : 1.7

---

[a] P.I.K.L. = Percent Initial Klason Lignin

[b] Vanillin fraction consists of vanillin + p-hydroxybenzaldehyde

Gibbs was able to show that a strong positive Mäule colour test is given by each of the above species, while Crocker had previously obtained positive tests with Podocarpus amarus and the Gnetales.

The observed chemical similarity of the lignins of the Gnetales with those of the angiosperms is of interest, in view of the fact that, although they show many characteristic features of the gymnosperms, they have pronounced vessels, a feature generally found only in the angiosperms. Holmberg (153) from the analysis of thioglycollic lignin isolated from Ephedra procera, found a similar chemical relationship of this species to the angiosperms. Thus the methoxyl values (calculated for a thioglycollic acid free lignin), isolated from the angiosperms and Ephedra procera were 20-23%, as compared with values of 15-17% for the gymnosperms.

The chemical similarity of the two species of Podocarpus and Tetraclinis to the angiosperms, is not as pronounced as with the Gnetales, the Klason lignin content of the two former, (although lower than that of other Podocarpus being that of a typical gymnosperm (Table III). The experimental ratio of syringaldehyde to vanillin (1:1) was also lower than that generally found in the aldehyde mixture obtained from the gymnosperms.

The isolation of syringaldehyde from Tetraclinis articulata, together with its positive behaviour (Mäule test), adds confirmation to the recent reclassification of this plant, in which it was separated from Callitris, three species of which were found by Gibbs (152) and one by Crocker (151) to give a negative Mäule test. On investigating Callitris rhomboidea (16, Table VI) with the nitrobenzene oxidation technique, only vanillin could be isolated.

The occurrence of the syringyl nucleus in both the *Gnetales* and certain species of *Podocarpus*, is of interest in the light of the results of Mez (144, 145) who, on the basis of the similarity of their serological reactions, has placed *Podocarpus*, *Ephedra* and *Gnetum*, on the same branch of his "family tree" (see page 54).

Gibbs (152) has obtained *weak* Mäule tests with a number of other species of *Podocarpus*, one of which, *P. macrophylla v. maki*, was studied by nitrobenzene oxidation, (Table VI). On fractional sublimation of the bisulphite fraction and fractional crystallization of the m-nitrobenzoylhydrazone from the last fraction of this sublimate, a trace (0.003 g.) of a hydrazone was obtained (m.p. 191-3°), which showed no depression on a mixed melting point with the m-nitrobenzoylhydrazone of syringaldehyde (m.p. 192-3°). On repeating this work, however, with larger amounts of material (from a different source), and fractionating the bisulphite fraction in a semi-micro Cooke-Bower fractionating column, no trace of syringaldehyde could be found.

Belliolum haplopus and *Zygogynum veiellardi* were the only notable exceptions found in the application of the nitrobenzene oxidation process to a wide variety of dicotyledons (Table VIIB). Both of these species were found to contain large amounts of Klason lignin (35 and 37% respectively) (Table III) while the yields of aldehydes obtained on oxidation were only 29.6 and 32.8% respectively, based on the Klason lignin. The ratio of vanillin to syringaldehyde in the isolated aldehyde mixture was found to be approximately 1:1. These two species belong to a group of primitive angiosperms, characterized by the absence of vessels, and comprise the families, Magnoliaceae, Winteraceae and Trochodendraceae. According to the most recent work on the classification of these groups, Belliolum and Zygogynum are included, with Drimys, in the Winteraceae, while Liriodendron and Trochodendron are placed in the Magnoliaceae and Trochodendraceae respectively. The very marked difference, in the chemical behaviour, of Belliolum and Zygogynum as compared with Drimys and also with Trochodendron and Liriodendron, (aldehyde ratio 1:3) suggests that further work is necessary to clarify the classification of this group of plants. Nitrobenzene oxidation studies on the other genera of all three families should be carried out, and might prove of valuable assistance in determining the true phylogenetic relationships involved.

Investigation of a number of monocotyledons (Table VIII) showed that the grasses (3-7) differ from the other monocotyledons (1,2) and the dicotyledons, in that they give on oxidation, low yields of aldehydes (16-30%) and, with the exception of sugar-cane (Saccharum officinarum), the low methoxyl values of the mixed m-nitrobenzoylhydrazones (Table IX) indicated the presence of a third, non-methoxyl-containing aldehyde. It was not found possible to separate this aldehyde from vanillin by sublimation, and the yield of vanillin reported in Table VIII is based on the total weight of the fraction subliming at 60°/1.0 mm. On reinvestigation of the bisulphite fraction, a new aldehyde was isolated by solvent fractionation from

Table IX

Methoxyl Values of m-Nitrobenzoylhydrazones of Mixed Aldehydes from Alkaline Nitrobenzene Oxidation of Monocotyledons

Plant Species	OCH_3 %
Dracaena sp.	14.5
Aloe abyssinica	14.3
Bamboosa sp.	10.8
Rye Straw	12.1
Corn (Cobs)	5.5
Corn (Stalks)	7.5
Sugar Cane	14.0

cold benzene, and identified as p-hydroxybenzaldehyde.

The isolation of p-hydroxybenzaldehyde from this monocotyledon is of great interest, since it has been found in no other group of plants, and may therefore, provide a useful means of distinguishing the grasses from other angiosperms.

The results obtained from the above investigation of various plant species by means of alkaline oxidation with nitrobenzene, have shown that there are characteristic differences in the chemical nature of lignins from different plant sources. That a comparison of the oxidation products from these lignins may serve, in some instances to differentiate between closely related species, is indicated by the marked difference in the nature and yields of the products isolated from Tetraclinis articulata, and Callitris rhomboideae, and from Trochodendron sp., Drimys winteri, Belliolum haplopus and Zygogynum vieillardi, (Table VI and VII) The results obtained in each of these cases indicates the necessity for a further study of these groups.

Due, in the case of many plant species, to the small amount of material available for study, the development of micromethods, for both the nitrobenzene oxidation and for the analysis of the resulting products appears to be desirable before the method can be applied to a truly comprehensive study of plant species. Again, many species of plants, such as Podocarpus macrophylla v. maki, were found by Gibbs (152) to give only very faint Mäule tests, while others, namely the leaves of certain Cycads and, in a preliminary investigation, certain species of Selaginella, were found to give a positive test only in the cortical fibers, while in each case the xylem gave only a negative test. Detection of the small amounts of syringaldehyde, that would be obtained from such restricted areas, could only be accomplished by micromethods.

Comparison of the results of the alkaline nitrobenzene oxidation of the various plant species studied, with those obtained by Gibbs (152) for the Mäule test, shows that a close correlation exists between the red colour given by the Mäule test, and the occurrence of the syringyl grouping in the lignin of these plants. Even in the case of Podocarpus macrophylla v. maki, from which the presence of syringaldehyde in its nitrobenzene oxidation reaction mixture was extremely doubtful,/and it must be remembered that the red colour given by the Mäule test was very faint, indicating at the most, only small amounts of syringyl derivatives.

The establishment of a direct connection between the red colour of the Mäule test and the presence of the syringyl grouping in the lignin of the plant, through the isolation and identification of the coloured compound formed, would be of great value, and would provide in the Mäule test, a micro-method for the detection of the syringyl grouping in lignin.

6. The Isolation of p-Hydroxybenzaldehyde from Corn-Stalks

The low methoxyl content found for the m-nitrobenzoylhydrazones of the "total aldehydes" from corn, and to a lesser extent from the other grasses, (Table IX) led to a careful reinvestigation of the aldehyde bisulphite fraction from corn-stalks.

(a) Fractional Distillation of the Bisulphite Fraction from Corn-Stalks

The combined bisulphite fractions from two large scale runs (50 g.) were fractionated in the semi-micro Cooke-Bower fractionating column. Crystalline fractions were obtained, the methoxyl analysis of which (Table X) showed that little or no separation of the vanillin and unknown aldehyde had been obtained. The last two fractions were identified by melting point and mixed melting point as syringaldehyde and amounted to 2.6% of the Klason lignin.

TABLE X

Fractionation of Aldehyde Bisulphite fraction from Corn-Stalks

Fraction No.	Methoxyl %
1	17.5
2	15.7
3	16.3
4	12.2
5	31.2
6	30.8

Refractionation of the first four fractions showed no well defined separation.

(b) Solvent Fractionation of Vanillin and p-Hydroxybenzaldehyde

Separation of the unknown mixture into vanillin and p-hydroxybenzaldehyde was accomplished finally by repeated solvent fractionation of the mixture in cold benzene, as indicated in Figure 1. The benzene soluble and insoluble fractions were identified by melting point and mixed melting point as vanillin and p-hydroxybenzaldehyde, and amounted to 4.5 and 1.2% of the Klason lignin respectively. The phenylhydrazone of the natural p-hydroxybenzaldehyde was prepared and a mixed melting point with the phenylhydrazone of a synthetic sample gave no depression.

The yields of vanillin, p-hydroxybenzaldehyde and syringaldehyde from corn-stalks are summarized in detail in Table XI.

Fig. 1

Solvent Refractionation of Aldehyde Fractions 1 - 4 (Table X)

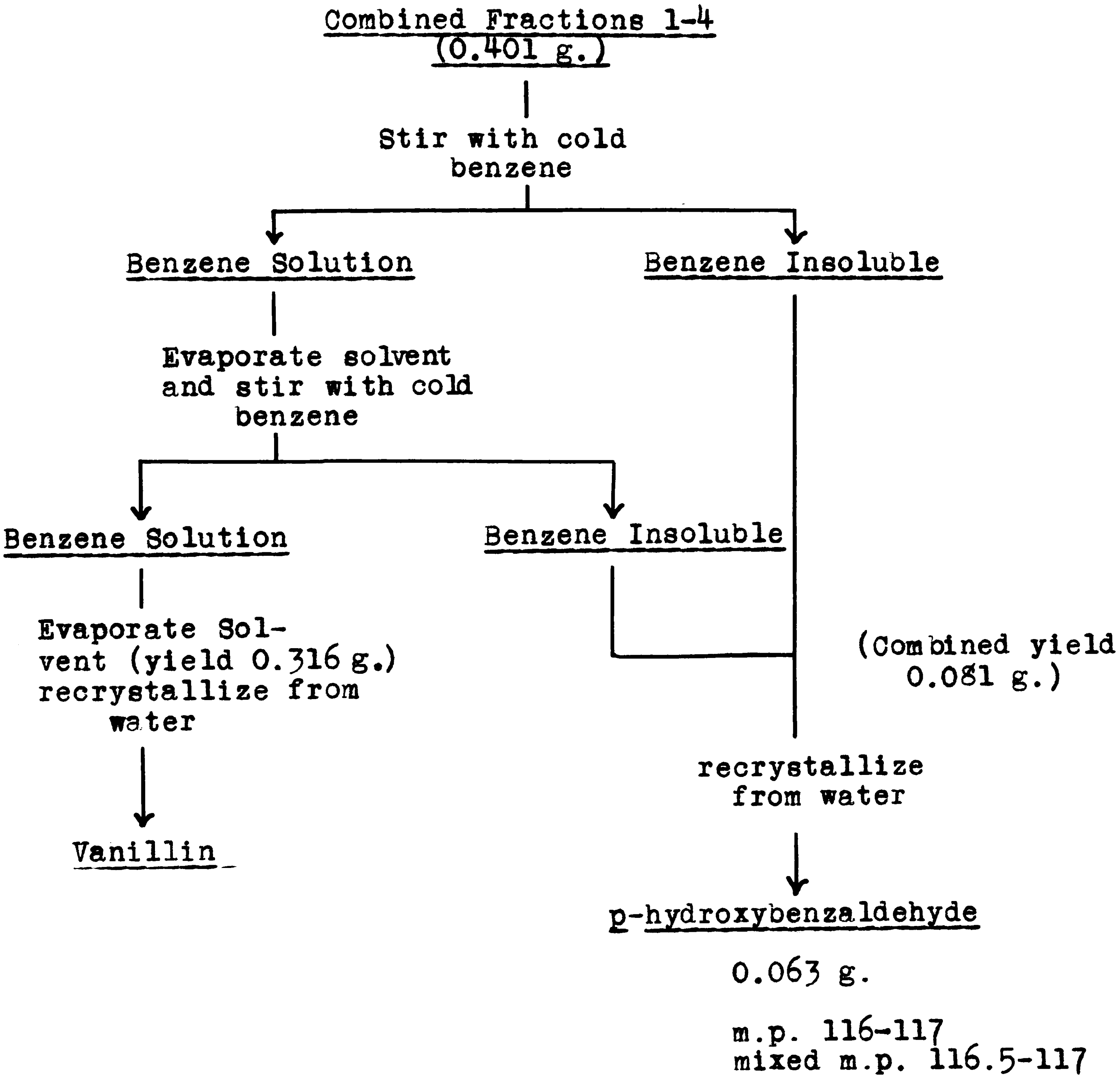

Table XI

Summary of Yields from Oxidation of Corn Stalks

Fraction	Crude Product		Recrystallized Products	
	% Total bisulphite fraction[a]	%Klason lignin	% Total bisulphite fraction[a]	% Klason lignin
"Total aldehydes" calc'd from weight of m-nitrobenzoyl hydrazone	-	18.7	-	-
Crude bisulphite fraction	-	16.7	-	-
Total distillable oils	83.3	10.3	69.2	8.5
Vanillin	43.0	5.3	36.3	4.5
p-Hydroxybenzaldehyde	14.7	1.8	11.7	1.4
Syringaldehyde	25.6	3.2	21.2	2.6

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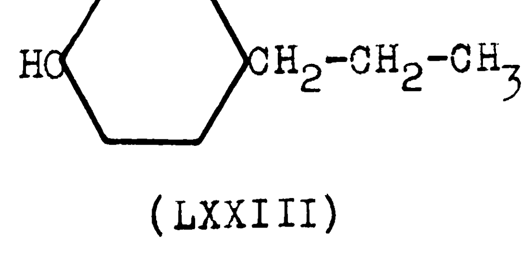
[a] Yields based on the weight of total "distillable" bisulphite fraction

B. Nitrobenzene Oxidation of Extracted Lignins and Related Compounds

The alkaline oxidation of isoeugenol with nitrobenzene to give vanillin in high yields, has been known for some time (46). In the present investigation, a similar treatment was carried out with a number of monomeric 1-(4-hydroxy-3-methoxyphenyl)-propane derivatives, and isolated lignins and lignin derivatives. Under conditions similar to those employed for the oxidation of woody tissues.

(a) Oxidation of 1-(4-Hydroxy-3-methoxyphenyl)-propane Derivatives, Table XII

Isoeugenol and ferulic acid (expts. 1 and 2, Table XII), gave vanillin yields of 68 and 50% respectively, indicating the relative ease of oxidation of an ethylenic bond adjacent to the benzene ring. With dibromisoeugenol, obtained by addition of a mole of bromine to isoeugenol, the yield of vanillin was lowered to 38% (3, Table XII) and decreased still further to 17.9% with 1-(4-hydroxy-3-methoxyphenyl)-propane (LXXIII), in which the ethylenic bond has been saturated (4,Table XII).



(LXXIII)

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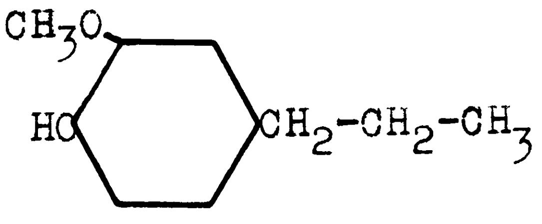


(LXXIII)

Table XII

Nitrobenzene Oxidation of Phenol Propyl Derivatives
(2 hours at 160°C.)

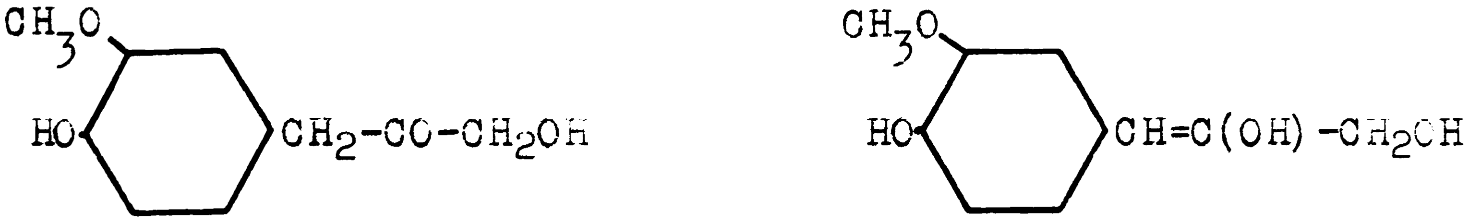
No.	Compound	Wt. used g.	Nitrobenzene cc.	2N NaOH cc.	Amorphous Product		Bisulphite fraction		Bicarbonate fraction g.	Alkali fraction g.
					g.	% OCH_3	g.	% Vanillin		
1	Isoeugenol	1.08	3	100	1.70	0.97	0.688	68.5	0.012	0.013
2	Ferulic acid	1.0	3	100	1.23	-	0.396	50.5	-	-
3	Isoeugenol dibromide	0.95	3	100	2.20	0.35	0.171	38.4	0.029	0.012
4	1-(4-Hydroxy-3-methoxyphenyl)-propane	1.0	3	100	0.57	-	0.164	17.9	-	-
5[a]	1-Acetoxy-1-(4-acetoxy-3-methoxyphenyl)-2-propanone	0.15	1	20	-	-	0.00	-	0.030	0.008
6[b]	1-(4-Hydroxyphenyl)-1-2-propanedione	0.78	8	150	-	-		11	-	-
7[b]	2-Hydroxy-1-(4-hydroxy-3-methoxyphenyl-1-propanone	2.0	8	150	3.77	-		14	-	-
8[b]	2-Ethoxy-1-(4-hydroxy-3-methoxyphenyl-1-propanone	2.0	8	150	3.13	-		20	-	-

[a] Reaction carried out at 100° for 1 hour.

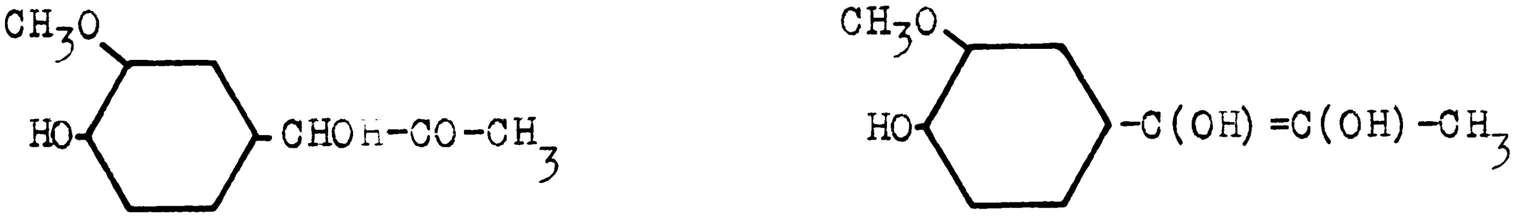
[b] Experiment of W. Hewson (159)

These results indicate that alkaline oxidation with nitrobenzene may proceed in one of two ways: (a) by direct oxidation of the double bond adjacent to the benzene ring, or (b) by the stepwise oxidation of the aliphatic side-chain.

In the light of the above results, it was of interest to investigate the action of nitrobenzene and alkali, on compounds capable of forming a double bond in the 1-2 position to the benzene ring by enolization. Such compounds are to be found in the postulated "lignin building units" (XX) and (XXXI) of Hibbert (4).



(XX)

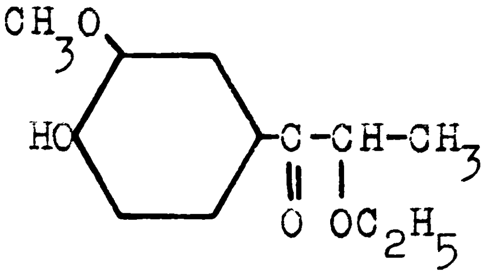


(XXXI)

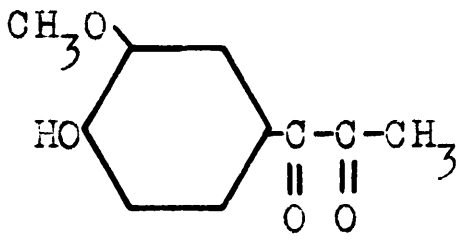
The action of alkali and nitrobenzene on benzoin in boiling xylene solution, according to Lyons and Pleasant (131), gives a quantitative yield of benzoic acid. None of the expected benzil could be isolated, leading the authors to conclude that the oxidation of benzoin occurred in two steps, benzil forming only as an intermediate and then being oxidized further to benzoic acid.

More recently Bergman (132) has converted benzoin to benzil, in quantitative yields, by heating the former with nitrobenzene for 3 hours in the absence of any reagent.

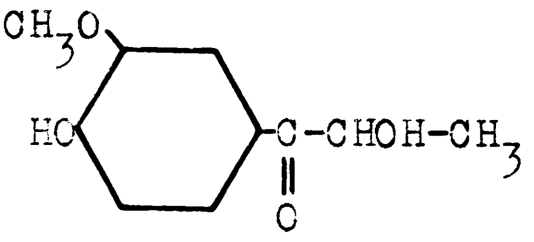
Hewson (159) investigated the action of nitrobenzene and alkali on a number of the ethanolysis products from wood. The diketone, 1-(4-hydroxy-3-methoxyphenyl)-1-2-propanedione (XIV) was found to yield only small amounts (up to 11%) of vanillin, while the treatment of 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (XXXIII) and the corresponding 2-ethoxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (XII), (both capable of enolization) gave slightly increased yields, 15 and 20% respectively. These results have been included in Table XII for the sake of comparison.



(XII)



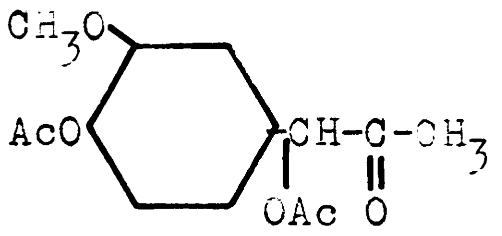
(XIV)



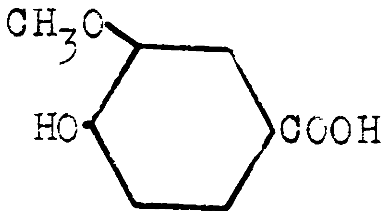
(XXXIII)

The low yields of vanillin obtained from the above ethanolysis products, compared with the high yields obtainable from wood, (up to 46% from maple, calculated on the Klason lignin content) and monomers of the isoeugenol type, lends support to Hibbert's view that such compounds merely represent stabilized end-products, rather than true "lignin building units" (4).

In an attempt to oxidize 1-acetoxy-1-(4-acetoxy-3-methoxyphenyl)-2-propanone (LXXIV) with nitrobenzene and aqueous alkali at reflux temperature, no vanillin could be isolated from the reaction mixture. Instead a 15% yield of vanillic acid (LXXV) was obtained, (5, Table XII).

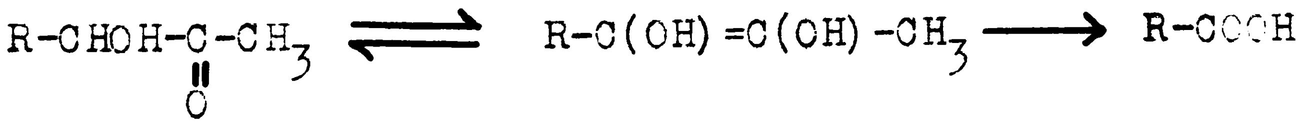


(LXXIV)



(LXXV)

Since vanillin has been shown to be stable under the conditions employed in these alkaline oxidations with nitrobenzene (159), the formation of vanillic acid must be assumed to take place by the cleavage, (under the influence of alkali alone), of the enediol of 1-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone (XXXI), formed from (LXXIV) by deacetylation in the alkaline medium.

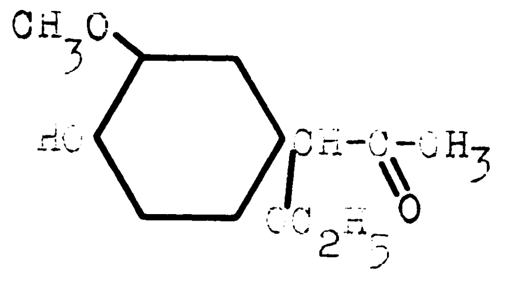


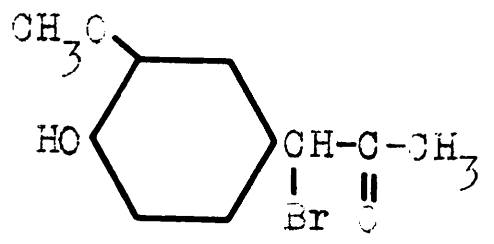
$$R\text{-}CHOH\text{-}\underset{\underset{O}{\|}}{C}\text{-}CH_3 \rightleftharpoons R\text{-}C(OH)\text{=}C(OH)\text{-}CH_3 \longrightarrow R\text{-}COOH$$

(XXXI)

R = guaiacyl.

The ease of formation of the enediol from (XXXI), is indicated by the results of Mitchell (163), who showed that 1-bromo-1-(4-hydroxy-3-methoxyphenyl)-2-propanone (LXXVI) was converted into 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (XII), when treated with dilute sodium hydroxide at 50°, in a yield of 70%. Further supporting evidence for the mechanism, is to be found in the results of West (70), who showed that on heating the corresponding 1-ethoxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone (XVIII) in 10% sodium hydroxide solution overnight at 50°, vanillic acid was formed in a yield of 6%.


(XVIII)


(LXXVI)

Investigation of the alkali-soluble-fraction from the alkaline nitrobenzene oxidation of (LXXIV), showed that conversion into (XII) had not occurred. Due to the small amount of starting material available (0.150 g.) a more complete analysis of the reaction products could not be carried out.

(b) The Nitrobenzene Oxidation of Isolated Lignins

In an investigation on the action of alkali and nitrobenzene on two ethanolysis products from maple wood, namely amorphous ethanol lignin and high boiling "lignin tars", Hewson (159) obtained yields of 7 and 14% of aldehydes (isolated as their m-nitrobenzoylhydrazones) respectively, (1 and 2, Table XIII). In the present investigation, a number of lignins isolated under various conditions, were subjected to alkaline oxidation with nitrobenzene.

Ozonized maple ethanol lignin in which one equivalent of

Table XIII

Nitrobenzene Oxidation of Isolated Lignins

No.	Lignin	Wt. used g.	Nitrobenzene cc.	2N NaOH cc.	Amorphous product g.	Bisulphite g.	Fractio %
1[a]	Maple Ethanol Lignin	7.83	12	400	4.6	-	7.3
2[a]	Maple "Lignin-Tars"	10.4	18	400	3.9	-	14.6
3	Ozonized Maple Ethanol Lignin	1.0	3	100	-	0.008	0.8
4	Hydrol Lignin-1[b]	1.17	3	100	-	0.316	27.2
5	Hydrol Lignin-2[b]	3.0	10	200	4.0	0.095	2.8

[a] Experiment of W. Hewson (159)

[b] Maple hydrol lignin, isolated under neutral conditions

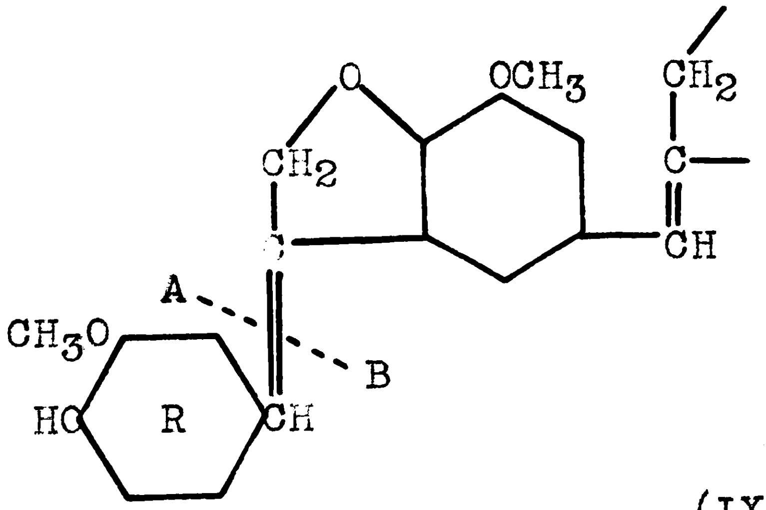
[c] Non-distillable residue left after rehydrogenation of spruce hydrol lignin isolated in the presence of alkali

ozone had been absorbed per C_6C_3 lignin unit, gave, on oxidation by the standard procedure, a yield of aldehydes amounting to less than 1% of the weight of the starting material.

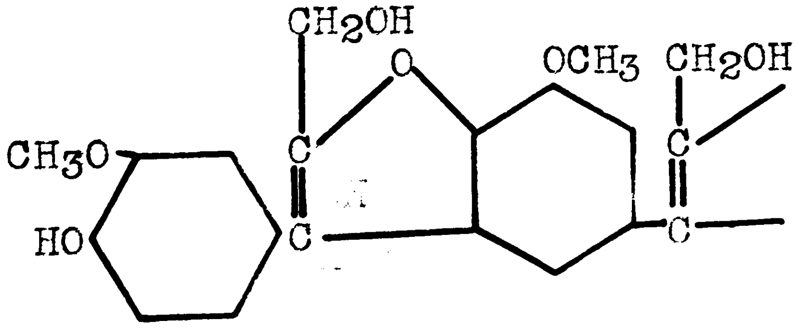
Hydrol lignin, isolated from maple wood by the action of neutral ethanol-water solution (1:1) at 165°, under 4000-5000 pounds pressure of hydrogen, in the presence of "Raney Nickel Catalyst", gave a yield of 27% of mixed aldehydes which were shown to consist of vanillin and syringaldehyde in the ratio of 1:2.5. The hydrol lignin apparently had undergone relatively little change during its isolation under the above approximately neutral conditions. In the case of a sample of "hydrol lignin" isolated from spruce wood by the action of alkaline ethanol-water solution at 190° in the presence of hydrogen and a "Raney Nickel Catalyst" followed by rehydrogenation at 200° over copper-chromoxide catalyst and subsequent fractionation of the hydrogenated product, it was found that the residual non-distillable portion gave no vanillin.

The relatively high yields of vanillin (11 and 18%) obtained on the alkaline oxidation of 1-(4-hydroxy-3-methoxy)-1-2-propanedione (XIV) and 1-(4-hydroxy-3-methoxyphenyl)-propane (LXXIII) respectively (Table XII), as compared with the low yields of aldehydes obtained from lignins, in which the presence of an active or potential ethylenic bond has been demonstrated, suggest that oxidation of the side chain possibly is prevented by the presence of a carbon to carbon linkage between the side chain of one unit and the aromatic nucleus of a second. The high yield of aldehydes obtainable from woody tissues may therefore, be regarded as supporting evidence for the presence in protolignin of active or potential ethylenic bonds.

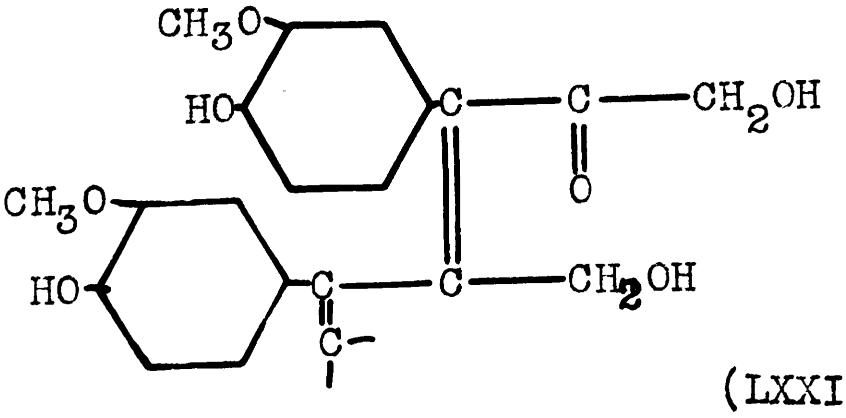
The condensation of propylphenol units of the type $R-CH_2-CO-CH_2OH$ $R-CH=C(OH)-CH_2OH$ (where R is 4-hydroxy-3-methoxyphenyl), can be visualized as occurring in three ways to give rise to theoretical lignin polymers of types (LXXVII, LXXVIII and LXXIX).



(LXXVII)



(LXXVIII)



(LXXIX)

Alkaline oxidation of a polymer of the type (LXXVII) with nitrobenzene, would be expected to take place at the double bond (A, B) to yield one mole of vanillin for each polymeric chain. With a polymer of the type (LXXVIII), however, this would not be expected to occur readily, since in each case the formation of vanillin would require the additional cleavage of the carbon-carbon bond between the side-chain and the adjacent aromatic nucleus (LXXVIII). The occurrence of a polymer of the type (LXXIX) in the guaiacyl series is regarded by Hibbert (4) as much less likely than either of the other two types. Alkaline oxidation of such a polymer, with nitrobenzene, would lead to the formation of one mole of vanillin for each C_6-C_3 unit.

The condensation of syringyl units, to give rise to polymers of the types (LXXVII) and (LXXVIII), is precluded by the blocking of the 5-position with a second methoxyl group. Instead, polymers of the styrene, or dicinnamyl ether type, or of the type (LXXIX) might be expected (page 41). Evidently, alkaline oxidation of a polymer of the type (LXXIX), would give syringaldehyde in high yields. Evidence for such a polymer, which should form a reversible system with alkalies and acids, is found in the difference in the yields of aldehydes obtainable from spruce and maple woods on alkaline oxidation with nitrobenzene. The maximum yield of 25% of vanillin from spruce wood (and other gymnosperms) suggests the presence of a tetramer of the type (LXXVII), since only the end group (R) would be oxidized to vanillin. It is assumed that the syringyl nucleus is present in maple lignin entirely in the form of (LXXIX) and that of the guaiacyl entirely as (LXXVII) then the theoretical yield of syringaldehyde would amount to 50% of the total Klason lignin, while that of vanillin (based on the yield from spruce, 25%) comes to only 12.5%, a result in approximate agreement with the experimental data.

C. Miscellaneous Oxidation Experiments

1. Alkaline Oxidation of Maple Wood with Organic Nitro Compounds other than Nitrobenzene

In an attempt to increase the yield of aldehydes above that obtainable by oxidation with nitrobenzene, the oxidizing action of several other organic nitro compounds was investigated.

(a) Alkaline Oxidation of Maple Wood with Nitrobenzaldehyde

The alkaline oxidation of maple wood with m- and p-nitrobenzaldehyde was carried out by the standard nitrobenzene oxidation technique (Table XIV). When the weight of m-nitrobenzaldehyde, used for the oxidation, was chemically equivalent to the amount of nitrobenzene employed in the standard oxidation procedure, the yield of aldehydes from 10 g. of maple wood (2.0 g. of Klason lignin) was only 15.5%. A large amount of amorphous product (9.9 g.) was obtained, however, containing 2.85% methoxyl, which suggested that the oxidizing agent had been removed by condensation with the lignin.

On repeating this experiment using a large excess of m-nitrobenzaldehyde (three times the corresponding amount of nitrobenzene) the yield of "total aldehydes" was 48.9%, the highest yield isolated from maple wood in these investigations (3, Table XIV). The yield of the amorphous product was correspondingly increased to 20.5 g. (OCH_3=1.58%). The total yield of

Table XIV

Oxidation of Maple Wood with Organic Nitro Compounds

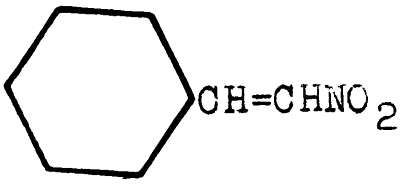
2 hours at 160°C.

Oxidizing Agent	Amt. used g.	Woodmeal g.	2N NaOH cc.	Wood Residue g.	Amorphous Product g.	Total Aldehydes %
Nitrobenzene	6	10.0	200	-	-	44.0
m-Nitrobenzaldehyde	7.3	10.0	200	4.1	9.90	15.5
m-Nitrobenzaldehyde	21.9	10.0	200	5.5	20.5	48.9
p-Nitrobenzaldehyde	7.3	10.0	200	4.9	10.2	10.5
-Nitrostyrene	4.4	4.7	100	2.0	1.20	6.4
Nitromethane	11.0	10.0	200	4.4	1.60	0.0

methoxyl, isolated as aldehydes (calculated from the methoxyl analysis of the mixed m-nitrobenzoylhydrazones), and as the amorphous product, amounted to 97.9% of the initial methoxyl in the wood.

(b) Alkaline Oxidation with ω-Nitrostyrene

The use of ω-nitrostyrene (LXXX) was suggested by the intermediate character of its nitrogroup, which, since it is conjugated with the benzene ring, should be between that of an aromatic and an aliphatic nitro group.



(LXXX)

Oxidation of maple wood with w-nitrostyrene gave 6.4% of aldehydes.

(c) Alkaline Oxidation with Nitromethane

Oxidation of maple wood with alkali and nitromethane (5, Table XIV) gave no aldehydes, and only very small amounts (4.5%) of benzene soluble oils.

The decreasing yields of aldehydes obtained on oxidation of maple wood with nitrostyrene and nitromethane respectively, show that the oxidizing action of the nitro group in this reaction is related to its attachment to the aromatic ring.

2. Oxidation of Maple Wood with Nitrobenzene in Pyridine Medium

The isolation of lignin by means of alkali brings about extensive changes in the proto-lignin during the reaction. The use of another, less drastic alkaline lignin extractant was therefore indicated in order to increase the yields of aldehydes obtained by nitrobenzene oxidation of plant material. Hewson (159) has employed milder inorganic alkaline reagents, such as trisodium phosphate and potassium carbonate in place of sodium hydroxide, in the oxidation of maple wood with nitrobenzene, and obtained yields of 23 and 10% of the Klason lignin respectively.

The use of pyridine as a medium for nitrobenzene oxidation might possibly be more effective in view of its basic properties and solubilizing effect, whereby the nitrobenzene might react in true solution.

On treating maple wood at 160° with nitrobenzene and pyridine, no aldehydes were obtained, less than 1% of the Klason lignin having been removed from the wood during the reaction.

3. Alkaline Oxidation of Maple Wood with Nitrobenzene in Ethanol

Treatment of maple wood, in ethanol at reflux temperatures, with nitrobenzene and alkali, gave no aldehydes. Instead, the ethanol was oxidized by the nitrobenzene to acetic acid.

4. The Alkaline Oxidation of Waste Sulphite Liquor

(a) Oxidation with Sodium Nitrite and Alkali

The action of sodium nitrite and alkali on waste sulphite liquor was investigated under various conditions of reaction (3-8, Table XV). The strong oder of ammonia, present at the end of each reaction, indicated that considerable oxidation had taken place. There was however, only a slight increase in the yield of vanillin, over that obtained by the action of alkali alone.

(b) Oxidation with Alkali and Nitrobenzene

The attempted alkaline oxidation of waste sulphite liquor with nitrobenzene, according to the Freudenberg procedure, but without agitation, gave no increase in the yield of vanillin over that expected by the action of alkali alone (4%) under similar conditions, but with good agitation throughout the course of the reaction, the yield of vanillin from waste sulphite liquor was increased to 16.5% (Table XV).

(c) Oxidation with m-Nitrobenzene Sulphonic Acid

Since it had been found that, due to its insolubility in water, nitrobenzene gave no increase in the yield of vanillin from waste sulphite liquor, unless considerable agitation were

Table XV

Alkaline Oxidation of Waste Sulphite Liquor

Oxidizing Agent	Amt. g.	2N Alkali cc.	Time of Reaction hrs.	Temp. °C.	Yield Vanillin %	
None	-	22.5	3	160	3.9	
"	-	22.5	6	100	2.4	
Sodium Nitrite	21	22.5	3	160	2.9	
" "	8.5	22.5	3	160	6.4	
" "	4.2	22.5	3	160	4.7	
" "	8.5	22.5	3	100	2.3	
" "	8.5	45	3	160	3.3	
" "	8.5	11.2	3	160	5.9	
Nitrobenzene	36	22.5	3	160	6.2	(no agitation)
"	36	22.5	3	160	16.5	(agitation)
m-Nitrobenzene Sulphonic Acid	47	32.3	3	160	10.5	(no agitation)

provided, m-nitrobenzene sulphonic acid was employed, to determine whether the increased solubility of its sodium salt over that of nitrobenzene would bring about the desired increase in the yield of vanillin. On heating waste sulphite liquor with alkali and m-nitrobenzene sulphonic acid for three hours at 160° without agitation, the yield of vanillin amounted to 10.5%, as compared to 3.9%, obtained by the action of alkali alone (Table XV).

EXPERIMENTAL

PART A The Nitrobenzene Oxidation of Plant Materials

1. Preparation of Woodmeal

All experiments in this investigation, unless otherwise stated, were carried out with solvent extracted woodmeal, prepared in the following manner:

> Air dried chips were passed through a 40 mesh screen in the Wiley Mill, and the resulting woodmeal extracted for 48 hours with ethanol-benzene (1:1), followed by 24 hours with ethanol, then washed for 12 hours with hot water and air dried.

2. Klason Lignin Analysis (Tables III, IV and V)

The moisture content of the air dried woodmeal was determined by drying a weighed sample overnight at 105°, and the Klason lignin analysis carried out on the oven dried sample by the method of Ritter, Seborg and Mitchell (163) involving the use of 72% sulfuric acid.

3. Methoxyl Analyses of Wood Samples (Tables III, IV and V)

0.050 g. samples of oven dried woodmeal were analyzed by the method of Peniston and Hibbert (163).

4. Oxidation of Plant Materials with Nitrobenzene and Alkali

The nitrobenzene-alkali pressure, oxidation reactions were carried out in a stainless steel pressure vessel (500 cc. capacity), fitted with a thermometer well and removable baffles, consisting of two strips of monel metal, perforated with 1/4" holes, and fitted together in the form of an X (Fig. 2.)

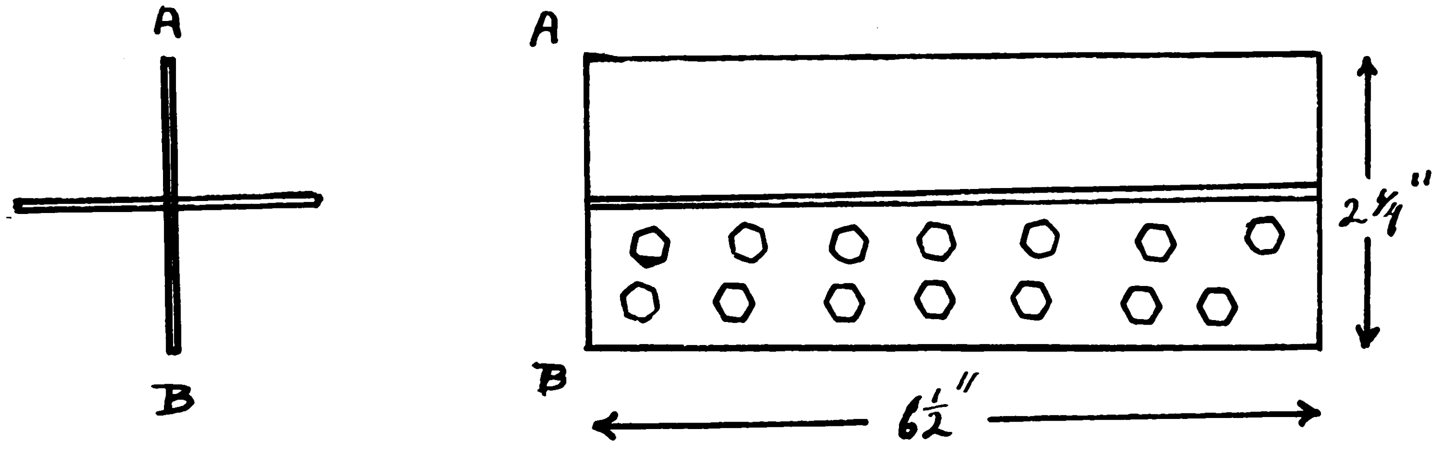


FIGURE II

Heating was electrical, and agitation was obtained by rotating the bomb in a horizontal position at 100-150 r.p.m., throughout the course of the reaction.

The standard oxidation procedure, developed by the writer, and based upon the method of Freudenberg (49) was as follows: The solvent extracted (see page 115) woodmeal in an amount equivalent to 8-9 g. of Klason lignin, nitrobenzene (24 cc) and aqueous sodium hydroxide (400 cc of 2N), were introduced into the pressure vessel and heated at 160°, with violent agitation, for 2 hours. After cooling, the alkaline reaction mixture was steam distilled until the distillate had become clear, in order to remove volatile nitrogenous products (nitrobenzene, azobenzene and aniline) and the residual mixture filtered. The wood residue was washed well with alkali (5%) then with water, dried at 105° and weighed. The combined filtrate and washings were made acid to Congo Red, and the resulting mixture (in which a large amount of solid amorphous product was present), continuously extracted with benzene for 48 hours. The benzene extract was shaken successively with five 50 cc. portions of each of the following reagents: (i) sodium bisulphite solution (10%); (ii) sodium bicarbonate solution (8%); (iii) sodium hydroxide solution (5%) and the three extracts then analyzed.

(i) Sodium bisulphite soluble fraction~~s~~ The sodium bisulphite extract was acidified, the sulfur dioxide removed under reduced pressure at room temperature, the solution filtered and diluted to 500 cc. The "total aldehydes" in an aliquot of this solution (50 cc) were determined as m-nitrobenzoylhydrazones by treatment of the solution at 60°, with m-nitrobenzoylhydrazine (0.5 g.) after first buffering with sodium acetate (10 g.). After half an hour at 60°, the mixture was cooled to room temperature and allowed to stand overnight. The resulting hydrazone was filtered on a tared sintered glass filter, then dried for one hour at 105° and weighed. The remainder of the solution (450 cc) was back extracted continuously with benzene for 24 hours. The benzene extract was dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure. After drying the residue for one hour in the Abderhalden at 60°, there was obtained a brown, often partially crystalline product. In the case of the gymnosperms this product could be purified either by sublimation, or directly by charcoaling and recrystallization from water, giving pure vanillin, m.p. 81-82°. With the angiosperms, the mixture of vanillin and syringaldehyde in the crude bisulphite fraction was separated as outlined in paragraph 8 of this section (p. 119).

(ii) Sodium bicarbonate soluble fraction: The sodium bicarbonate extract was acidified, extracted with ether, dried with anhydrous sodium sulphate and the ether removed. The resulting, partially crystalline residual product was dried in the Abderhalden at 60° and weighed.

(iii) Sodium hydroxide soluble fraction: The sodium hydroxide extract was acidified, and extracted with chloroform or ether. The resulting extract was dried over anhydrous sodium sulphate, the solvent removed and the residual product dried and weighed.

5. Nitrobenzene Oxidation of Spruce and Maple Woods (Table I)

Spruce and Maple Woods were oxidized and the reaction mixtures analyzed as detailed above.

6. The Effect of Time, Amount of Woodmeal and Reagents on the Yield of Aldehydes from Maple Wood

Solvent extracted maple wood was oxidized under a variety of conditions as detailed in Table II .

7. Modified Oxidation Procedure for Smaller Amounts of Plant Materials

Extracted woodmeal (containing approximately 1.0 g. of Klason lignin), nitrobenzene (3 cc) and aqueous sodium hydroxide (100 cc of 2N) were heated at 160° with agitation for 2 hours. After cooling, the alkaline reaction mixture was filtered, the wood residue washed with alkali and water, and the combined filtrate and washings made acid to Congo Red. The acid solution was extracted continuously with benzene for 36 hours, and the benzene extract then shaken with five successive 30 cc portions of 10% sodium bisulphite solution. After acidification and removal of SO_2 in the usual manner, the volume of the solution was made up to 250 cc and the "total aldehydes" determined in a 50 cc aliquot of this solution. The remainder (200 cc) was back extracted with benzene and worked up in the usual manner.

8. Separation of Vanillin and Syringaldehyde

The separation of vanillin and syringaldehyde from the bisulphite fraction obtained from the angiosperms was carried out as detailed below for maple wood.

(a) Precipitation of the Syringaldehyde Obtained from Maple Wood as the Ammonium Salt.

The residual crude bisulphite fraction (0.70 g.) left after removal of the solvent, was dissolved in anhydrous ethanol (50 cc), mixed with 3 cc of a solution (7%) of dry ammonia in anhydrous ethanol, and the mixture allowed to stand for one hour at 0°. The precipitated ammonium salt was filtered, washed with anhydrous ethanol, then with ether, and dried in a vacuum desiccator for one hour; Yield: 0.631 g. This ammonium salt was suspended in 2 cc of water, decomposed with sulfuric acid (2 cc of 15%), the syringaldehyde removed by filtration, washed with water and dried in the Abderhalden at 60°; Yield 0.518 g. (91% of theoretical from the ammonium salt) m.p. 105-110°. The crude syringaldehyde was recrystallized twice from water, and dried, giving 0.390 g. of a pale yellow crystalline product; m.p. 110.5-112°. A mixed melting point with an authentic sample showed no depression. OCH_3: found: 33.9 and 34.0%; calculated: 34.1%. The yield of vanillin was obtained by subtracting this value from the yield of "total aldehydes", determined as their m-nitrobenzoylhydrazones.

(b) Fractional Sublimation of Vanillin and Syringaldehyde

An aliquot of the crude bisulphite fraction (0.20 g.) was fractionally sublimed by the method of Hawkins, Wright and

Hibbert (161). To ensure complete removal of the vanillin, it was found necessary to allow the sublimation to proceed at 60°/10 mm for from 8 to 10 hours. After washing the sublimed vanillin from the cold finger, sublimation was then continued at 100°/0.3 mm until no further material could be sublimed. The crude vanillin sublimate was purified by conversion into the m-nitrobenzoylhydrazone (m.p. 207-9°) and the yield of vanillin based upon the weight of this crude hydrazone. On recrystallizing from methanol, the melting point of the hydrazone could be increased to 210-12°. A mixed melting point with an authentic sample showed no depression. The crude syringaldehyde was purified by recrystallization from water giving a product with the correct melting point (111-112°).

9. Taxonomic Investigation of Plant Species by Means of Alkaline Nitrobenzene Oxidation

A wide variety of plant species were oxidized with nitrobenzene and alkali, and detailed results are shown in Tables VI to VIII.

10. Isolation of p-Hydroxybenzaldehyde from the Bisulphite Fraction of Corn Stalks

In duplicate runs, extracted corn-stalk woodmeal (25 g. containing 5.0 g. of Klason lignin), nitrobenzene (15 cc) and aqueous sodium hydroxide (300 cc of 2N) were heated at 160°, with agitation, for 3 hours. The combined alkaline reaction mixtures were steam distilled and the aldehydes recovered by the standard procedure. A yield of 1.503 g. of a viscous oil was obtained from the bisulphite soluble fraction.

Fractional distillation of the crude bisulphite fraction: The fractionation was carried out in a semi-micro Cooke-Bower fractionating column. The first four crystalline fractions, distilling at a bath temperature of 130 to 160°/0.2 mm and amounting to 0.825 g, OCH_3-12-17% (Table IX page 90), suggested the product was probably a mixture of vanillin and p-hydroxybenzaldehyde. The remaining two fractions, b.p. 210-30°/0.25 mm, (0.284 g.) consisted of syringaldehyde. Recrystallized from water, m.p. 110-112°; yield 0.235 g. A mixed melting point showed no depression.

Solvent separation of vanillin and p-hydroxybenzaldehyde: The combined fractions 1 to 4 (Table X) (0.401 g.) were suspended in cold benzene (5 cc), the mixture stirred for one hour and then centrifuged. The benzene insoluble residue was washed with cold benzene (1 cc) and dried (A). The benzene solution was taken to dryness under reduced pressure, and the residue again treated with cold benzene (3 cc), and the insoluble fraction centrifuged and washed as before (B). The benzene solution was evaporated, and the residue dried in the Abderhalden; yield 0.316 g. This crude product was recrystallized twice from water yielding vanillin (0.207 g.); m.p. 81-2°. The combined benzene insoluble fractions (A) and (B) (0.081 g.) were recrystallized twice from water giving a white crystalline product (0.063 g.); m.p. 116-7°. A mixed melting point with p-hydroxybenzaldehyde gave no depression.

Preparation of p-hydroxybenzaldehyde phenylhydrazone: To a solution of the natural p-hydroxybenzaldehyde (0.050 g. in 2 cc of water), a solution of phenylhydrazone hydrochloride (0.100 g. in 2 cc) was added, and the mixture digested on the steam bath for 20 minutes. After standing one hour in the cold room, the mixture was filtered and the product washed with cold water. The crude material (0.095 g.) was recrystallized twice from ethanol-water (1:1), yielding colourless crystals which darkened upon standing exposed to the light for a few days; m.p. 175-77°. A mixed melting point with the phenylhydrazone of synthetic p-hydroxybenzaldehyde gave no depression.

A summary of the yields of p-hydroxybenzaldehyde, vanillin and syringaldehyde is given in Table XI.

B. Alkaline Nitrobenzene Oxidation of Isolated Lignins and Related Compounds

1. Alkaline Oxidation of Propylphenol Derivatives with Nitrobenzene (Table XII)

(a) Oxidation of Isoeugenol and Related Compounds

The oxidation of a number of 1-(4-hydroxy-3-methoxyphenyl)-propane derivatives was carried out as detailed in experiments 1-4, Table XII, at 160° for 2 hours. After cooling, the reaction mixture was steam distilled, the residual solution acidified to Congo Red, and the mixture filtered to remove the resulting amorphous precipitate. The filtrate was extracted with benzene, and the benzene extract analyzed as in paragraph 4, page 116 . Detailed results are given in Table XII, page 98.

(b) Oxidation of 1-Acetoxy-1-(4-acetoxy-3-methoxyphenyl)-2-propanone

1-Acetoxy-1-(4-acetoxy-3-methoxyphenyl)-2-propanone (0.150 g.), nitrobenzene (1 cc) and aqueous sodium hydroxide (10 cc of 2N) were refluxed for one hour with stirring. The alkaline reaction mixture was extracted with ether to remove the nitrogenous product, acidified and filtered. The filtrate was extracted with ether, and the ether solution shaken with sodium bisulphite solution (10%), sodium bicarbonate (8%) and sodium hydroxide (5%). The bisulphite solution was analyzed with m-nitrobenzoylhydrazine in the usual way, and gave no aldehydes.

The bicarbonate solution was acidified, extracted with ether and, after drying with anhydrous sodium sulphate, the ether solution evaporated to dryness to give 0.030 g. of a crystalline product. Recrystallization from water yielded 0.018 g. of a white crystalline product; m.p. 205-6°. A mixed melting point with vanillic acid gave no depression.

The sodium hydroxide solution was acidified and extracted with ether as above. On evaporation of the ether solution, 0.008 g. of alkali-soluble oils were obtained.

2. Alkaline Oxidation of Isolated Lignins (Table XIII)

The alkaline oxidation of a number of isolated lignins with nitrobenzene, was carried out by the procedure employed for the oxidation of woodmeal. The experimental details and results are given in Table XIII, page 103.

C. Miscellaneous Oxidation Experiments

1. Oxidation of Maple Wood with Organic Nitro Compounds other than Nitrobenzene

Unless otherwise indicated, each oxidation was carried out using the equivalent quantity of nitro-derivative, and the same amount of alkali (2N), as was used in alkaline oxidation with nitrobenzene, at 160° for 2 hours. The reaction mixture was analyzed by the method already outlined for nitrobenzene (page 116) with the exception that the amorphous precipitate, formed on acidification of the alkaline reaction mixture, was filtered off prior to extraction with benzene. Detailed results are given in Table XI

(a) Alkaline Oxidation of Maple Wood with Nitrobenzaldehyde

Both m- and p-nitrobenzaldehyde were used, in place of nitrobenzene for the oxidation of maple wood. Steam distillation of the alkaline reaction mixture was omitted and the excess nitrobenzaldehyde, after filtering to remove the residual woodmeal, extracted from the alkaline reaction mixture with ether.

(b) Alkaline Oxidation with ω-Nitrostyrene

The oxidation of maple wood with ω -nitrostyrene was carried out by the standard procedure, and the results are given in Table XIV, page 109.

(c) Alkaline Oxidation with Nitromethane

Maple wood was oxidzied with a large excess (three times the equivalent amount of nitrobenzene) of nitromethane. After filtering to remove the wood residue, the alkaline reaction mixture was acidified to Congo Red, and the amorphous precipitate filtered off, washed and dried. The filtrate and washings were extracted by hand with benzene (100, 75, 50, 50 cc)(a strong emulsion was formed which was broken by the addition of small amounts of methanol) and the benzene extract worked up in the usual manner.

2. Oxidation of Maple Wood with Nitrobenzene in Pyridine Medium

Extracted maple wood (10.0 g.), nitrobenzene (6 cc) and pyridine (200 cc) were heated at 160° for 2 hours with agitation. After cooling, the reaction mixture was filtered and the residual woodmeal washed with pyridine and water and dried; yield: 8.75 g. The filtrate and pyridine washings were concentrated under reduced pressure, and the concentrated solution poured into water (400 cc). The aqueous solution was decanted from the small amount of tars which precipitated out, acidified to Congo Red with hydrochloric acid and extracted with benzene. The benzene extract was shaken with five successive 30 cc portions of 10% sodium bisulphite solution, and after acidification and removal of sulfur dioxide, a portion of the aqueous extract was treated with 2,4-dinitrophenylhydrazine hydrochloride solution. No hydrazone was formed.

3. Nitrobenzene-Alkali Oxidation of Maple Wood in an Ethanol Medium

Maple woodmeal (38.5 g.) nitrobenzene (25 cc), sodium hydroxide (40 g.) and ethanol (500 cc) were refluxed, with stirring, for 12 hours. After cooling, the reaction mixture was filtered and the wood residue washed with ethanol. The filtrate and washings were neutralized with ethanolic hydrogen chloride solution, concentrated under reduced pressure to 150 cc, and poured into water (1000 cc). The water-insoluble products were removed by centrifuging and the aqueous solution continuously extracted with benzene for 48 hours. The benzene extract was shaken with five successive 30 cc portions of sodium bisulphite solution (10%) and after acidification and removal of sulfur dioxide, the bisulphite extract treated with 2,4-dinitrophenylhydrazine hydrochloride solution. No hydrazone was formed on standing overnight.

4. Alkaline Oxidation of Waste Sulphite Liquor

Concentrated neutralized waste sulphite liquor (82 g. containing 11 g. of lignin) was treated, in alkaline solution, with a variety of oxidizing agents as detailed in Table XV.

(a) Oxidation with Sodium Nitrite and Alkali

Oxidation was carried out under a variety of conditions, as shown in Table XV and the acidified reaction mixture containing an amorphous precipitate, extracted with benzene. The benzene solution was extracted with sodium bisulphite solution (10%) and the vanillin in the bisulphite extract determined as the m-nitrobenzoyl hydrazone in the usual way.

(b) Oxidation with m-Nitrobenzenesulphonic Acid

Waste sulphite liquor (82 g.) nitrobenzenesulphonic acid (47 g.), sodium hydroxide (22.5 (+9.8 g. required to neutralize the sulphonic acid)), and water (118 cc) were heated for 3 hours at 160° without agitation. The reaction mixture was steam distilled, filtered, and worked up by the procedure outlined for the oxidation of woodmeal with nitrobenzene.

(c) Oxidation with Nitrobenzene

In two experiments, as detailed in Table XV, waste sulphite liquor was oxidized with nitrobenzene and alkali, with and without agitation, respectively. The reaction mixture was worked up by the standard procedure.

SUMMARY

The alkaline oxidation of maple wood and other angiosperms with nitrobenzene, at high temperatures and pressures, results in conversion of the lignins into a mixture of vanillin and syringaldehyde in a yield of 40-50%, the ratio of the aldehydes being approximately 1 : 3. With gymnosperms, treated similarly, vanillin only was formed in yields of 20-25% of the Klason lignin. This serves to establish the similarity of the lignin present in all plants as of an essentially aromatic type, the nuclei present being either of the guaiacyl or syringyl types or both.

With corn stalks this oxidation procedure resulted in the isolation of an additional product, namely, p-hydroxybenzeldehyde. Preliminary evidence has indicated its presence in the oxidation products from other monocatyledons.

Investigation of fifty plant species using this oxidation technique, has shown that a close correlation exists between the positive Mäule test shown by angiosperms and certain other plants, and the occurrence of the syringyl grouping in the lignin present. With gymnosperms only vanillin is formed on oxidation, although a few exceptions, notably, _Podocarpus_ _amarus_, _P_. _pedundulata_, _Tetraclinis_ _articulata_ and the _Gnetales_ yielded both vanillin and syringaldehyde. The isolation of the latter from _Tetraclinis_ _articulata_, and not from _Callitris_

rhomboidea, supports their recent reclassification, involving séparation of Tetraclinis from Callitris. The results obtained with the Gnetales are of marked significance as indicating a definite chemical relationship of these plants to the angiosperms.

The alkaline oxidation of propyl-phenol derivatives with nitrobenzene has been shown to take place either by oxidation of an ethylenic bond adjacent to the aromatic ring, with resulting high aldehyde yields, or, where this bond is absent, by oxidation of the C_3 side chain with lower aldehyde yields.

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