STRUCTURE OF LIGNIN: ISOLATION OF NEW ETHANOLYSIS PRODUCTS FROM MAPLE WOOD

by

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CLAIMS TO ORIGINAL RESEARCH

1. A method, for the isolation of vanilloyl and syringoyl methyl ketones as pure crystalline compounds from the bisulfite-soluble fraction of the maple-wood-ethanolysis oils, has been developed.

2. The presence of vanillin and syringaldehyde in the bisulfite-soluble fraction of the maple-wood-ethanolysis oils has been confirmed by isolation of the two aldehydes as free compounds.

3. Two new compounds, namely, (4-hydroxy-3,5-dimethoxyphenyl)-propanone-2 and (4-hydroxy-3-methoxyphenyl)-propanone-2, have been isolated from the bisulfite-soluble fraction of the maple-wood-ethanolysis oils, and identified by direct syntheses.

4. Some properties of the diketones and of the new compounds have been determined.

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Part A. Historical Review

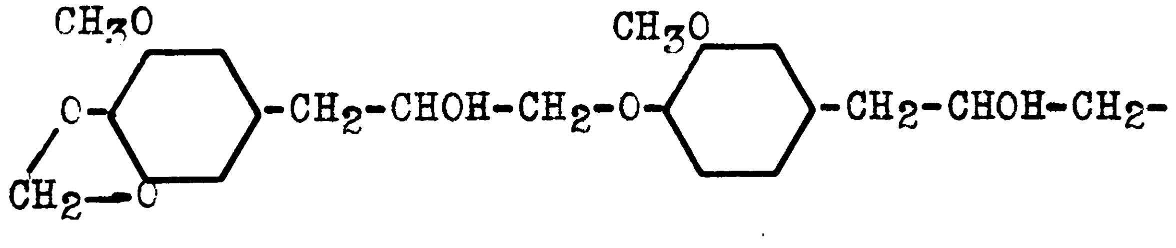
Early Developments in Lignin Chemistry

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 tissue (7), (ii) on data derived from the ethanolysis of the spruce lignin sulfonic 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 lignins. 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 chlorhydrin.

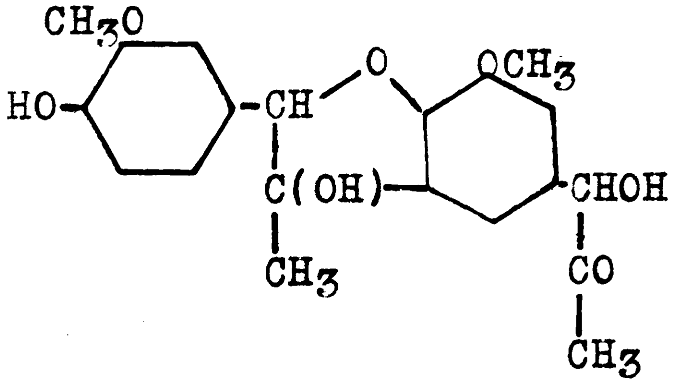
Freudenberg's earlier theory of the structure of lignin (12) was based essentially on the isolation of small amounts of catechol, protocatechuic acid and formaldehyde from spruce hydrochloric acid lignin, and on analyses of this lignin. These results indicated (a) aromatic character and absence of free phenol groups: (b) attachment of the methoxyl groups to aromatic nuclei; and (c) presence of aliphatic hydroxyl groups in side chains attached to the benzene nucleus. Freudenberg's modification 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 a "linear type" condensation polymer (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,



(I)

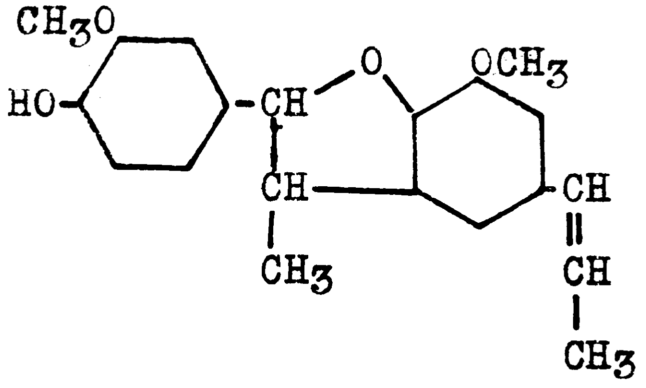
page 135). The assumed presence of the dioxymethylene group was based on the liberation of a small amount of formaldehyde (up to 1.2 per cent) (13,14,15). The side chains visualized by Freudenberg were $R-CHOH-CHOH-CH_2OH$, $R-CH_2-CHOH-CH_2OH$, $R-CHOH-CH_2-CHO$ and $R-CHOH-CO-CH_3$ (12, page 135). 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 they are in equilibrium with each other, at least <u>in vivo</u>.

On the basis of the studies on alkali degradation of spruce lignin, followed by methylation and oxidation (16,17,18), Freudenberg has 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 (19)).

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 on (i) alkaline oxidation of lignin, wood, and lignin sulfonic 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

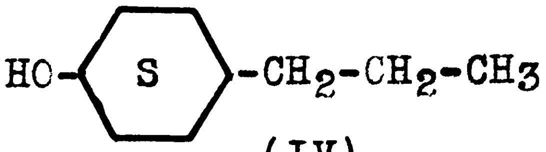
The action of alkali on lignin sulfonic acids has been investigated extensively following earlier observations (4,page 37) regarding the presence of vanillin, at least in small quantities, in the reaction mixture. With spruce lignin sulfonic acid a yield of 6-7 per cent of vanillin (20) and a smaller amount of acetovanillone (21) and guaiacol (22) are obtained, while, with oak lignin sulfonic acid, in addition to these there are present syringaldehyde (23), acetosyringone (24), and 1,3-dimethoxypyrogallol (23). The addition of an oxidizing agent such as metanitrobenzene sulfonic acid, in small quantity, to the alkaline spruce lignin sulfonic acid mixture gave a lower yield of aldehyde (25). More recently Freudenberg and co-workers have developed an alkali-nitrobenzene oxidation technique by which yields of 20-25% of vanillin from spruce wood and spruce lignin sulfonic acid are obtained. (26).

Although yields of 25% vanillin appear to be the maximum obtainable from soft woods, an extension of this method to maple and aspen woods gave yields of 46 and 48% respectively of mixtures of vanillin and syringaldehyde (27). If it be assumed that these aldehydes are derived from propylphenol units similar to those obtained by the ethanolysis of maple wood, these yields indicate that approximately 58-62% of the protolignin in angiosperms is aromatic in nature, and, thus the validity of Klason's assumption that lignin has a fundamentally aromatic structure is established.

Lautsch and Piazolo (28) oxidized a brominated spruce lignin with alkali and nitrobenzene and obtained 6-bromovanillin (8 per cent), a product which cannot be prepared directly from vanillin. This result is assumed to prove that the units in lignin are united with each other by etherification at the 4-position. Attempts to increase the yield of aromatic lignin oxidation products by substituting certain metallic oxides (29) for the nitrobenzene were unsuccessful.

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 (30), the protolignin being converted, in part, to 4-*n*-propylcyclohexanol (IV) and 3-(4-hydroxycyclohexyl)-propanol-1 (V) in yields of 19.5 and 5.8 per cent respectively (based on the Klason lignin content of wood). Using the carbon content of these isolated units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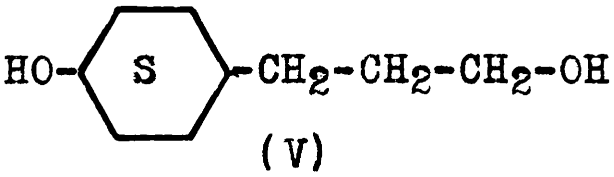

(V)

and of the "methoxyl-free protolignin" in the wood as a basis for calculation, the combined yield of propylcyclohexane derivatives represents a recovery of 36 per cent (30).

Examination of the yields of hydrogenation products from various amorphous fractions of a maple lignin isolated by ethanolysis (31) 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 the prevalence of -C-O-C- bonds between the propylphenol "lignin building-units" in those lignin 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.

Hatihama et al. (32) hydrogenated hydrochloric acid lignin in the presence of several less active catalysts, particularly nickel, and obtained a 50% yield of ether-soluble aromatic oils containing pyrocatechin and n-propylguaiacol. Similar results have been reported by Freudenberg and co-workers (33) who, following earlier work by Bobrov and Kolotova (34), extended the investigations to sulfite liquor. More recently, Freudenberg and Adam (35) 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. In this manner ether-soluble, tarry fractions (20-50% yield) containing among other products, phenols and phenolic ethers were obtained.

In addition to establishing the foregoing evidence for the presence of the propylphenol unit in lignin, hydrogenation studies have shown that a relatively large proportion of the propylcyclohexane derivatives obtained from wood (30) and certain lignins (31,36,37) contain oxygen atoms attached to the terminal carbon atom in the propyl side chain. This result provides the only experimental proof of the presence of oxygen in that position in protolignin.

A further contribution of the hydrogenating technique to lignin chemistry has been the classification of lignins according to their relative complexities (based on the yield and nature of hydrogenation resins). The results of Adkins and co-workers (37,38) have shown that sulfuric acid, soda and alkali lignins are more complex than either protolignin (30) or alcoholysis lignins (31,36).

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.

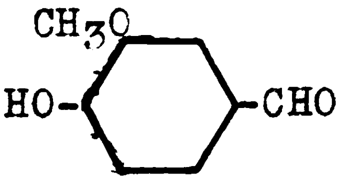
The belief that proto- and extracted lignins exist only as highly polymerized, complex substances led investigators to conclude that the amorphous water-insoluble product obtained by customary extraction methods was the only form in which lignin could be isolated. Precipitation into water from concentrated alcohol, acetic acid, or other solutions has been almost an invariable step in the isolation or 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 on only two occasions, namely, by Friedrich and Brüda (39) (methanolysis of beech) and by Brauns and Hibbert (40) (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 of this was found when Cramer, Hunter and Hibbert (41) 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 proportion (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 fraction; (b) the factors involved in their isolation, especially those relating to their actual presence as such in the wood or their appearance as 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 protolignin.

To date, approximately one-third (12% of Klason lignin content) of the water-soluble oils from maple wood has been shown to be composed of α-ethoxypropiovanillone (VIII) (41a) and its syringyl analog (IX) (41b), vanilloyl methyl ketone (X) (43a) and its syringyl analog (XI) (43b), vanillin (VI) (42) and syringaldehyde (VII) (42).



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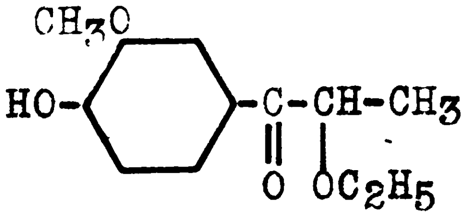

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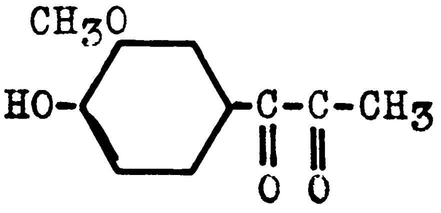
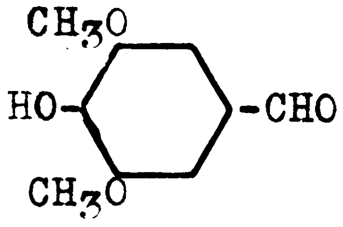


(X)



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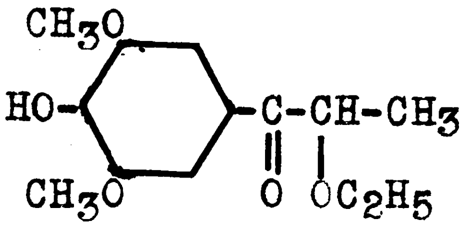

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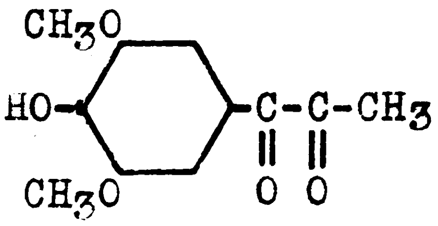


(XI)

An actual relationship between these monomeric units and extracted lignins has been established by Peniston, McCarthy and Hibbert (44). 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 (V) (by means of hydrogenation) interest has been centered on the potential significance of related compounds such as β-hydroxypropiovanillone (XII), 1-guaiacyl-3-hydroxy-propanone-2 (XIII) and their corresponding syringyl derivatives.

$R\text{-}CO\text{-}CH_2\text{-}CH_2\text{-}OH$

(XII)

$R\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}OH$

(XIII)

4. Miscellaneous Experiments

The effect of variables in the Klason lignin determination has been carefully studied by Freudenberg and Ploetz (45,46).

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 hardwoods. The use of hydrogen fluoride as a solvent for lignin has been suggested by Wiechert (47,48). 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 (49); in particular with respect to (i) the apparent condensation of lignin with protein material and (ii) the higher values found when the plant material is pre-dried at a relatively high temperature (100°C.).

Benson and co-workers (50,51), in an attempt to establish a lignin research program of industrial significance, have commenced a study of desulfonated (with sodium hydroxide) calcium lignin sulfonate. Their recent results (52) indicate that nitrated desulfonated lignin closely resembles nitrated butanol lignin.

Butanol lignin, obtained by treating wood with butanol-water and butanol-water-alkali at 160°C., has been studied extensively by Bailey (53). He concludes (54) from comparative butanolysis studies of aspen and jack pine that a portion of the lignin in softwoods (but not in hardwoods) is chemically bound to cellulose. The presence of glucosidic linkages involving phenolic hydroxyl groups of the lignin is rendered doubtful by the results of investigations (55) on model substances (glucosides of the ethanolysis lignin units).

The necessity for careful classification of lignin sources is re-emphasized in results obtained by Ritter (56) 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% at the center to 19.5% at the periphery.

The similarity of the behavior of coumarin and of acetic acid lignins towards diazomethane has led Wright (57) to suggest that lignins contain a coumarin-type of lactone linkage. von Wacek and Nittner (58) subjected beechwood tars to ozonolysis and from analyses of the reaction products concluded that substituted coumarones were present in the tars. The presence of two pyran rings in each "lignin building unit" (60) is assumed from absorption spectra studies (59) on spruce native lignin (60), 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 Synthesis of Propylphenol Derivatives

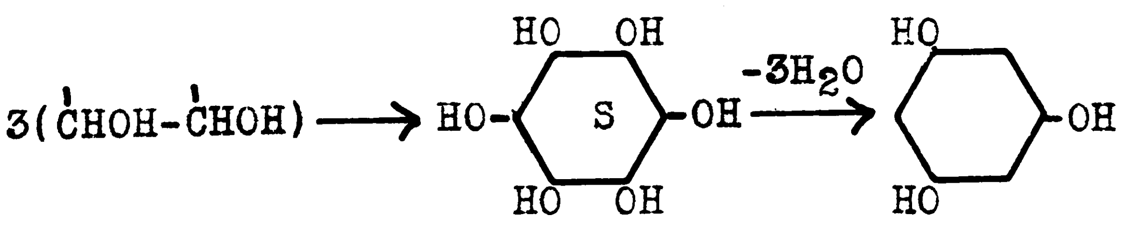
(1) Synthesis of Simple Phenols- Various theories concerning the synthesis of phenolic compounds in plants have been based on assumed transformation of hexoses (61,62), while a more recent theory (4) considers the phenolic substances as being formed from intermediate photosynthetic and/or plant respiratory products.

(i) Free Radical Theory (63)- Specific types are assumed to be present in the active stages of plant growth (formic acid (XIV), glycollic aldehyde (XV), and acetaldehyde (XVI)) in equilibrium with the corresponding enol-radicals:

$$HCOOH \rightleftharpoons \overset{|}{\underset{|}{C}}(OH)_2 \qquad CH_2OH.CHO \rightleftharpoons \overset{|}{C}HOH.\overset{|}{C}HOH \qquad CH_3.CHO \rightleftharpoons \overset{|}{C}H_2.\overset{|}{C}HOH$$

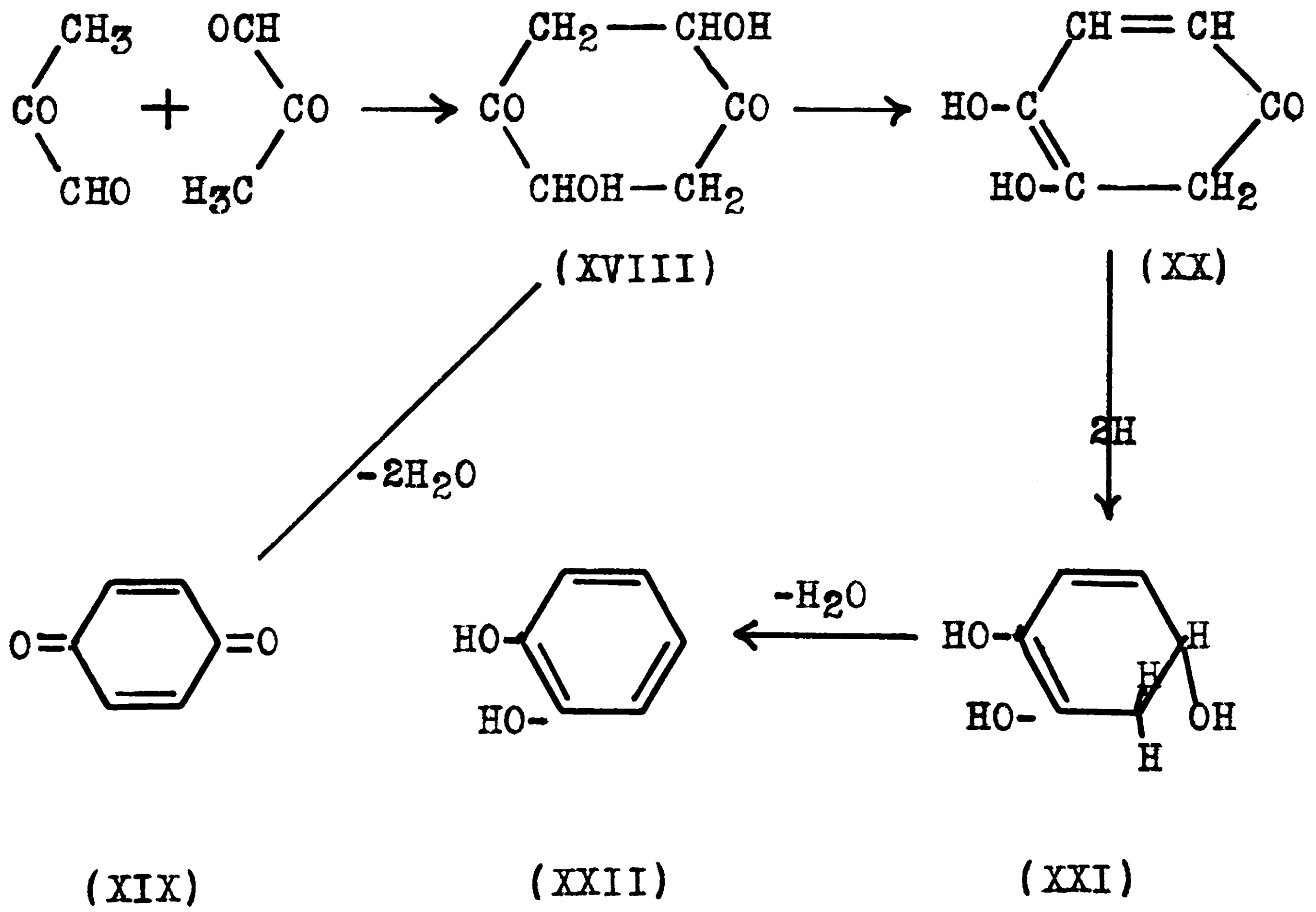
(XIV) (XV) (XVI)

Union of the free radicals may give rise to hydroaromatic derivatives which, by loss of water, could yield phenols; for example, phloroglucinol (XVII) from (XV):



(XV) Inositol (XVII)

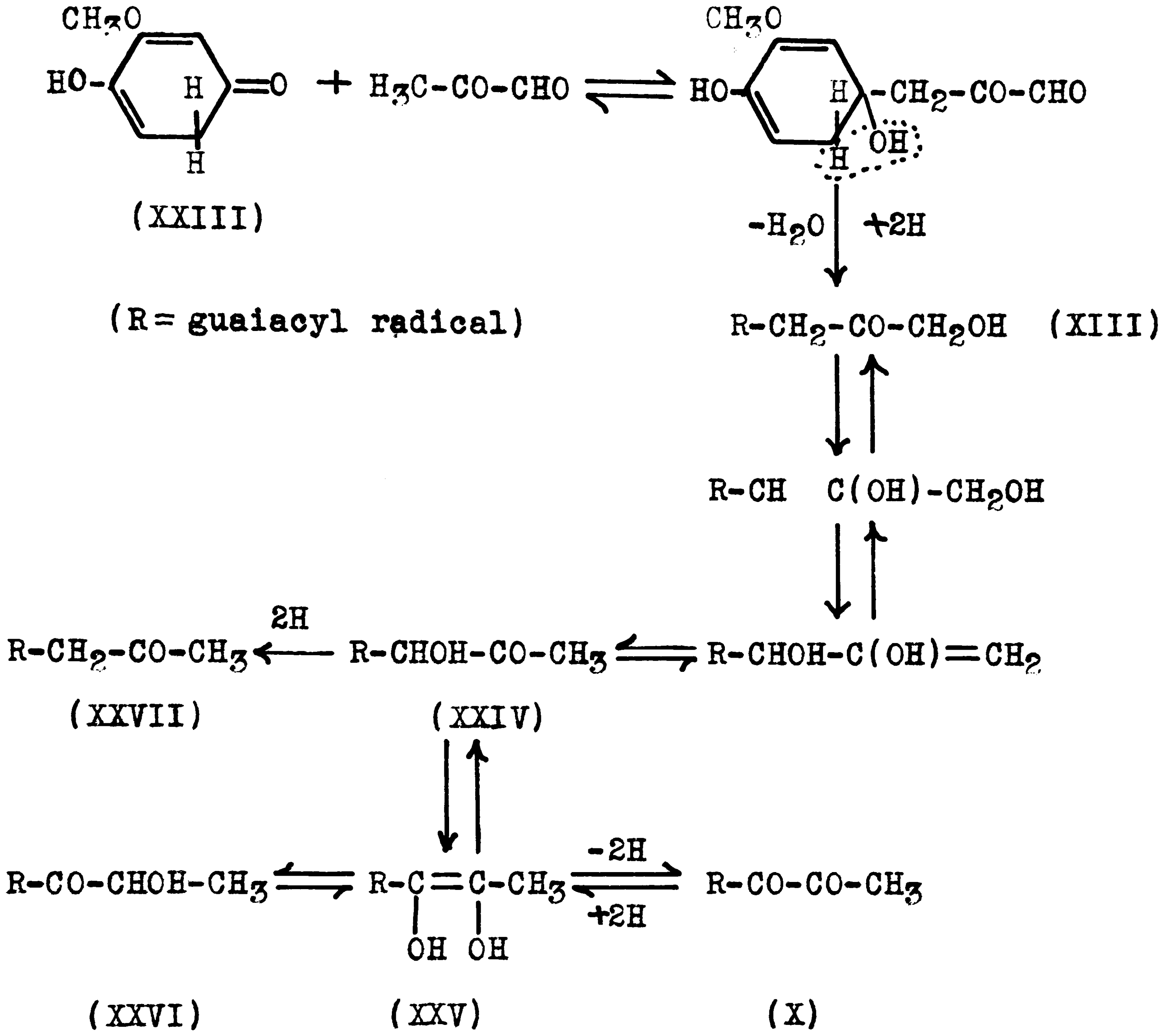
(ii) Methyl Glyoxal Theory of Phenol Formation (64) - Methyl glyoxal, which is a well-recognized intermediate in animal cell respiratory processes (65, page244), presumably occupies a somewhat analogous position in plant carbohydrate metabolism (66, page 264) and has actually been isolated from a number of higher plants (67). Hibbert suggests the possibility of two molecules of methyl glyoxal polymerizing to yield a cyclic dihydroxydiketone (XVIII) capable of undergoing the indicated reactions to give quinone (XIX) and 1,2-dihydroxy-4-ketocyclohexadiene (XX). This ketohexadiene,(XX),



on reduction could give rise to a hydroxy-enediol, (XXI) which could yield catechol (XXII) upon the loss of a mole of water.

Pyrogallol (the precursor of the syringyl nucleus) could be formed by enzymatic oxidation of catechol (XXII) just as the latter is formed from phenol (68).

(11) Suggested Plant Synthesis of Propylphenol Derivatives - Condensation of the "methoxylated" ketocyclohexadiene, (XXIII), with a third mole of methyl glyoxal, followed by the loss of a mole of water and reduction would yield (XIII). An intramolecular change involving an allyl shift in (XIII) would give the primary dismutation isomer, (XXIV), (not yet isolated) which could then yield the ene-diol (XXV), this in turn giving the benzoin derivative (XXVI). The benzoin derivative (XXIV) on reduction could yield the desoxybenzoin (XXVII). Moreover the ene-diol, (XXV) is a dihydro derivative of the 1,2-diketone (X).



The only reaction of this series which conceivably could be open to question is concerned with the rearrangement of (XIII) to (XXIV). Apparently there are no analogous reactions described in the literature, although, if it is assumed that (XIII) is in equilibrium with its aldehydo form ($R-CH_2-CO-CH_2OH$ $R-CH_2-CHOH-CHO$), then the reaction undergone by its analog, benzyl glycollic aldehyde, (XXVIII), in the presence on ethanol and sulfuric acid is highly significant (69), in view of its conversion, by this means, into a mixture of phenyl acetyl carbinol and benzoyl acetone. Furthermore, since it has been

$$2\ C_6H_5\text{-}CH_2\text{-}CHOH\text{-}CHO \longrightarrow C_6H_5\text{-}CHOH\text{-}CO\text{-}CH_3 + C_6H_5\text{-}CO\text{-}CO\text{-}CH_3$$

(XXVIII)

shown experimentally (70) that the veratryl derivative of (XIII) is converted into the ethyl ether of (XXVI) by ethanolic hydrogen chloride, the postulation of (XXIV) and (XXV) as intermediates would appear to be justified.

There is ample support in the literature for the postulated equilibrium (XXIV) $\rightleftharpoons$ (XXVI). The simplest, and best known examples of such dismutation reactions, in the case of 1,2-hydroxy ketones 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 of glyceric aldehyde $\rightleftharpoons$ dihydroxyacetone, etc. (71). A detailed review of this type of dismutation transformation is given in part C of this introduction (page 52). In this review it is pointed out that in the equilibrium system $R\text{-}CHOH\text{-}CO\text{-}CH_3 \rightleftharpoons R\text{-}C(OH){=}C(OH)\text{-}CH_3 \rightleftharpoons R\text{-}CO\text{-}CHOH\text{-}CH_3$ where R is a para substituted benzene radical, $R\text{-}CO\text{-}CHOH\text{-}CH_3$ 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 $R\text{-}CO\text{-}CHOH\text{-}CH_3$, namely, α-ethoxypropiovanillone (VIII) and -syringone (IX). In the light of the above literature references (69,70), it is probable that these substances (VIII) and (IX) represent only stabilized end products originating from the assumed lignin progenitors, 1-guaiacyl-3-hydroxypropanone-2 (XIII) and its syringyl analogue respectively; (see also page 30) in other words, the first members of the proposed new aromatic system of plant respiratory catalysts (4).

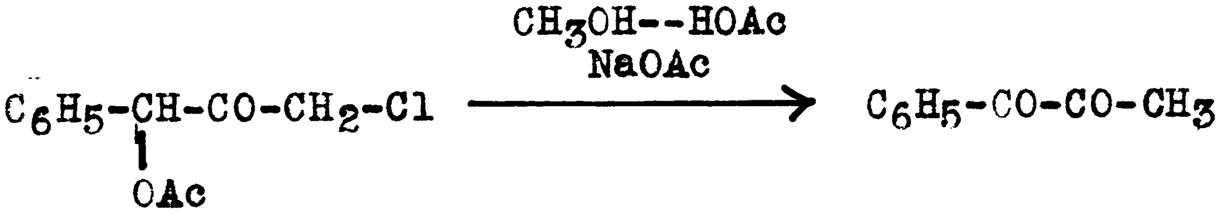
It is therefore of great importance to consider to what extent members of the second type of ethanolysis products isolated, namely, vanilloyl- (X) and syringoyl methyl ketones (XI), represent also stabilized end ethanolysis products derived from more reactive lignin progenitors. A consideration of a series of products, namely coniferyl alcohol (XXIX) ($R-CH=CH-CH_2OH$), oxyconiferyl alcohol (XXX) ($R-CH_2-CO-CH_2OH \rightleftharpoons R-CH=C(OH)-CH_2OH$) and dioxyconiferyl alcohol (XXXI) ($R-CO-CHOH-CH_2OH \rightleftharpoons R-C(OH)=C(OH)-CH_2OH$), points to the possibility of the existence of a similar dismutation equilibrium involving the dioxy-ketone (XXXI) as one constituent:

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

Examination of the enediol (XXXI), 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 $-CH_2OH$, and by analogy this dioxyconiferyl alcohol type of compound might be expected to yield vanilloyl methyl ketone 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 behavior of analogously constituted chain and cyclic derivatives. Thus Bradley and Eaton (194) observed that chloromethyl α-acetoxybenzyl ketone (XXXII), when heated with a solution of sodium acetate in glacial acetic acid and methanol, was converted in almost quantitative yield into benzoyl methyl ketone (XXXIII). The same chloroketone (XXXII) when left standing at room temperature for one year decomposed

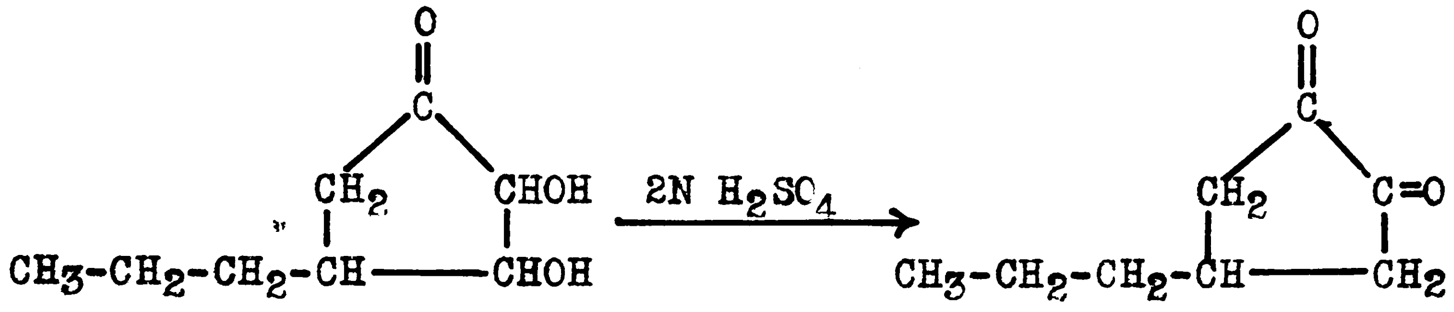


$$C_6H_5\text{-}\underset{\substack{|\\ OAc}}{CH}\text{-}CO\text{-}CH_2\text{-}Cl \xrightarrow{\substack{CH_3OH\text{--}HOAc\\ NaOAc}} C_6H_5\text{-}CO\text{-}CO\text{-}CH_3$$

(XXXII) (XXXIII)

spontaneously into (XXXIII). It seems justifiable to conclude that during this period, (XXXII) 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 coworkers (101) 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 (LXVIb), and this in the presence of acid underwent rearrangement to a cyclic diketone (LXVIc). It is evident that the molecule of

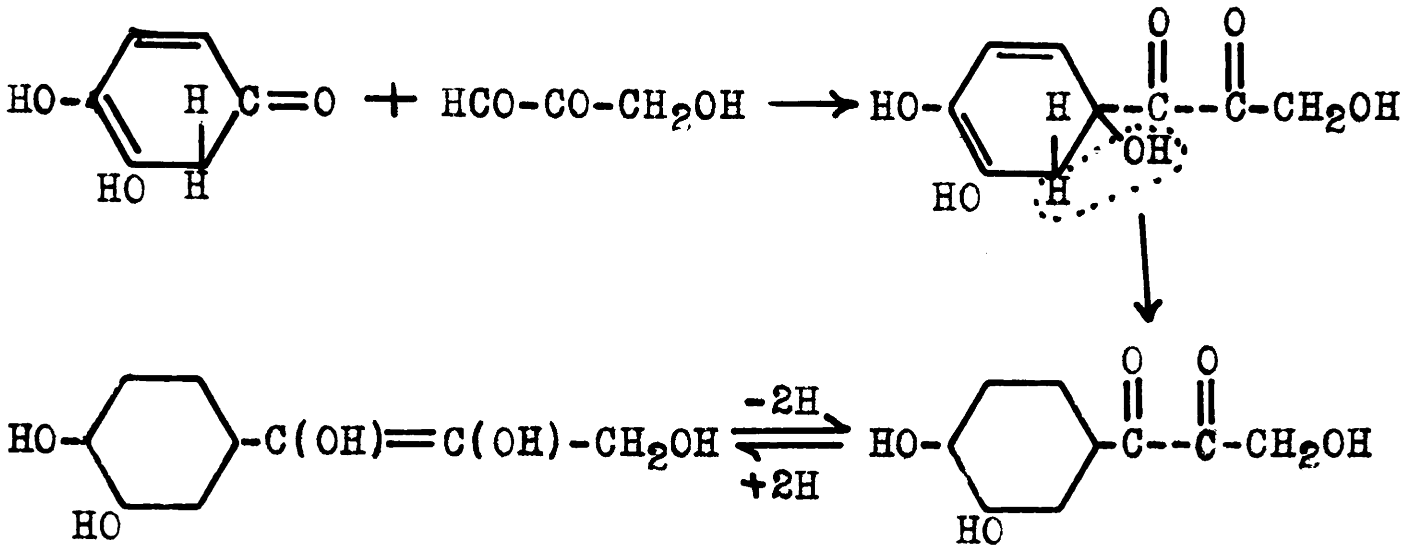


(LXVIb) (LXVIc)

tetrahydroterrein contains a grouping analogous to that in dioxyconiferyl alcohol (XXXI) 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.

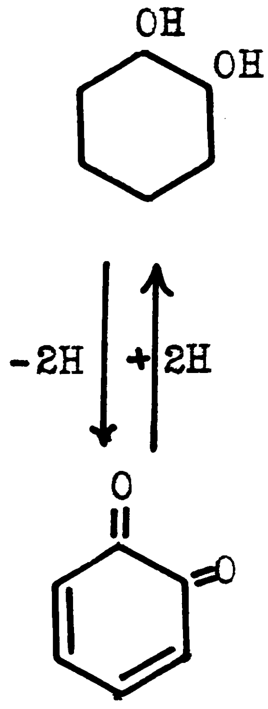
The plant origin of these dioxyconiferyl alcohol derivatives is, as yet, unknown, but it is interesting to note that their plant synthesis can be visualized as consisting of a primary condensation of two moles of methyl glyoxal, with the loss of a mole of water, followed by a further condensation with a third molecule, in this case hydroxymethyl glyoxal (instead of methyl glyoxal as in the case of the end methyl group derivatives)-(page 17).



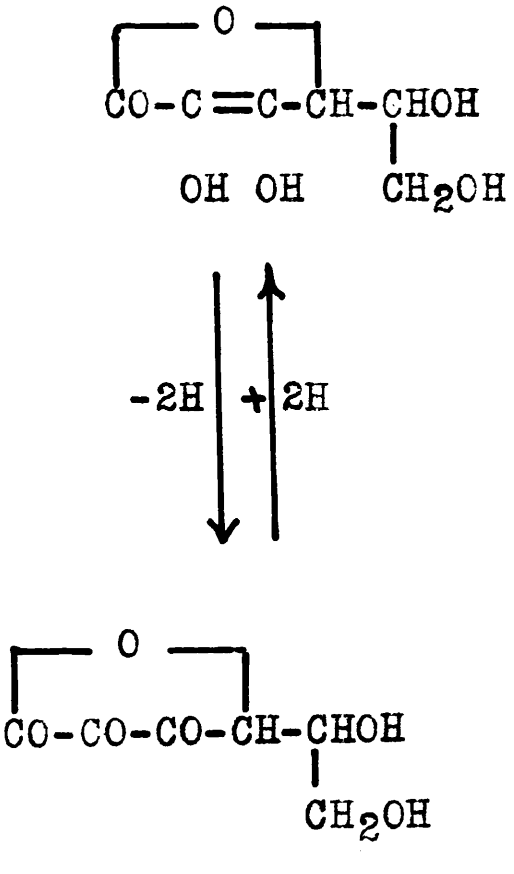
(6) Possible Significance of Lignin Progenitors in Plant Oxidase Systems

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 (65) 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 known as a "carrier" or "hydrogen transporter". Thus hydrogen is transferred 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.

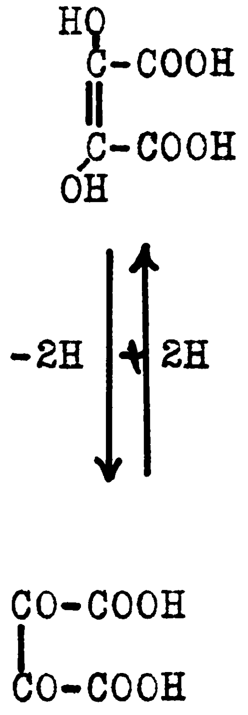
Szent-Györgyi has demonstrated the presence of a number of dehydrogenase-catalyst systems in various forms of plants; such as those of catechol (XXXIV) (73), ascorbic acid (XXXV) (74), and dioxymaleic acid (XXXVI) (75).



(XXXIV)



(XXXV)

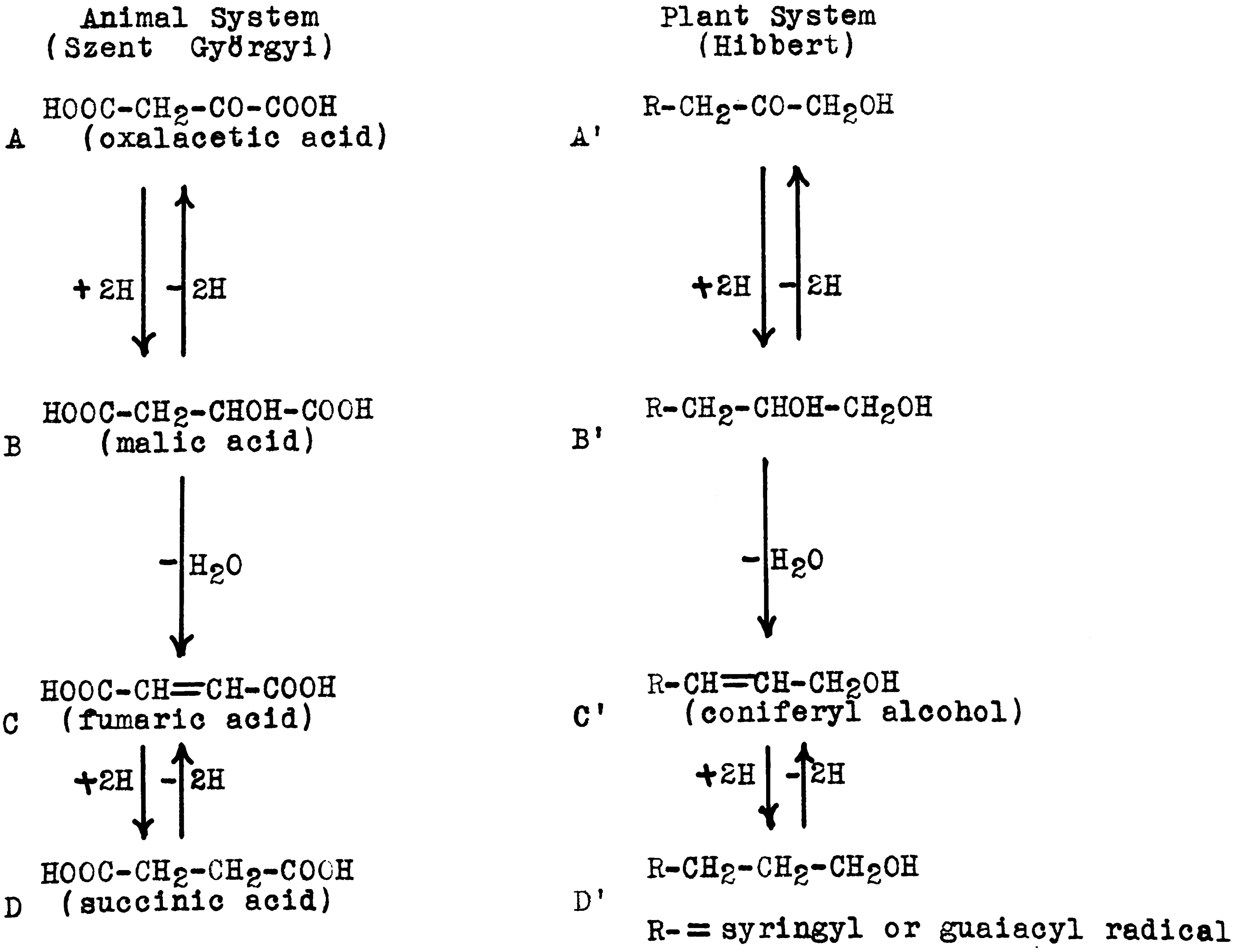


(XXXVI)

Each of these can function as an oxidation-reduction system, the oxidized molecule being a 1,2-diketone and the reduced molecule 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 (XXVI) and((X). As discussed above (page 19), these presumably represent stabilized end products, their forerunners, being 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 him (4) to propose a plant respiratory catalyst system similar to the C_4-dicarboxylic acid system of Szent-Györgyi (76).



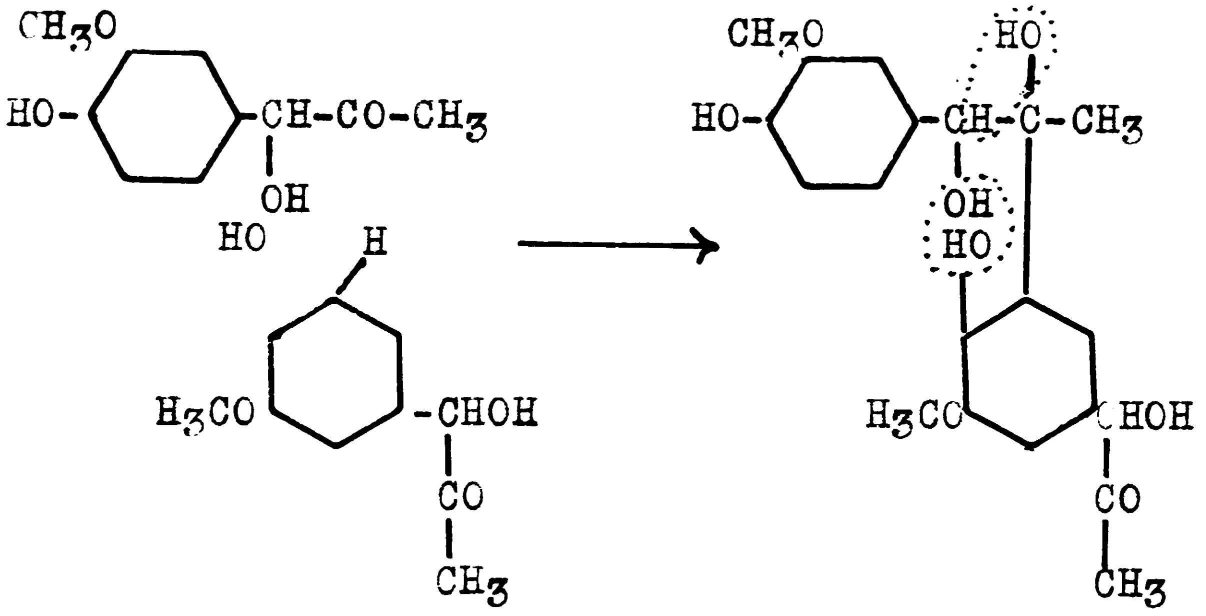
The first member (A') of the plant system, corresponding to oxalacetic acid (A), is the keto alcohol believed to be obtained from the condensation of three mols of methyl glyoxal (page 17). It is seen that the third member of the new system is coniferyl alcohol corresponding to fumaric acid in the C_4 system. The isomeric form of (A'), $R-CO-CH_2-CH_2OH$, 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 the components of the Krebs animal cell oxidation system (77). The passage from Hibbert's new system to the ene-diol-1,2-diketone system can take place by an allylic rearrangement of the oxyconiferyl or oxysyringyl alcohol isomer, as represented on (page17).

(7) Formation of Protolignin from Propylphenol Units

(a) Polymerization Mechanisms of Guaiacyl Units

Prominent workers in the field of lignin chemistry believe that lignin originates from propylphenol units of the type isolated from wood by Hibbert and coworkers, or from closely related compounds, and Freudenberg, Erdtmann, 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 of part A).

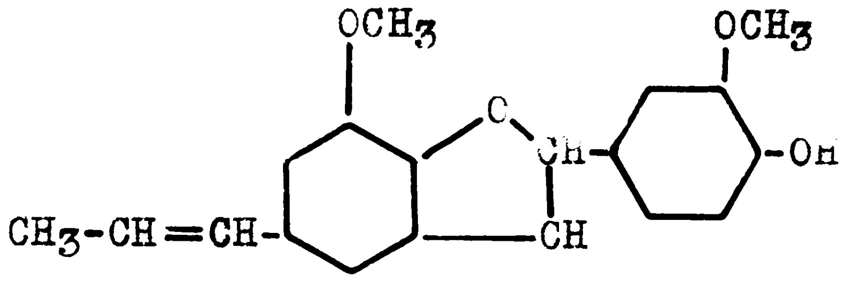
Hibbert (4) points out that the "lignin building units" containing a phenol group together with a carbonyl or an unsaturated linkage in the side chain would have a strong tendency to undergo para- and ortho -nuclear condensations both individually and collectively, under mild conditions, with formation 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 C_6H_5-C-C-C units .



(XXXVII)

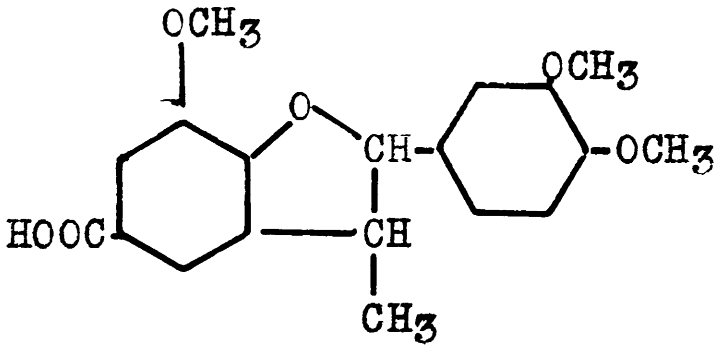
The condensation of the ortho-hydrogen atom of one unit with the carbonyl of another, followed by the elimination of two molecules of water as indicated would result in formation of a benzofurane derivative. Condensation with a third molecule would yield a trimer and so on. It is seen that this mechanism 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 (88) isoeugenol forms a dehydrogenated dimer, dehydrodiisoeugenol considered by Erdtmann (3) to have the formula (III).



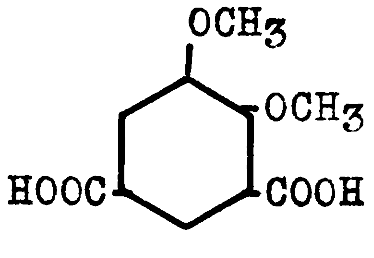
(III)

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

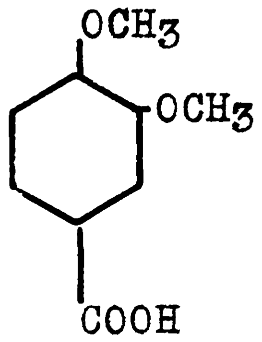


(XXXVIII)

Freudenberg found that (XXXVIII), after degradation with alkali, methylation, and permanganate oxidation, yielded isohemipinic acid (XXXIX) and veratric acid (XL). By the same treatment of spruce lignin Freudenberg obtained 2% of isohemipinic acid and 12% veratric acid, and traces of trimethyl gallic acid, and



(XXXIX)

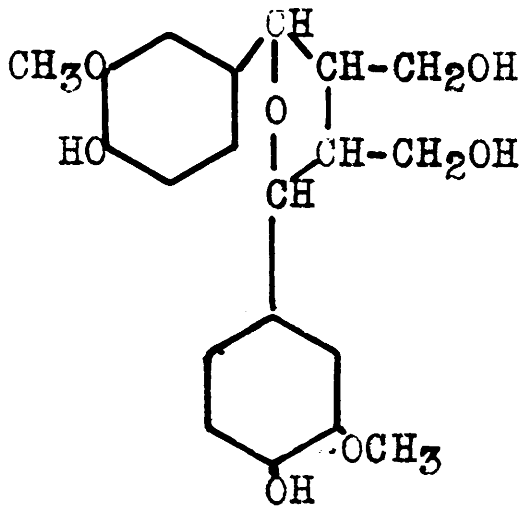


(XL)

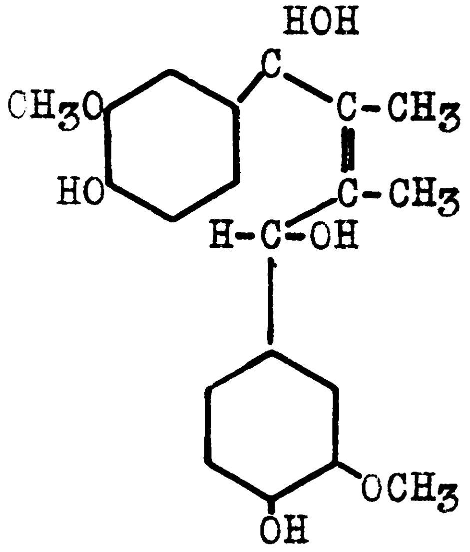
concluded (89) that some of the lignin molecules are of a type capable of yielding isohemipinic acid on alkali degradation, followed by methylation and oxidation. It is apparent that this observation supports the theory of Erdtmann (3) and of Hibbert (78) that protolignin is made up of dimers of the dehydrodiisoeugenol type (III).

Erdtmann in his recent review (3) points out that the structure of a wide variety of plant resins, e.g. olivil (XLI), lariciresinol (XLII), conidendrin (XLIII) 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.

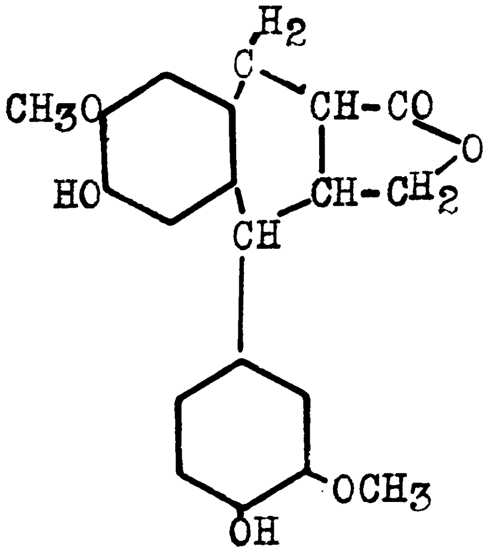
The similarity between these simple natural products and dehydrodiisoeugenol, and the analogies between lignin and dehydrodiisoeugenol have led Erdtmann to the belief that the phenomenon of dehydrogenative coupling of molecules probably plays a great role in the biogenesis of plant products.



(XLI)



(XLII)

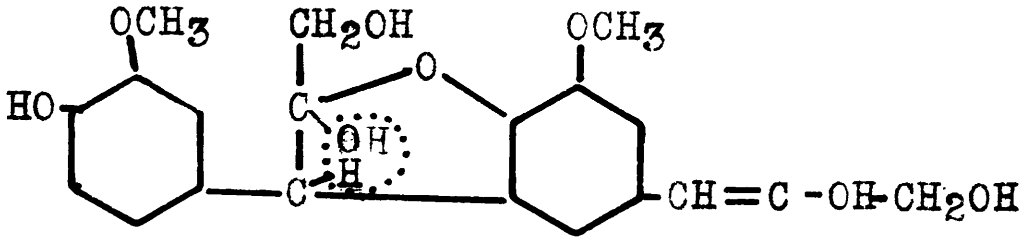


(XLIII)

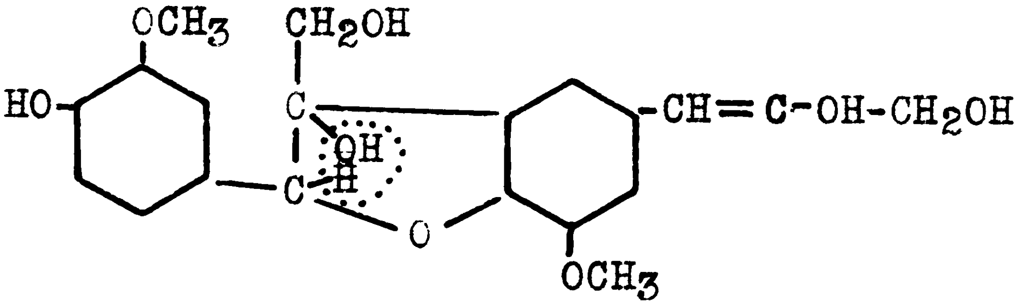
He states that the ethanolysis products α-hydroxypropiovanillone, (XXVI) and vanilloyl methyl ketone (X) and their syringyl analogues are not necessarily true lignin building units, since they may represent stabilized end products originating from dimeric types similar to dehydrodiisoeugenol. That such may well be the case has been recognized by Hibbert (78). (see also page 19)

There is a close relationship between the enolic forms of the side chains of certain propylphenol derivatives $R-C(OH)=C(OH)-CH_3$ (XXV); $R-CH=C(OH)-CH_3$ (XXVII); $R-CH=C(OH)-CH_2OH$ (XIII); and the side chains of isoeugenol $R-CH=CH-CH_3$ and coniferyl alcohol $R-CH=CH-CH_2OH$. 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 (XIII). These reactive dimers during the course of the ethanolysis could partially polymerize and partially undergo transformation to the stabilized ethanolysis units, as shown below.

Application of the dehydrodiisoeugenol type of polymerization to the enolic forms of certain propylphenol units, for example oxyconiferyl alcohol (XIII), as pointed out by Hibbert (78), could give rise to two general types of lignin polymers: (XLIV) and (XLV).

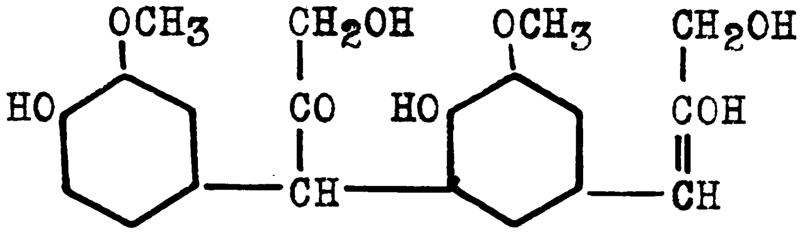


(XLIV)

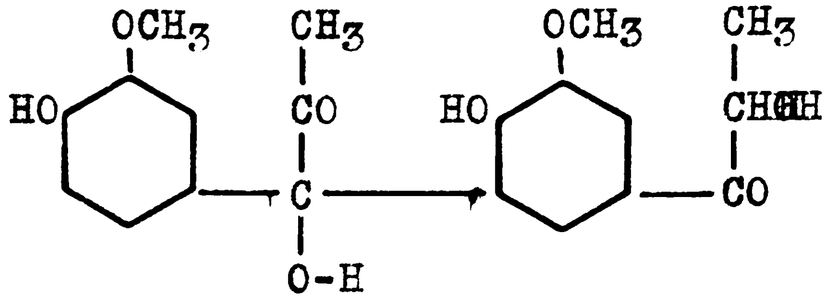


(XLV)

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 (XLIV) could give rise to the (XLVI) form, from which, by a double allylic shift, and by cleavage, one mole each of the diketone (X) and of the ketol (XXVI) could be obtained. Such a cleavage would only be possible with a reversible type polymer and not with the benzofurane irreversible type. The



(XLVI)



(XLVII)

same dehydrogenative-coupling type of polymerization and depolymerization can be applied also to two moles of the enediol of α-hydroxypropiovanillone (XXV), or to two moles of the enol of (4-hydroxy-3-methoxyphenyl)-propanone-2 (XXVII).

(b) Polymerization Mechanism of Syringyl Units

It is apparent that the syringyl analogs, 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" (XXVI),(XXVII) and (XIII) in their enediol forms are derivatives of styrene, they could possibly undergo the styrene type of 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 (79) in high yield, 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 (80) 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 (XIII) and the keto-alcohol(XXVI) a result in harmony with the above theoretical speculations. (page 31)

The reactions of lignin with mercaptans, particularly with thioglycollic acid, have been investigated over a period of years by Holmberg (81) and are of particular importance in any appraisal of the various suggested polymerization mechanisms. The mercaptans, R-SH, are thio-alcohols, and in the presence of acids, react in the same way (namely through the -SH-group) as alcohol with lignin in the 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 (82). 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 (81) as well as the related experiments carried out by Freudenberg (83), by Hägglund (84) and by Richtzenhain (85) were all designed to throw light upon the question of the actual groupings present in proto- and isolated lignins. Thus thioglycollic acid condenses with cinnamyl alcohol (86) with the loss of water to give cinnamyl thioglycollic acid.

$C_6H_5\text{-}CH{=}CH\text{-}CH_2OH + HS\text{-}CH_2\text{-}COOH \longrightarrow C_6H_5\text{-}CH{=}CH\text{-}CH_2\text{-}S\text{-}CH_2\text{-}COOH + H_2O$.

A similar reaction occurs with butyl alcohol, α-phenylethyl alcohol, and, more significantly with benzyl alcohol (87), its derivatives and the ethers of it and its derivatives, e.g. phenyl methyl carbinol (XLVIII), benzohydrol (XLIX), α-ethoxyphenylethane (L) and benzyl ethyl ether (LI).

$C_6H_5\text{-}CH(CH_3)\text{-}OH + HS\text{-}CH_2\text{-}COOH \longrightarrow C_6H_5\text{-}CH(CH_3)\text{-}S\text{-}CH_2\text{-}COOH + H_2O$

(XLVIII)

$C_6H_5\text{-}CH(CH_3)OEt + HS\text{-}CH_2\text{-}COOH \longrightarrow C_6H_5\text{-}CH(CH_3)\text{-}S\text{-}CH_2\text{-}COOH + EtOH$

(L)

$(C_6H_5)_2\text{-}CHOH$

(XLIX)

$C_6H_5\text{-}CH_2\text{-}OEt$

(LI)

(XLIX) and (LI) react in the same way as (XLVIII) and (L). Thioglycollic acid does not condense with such alcohols as phenylpropanol ($C_6H_5\text{-}CH_2\text{-}CH_2\text{-}CH_2OH$), and phenyl ethanol ($C_6H_5\text{-}CH_2\text{-}CH_2\text{-}OH$) (82).

From the foregoing results, the following conclusion is 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 with thioglycollic acid very readily, would suggest that the former is a derivative of benzyl alcohol. Examination of the various structures proposed for lignin (II), (XLV) and (XLVII) shows that they are such derivatives, hence Holmberg's work lends support to the proposed theories of lignin structure.

Part B. Metabolic Products formed by Molds from Carbohydrates

The application of fermentation reactions to the arts, such as brewing, production of vinegar and tanning, has been practised for a long time, though only during the nineteenth century was it definitely recognized that these processes are accomplished by the aid of living organisms. The formation of acid by molds is so easily recognized by simple titration that it was only natural that acid production early excited attention and most of the pioneer work in mold products dealt with the formation of carboxylic acids. The following acids have been recognized as metabolic products: oxalic, citric, fumaric, malic and succinic. Other metabolic products are ethyl alcohol, acetaldehyde, mannitol, fats and polysaccharides.

In 1922 Raistrick and co-workers commenced a comprehensive program of work on the general biochemistry relating to action of micro-organisms, and the "molds", a family of the Eumycetes, were chosen as the first group of micro-organisms for investigation. After twenty years of intensive work, many new products formed from different genera of molds were isolated and identified. Of these the anthraquinones, benzoquinones, quinols, benzoins, diketones, desoxybenzoins, and pyrones are of special interest and appear to be of marked biochemical importance.

The molds were grown on the Czapek-Dox medium which is a 5% glucose (the sole organic substrate) solution containing mineral salts. The sterilized medium was inoculated with the spores of the mold and incubated. The time of incubation, temperature and pH were varied to suit the particular species or strain of the mold. Both the reaction medium and the mold mycelium contained metabolic products (aromatic and aliphatic) which Raistrick and co-workers undertook to isolate and identify.

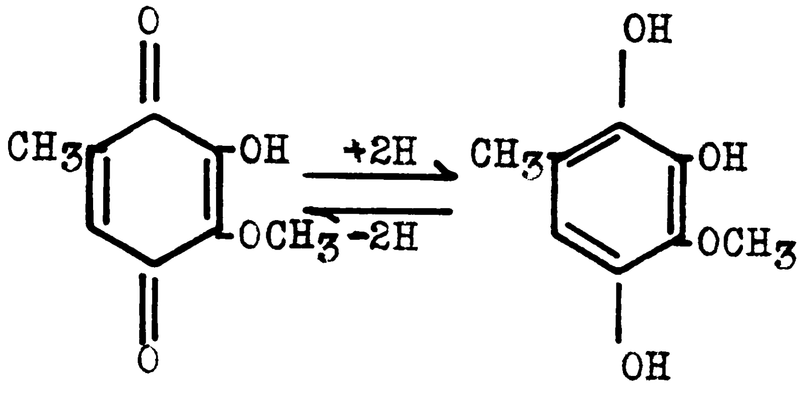
Micro-organisms apparently have their own biochemical characteristics. In general, the metabolic products isolated from one genus or species differ from those of another. Thus fifteen species or strains of Penicillium when grown on the Czapek-Dox medium, all yielded the same three phenolic acids (LXIII), (LIV) and (LXV) (page 41); in addition twelve of them produced mycophenolic acid whose structure has not yet been established. So far as is known these compounds are not produced by any other series of organisms. Their specific grouping on morphological grounds in one series is thus supported by their biochemical characteristics.

Another example in support of the specificity of micro-organisms is the fact that most of the species of the genus Helminthosporum when grown on the Czapek-Dox medium produce anthraquinones which are formed only by a limited number of species of other genera. It is interesting to note that Hibbert and co-workers, have found in the ethanolysis products from different woods, (gymnosperms) aromatic derivatives having a three-carbon side chain identical with those present in (LXIII), (LXIV) and (LXV) in which the aromatic nucleus is represented by the guaiacyl radicle. In the case of angiosperms, in addition to these, the syringyl analogs also have been obtained.

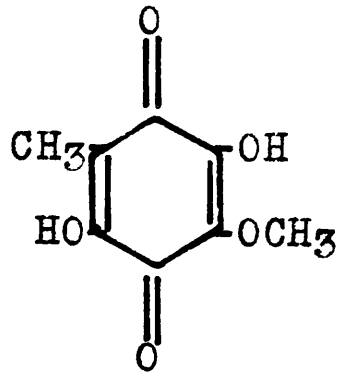
In the present investigation the author has been able to prove the presence of desoxybenzoins, analogs of (LXV) (both guaiacyl and syringyl types) in the ethanolysis products from maple wood. These results are of marked interest from the point of view that molds are lower forms of plant life.

Raistrick and co-workers have examined the metabolic products obtained by use of many strains and species of several genera of molds, and have established the structures of their products by direct syntheses.

Two derivatives of benzoquinone and a quinol of one of these were isolated (90) from different cultures grown on the Czapek-Dox medium. Fumigatin (LII) and spinulosin (LIII) were each formed by different strains of Aspergillus fumigatus Fresenius (91) and spinulosin (LIII) was also found to be a metabolic product of Penicillium spinulosum Thom. (91a). This represents one of the few examples where molds of different genera give rise to the same metabolic products. The structures of (LII) and of (LIII) have been confirmed by direct syntheses (92,93).



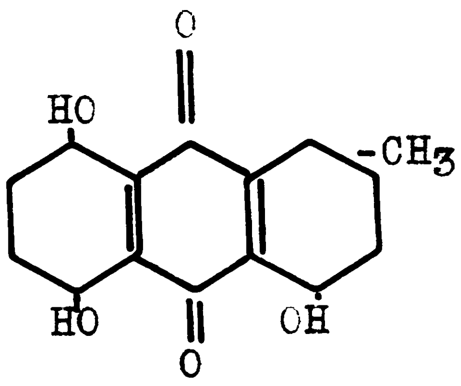
(LII) (LIV)

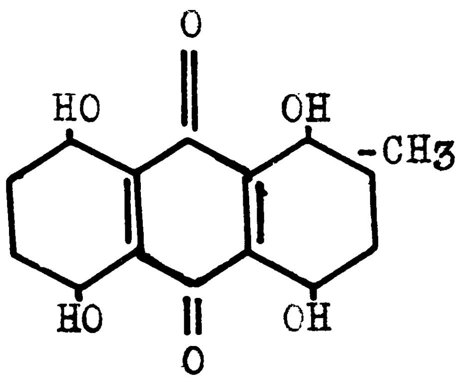


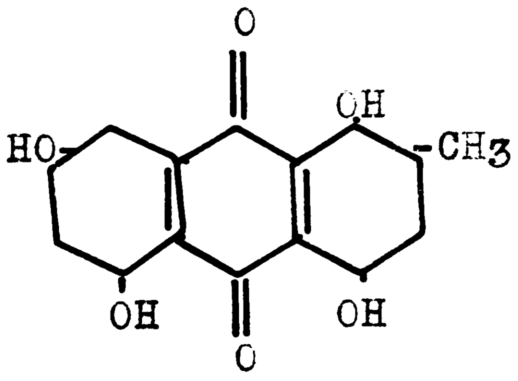
(LIII)

From freshly-separated mold metabolism reaction mixtures, Raistrick and co-workers (91)were able to isolate the quinol, 3-hydroxy-4-methoxytoluquinol (LIV). It was found to be unstable in air and rapidly oxidized to fumigatin (LII). Also (LII) was readily reduced to (LIV). Since (LIV) and (LII) are readily interconvertible and since they are both metabolic products, Raistrick was led to believe that they function as an oxidation-reduction system in the life processes of the mold.

Different species of the genus Helminthosporum and a few of the species of the genera Penicillium and Aspergillus when grown on a modified Czapek-Dox medium produce in the mycelium, a pigment consisting of different polyhydroxyanthraquinones. These were obtained from dried and powdered mycelium by extraction with chloroform. Helminthosporin (4,5,8-trihydroxy-2-methylanthraquinone) (LV) (94), cynodontin (1,4,5,8-tetrahydroxy-2-methylanthraquinone) (LVI) (95), catenarin (1,4,5,7-tetrahydroxy-2-methylanthraquinone) (LVII) (96) and tritisporin (6-(or 7)-hydroxymethyl-1,3,5,8-tetrahydroxyanthraquinone) (LVIII) (97) were isolated from the mycelium of the different species of the genus Helminthosporum. The yields were variable and as high as 30% of the dried mycelium. It is to be noted that many of the anthraquinones are isomeric, and that all are very closely related.


(LV)


(LVI)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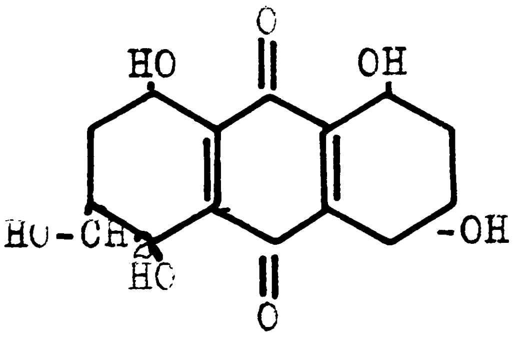
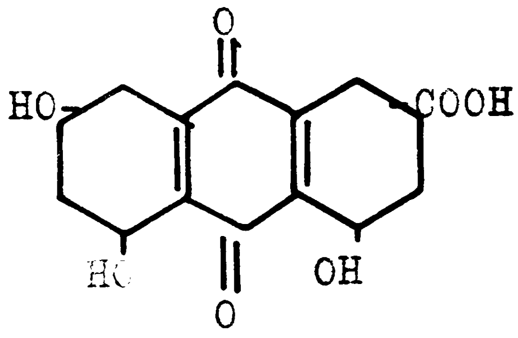
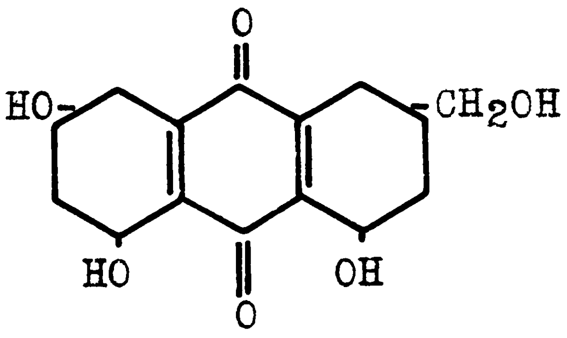
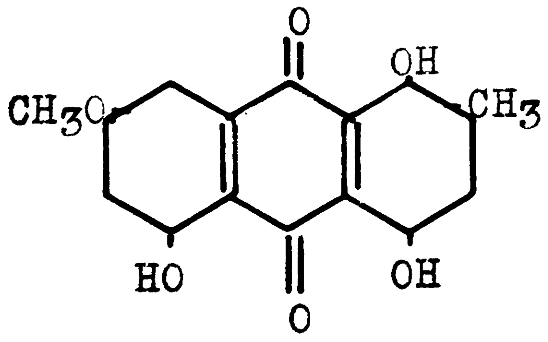
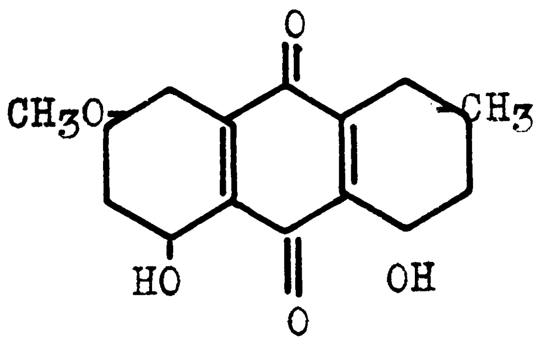

(LVIII)


(LIX)


(LX)

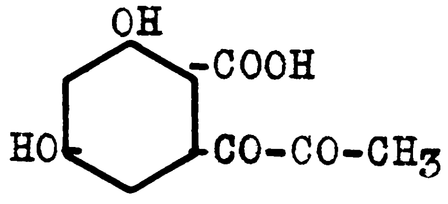

(LXI)


(LXII)

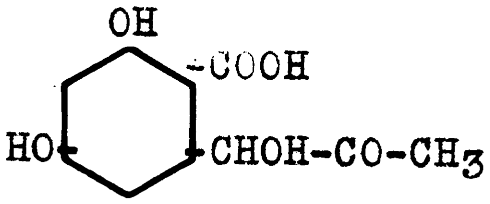
The coloring matters present in the mycelium of a strain of Penicillium cyclopium Westling grown on a glucose solution were also investigated.(98). Two anthraquinones, emodic acid (4,5,7-trihydroxyanthraquinone-2-carboxylic acid) (LIX), and ω-hydroxyemodin (4,5,7-trihydroxy-2-(hydroxymethyl)-anthraquinone) (LX) were isolated and identified. (LX) is easily convertible to (LIX) by acetylation, followed by oxidation, and hydrolysis. The structure of emodic acid was confirmed by synthesis. The coloring matters produced by two different species of Aspergillus contained partially methylated quinones: erythroglaucin (1,4,5-trihydroxy-7-methoxy-2-methylanthraquinone) (LXI) (96) and physcion (4,5-dihydroxy-7-methoxy-2-methylanthraquinone) (LXII) (99). Erythroglaucin is easily prepared by methylating catenarin (LVII), while physcion (LXIII) is a close relative of partially methylated (LX).

Since the only source of carbon was the glucose used as a substrate, these aromatic substances must have originated from this carbohydrate. Their formation by the mold is difficult to explain, but Raistrick believes they are formed from the sugars by a complicated series of oxidation-reduction processes (93).

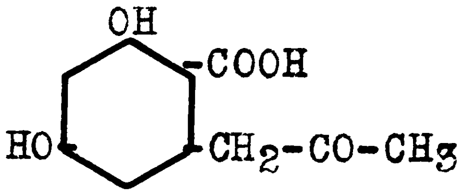
A very interesting series of compounds is that of the phenolic acids formed by the action of Penicillium brevi-compactum Dierckx and related species (100), on 5% glucose solution containing mineral salts. These are 3,5-dihydroxy-2-carboxybenzoylmethyl ketone (LXIII), 3,5-dihydroxy-2-carboxyphenylacetylcarbinol (LXIV) and 3,5-dihydroxy-2-carboxybenzylmethylketone (LV) respectively.



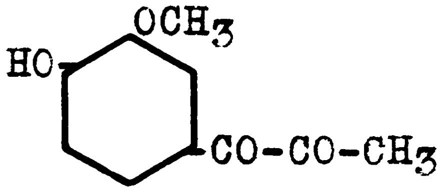
(LXIII)



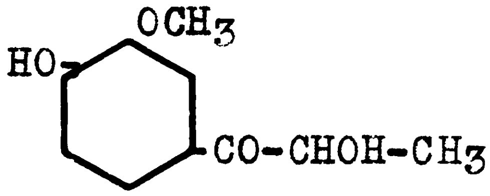
(LXIV)



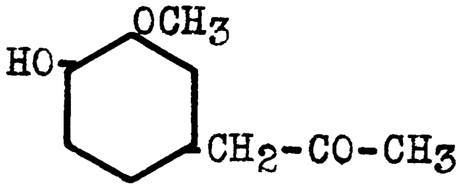
(LXV)



(X)

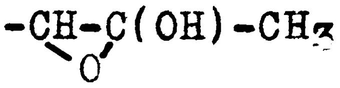


(XXVI)

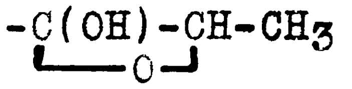


(XXVII)

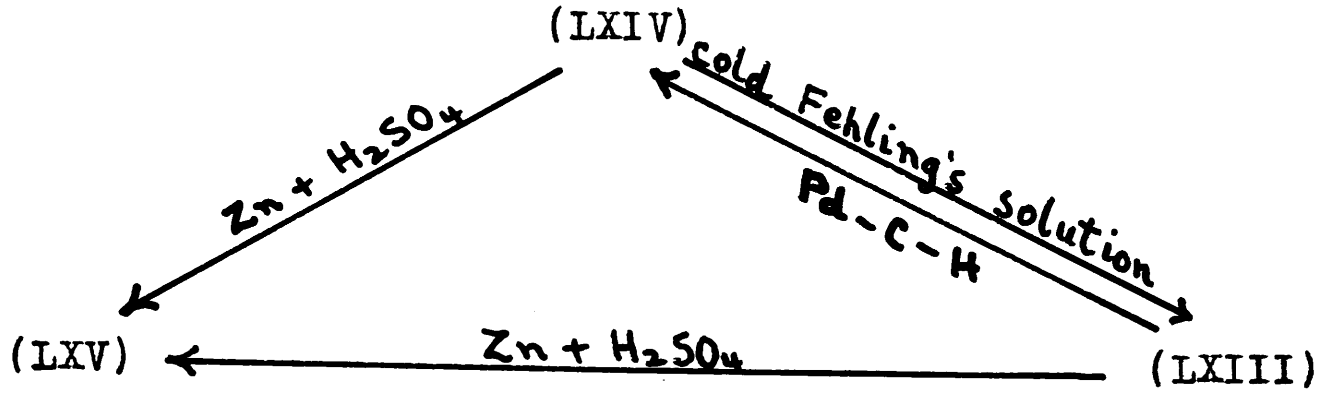
Oxford and Raistrick point out that the structure of the side chain of (LXIV) is uncertain and that it may be either one or other of the following: -CO-CHOH-CH$_3$, -C(OH)=C(OH)-CH$_3$,


$-\underbrace{CH-C}_{O}(OH)-CH_3$

or


$-\underbrace{C(OH)-CH}_{O}-CH_3$

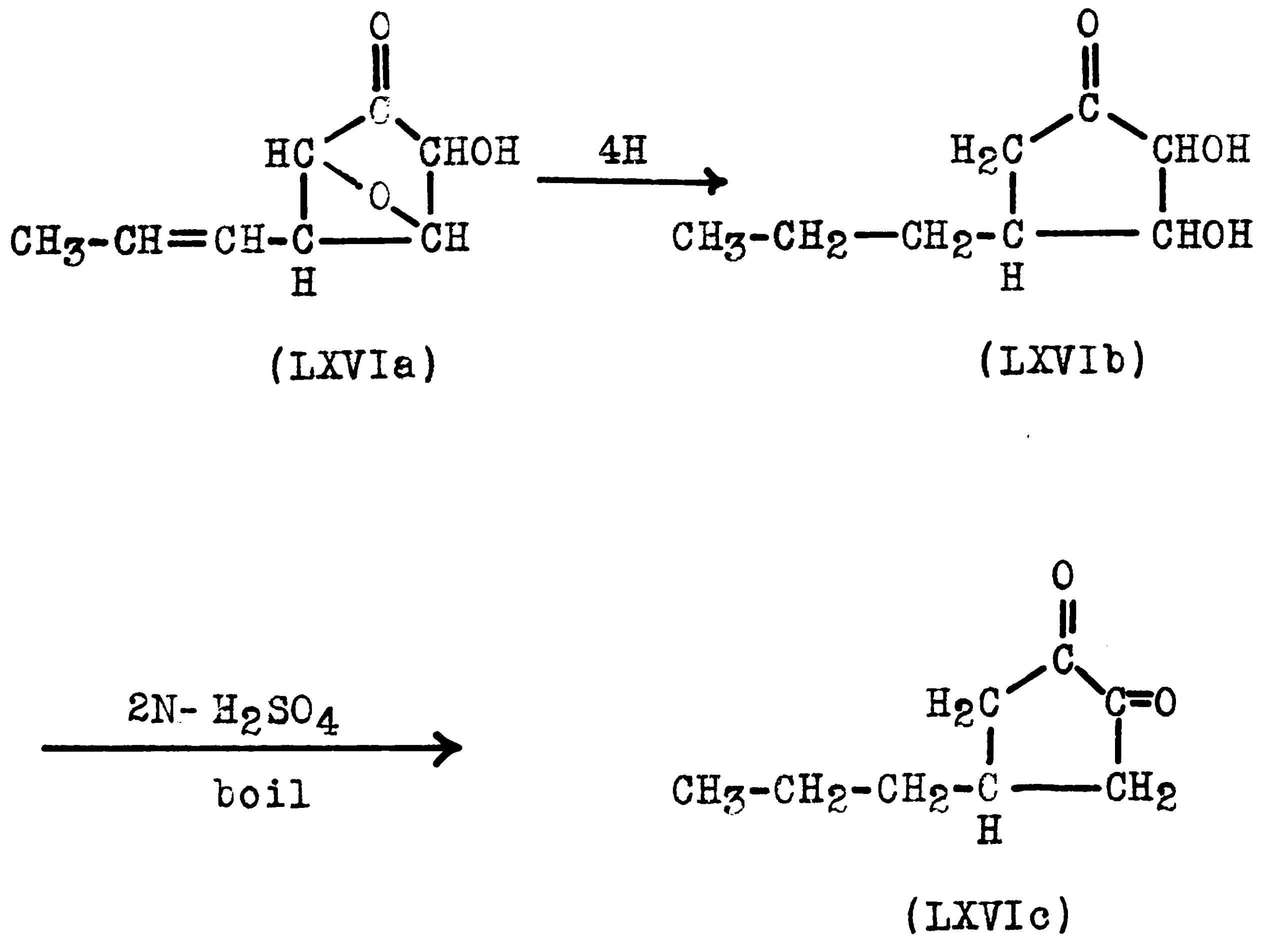
or an equilibrium mixture of several of the possible isomerides. They found that (LXIV) was optically inactive and explained this as due to the presence of an equilibrium with its enediol form. The three compounds were found to be readily interconvertible, i.e.



In most cases the principal constituent of the metabolic mixture was the diketone (LXIII). The yield of (LXV) was in general quite small - about 3% of the mixture. In the case of a few species, the benzoin (LXIV) was apparently the chief constituent.

These three metabolic products (LXIII),(LXIV) and (LXV) show a remarkable parallelism both in regard to side chain structure and interconvertibility with certain aromatic derivatives isolated from the ethanolysis products of wood, namely vanilloyl methyl ketone (X), α-hydroxy-propiovanillone (XXVI), and (4-hydroxy-3-methoxyphenyl)-propanone-2 (XXVII). The interesting question of their possible function as oxidation-reduction systems in the life of the higher plants, is thus raised. Inasmuch as they have been shown to be derived from lignin, this similarity in type would seem to provide support for Hibbert's view of lignin as a mixture of products arising from a group of monomolecular compounds of C_6-C-C-C type whose primary function is that of respiratory catalysts (78). It is of great importance to note that presumably Raistrick's and the analogous wood ethanolysis products actually represent stabilized end products rather than primary substances. This is rendered highly probable by the recently discovered remarkable ease of transformation of Hibbert's probable lignin respiratory catalyst R-CO-CH_2-CH_2OH, under the influence of acids, into the corresponding benzoin, R-CO-CHOH-CH_3 (70), isolated as the ethyl ether.

One metabolic product synthesized by several strains of <u>Aspergillus terreus Thom</u> may be classed as a keto-alcohol (LXVIa) (101). Of further interest is the fact that it contains a propenyl side chain. From a study of its properties, derivatives and breakdown products it has been shown to be 4-propenyl-2-hydroxy-3,5-oxidocyclopentane-1-one (LXVIa). It is also known as terrein.



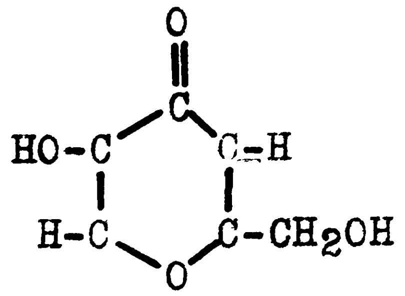
(LXVIa)

(LXVIb)

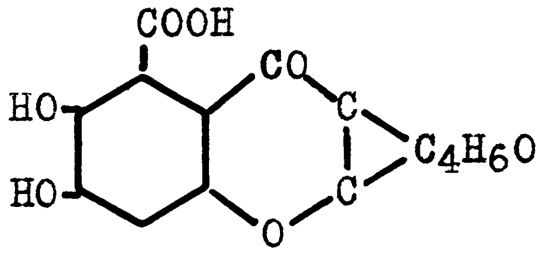
(LXVIc)

Terrein is readily hydrogenated to tetrahydro-terrein (LXVIb) which on boiling with 2N sulfuric acid yields the interesting compound (LXVIc),- a cyclic 1,2-diketone with a propyl side chain.

Kojic acid (5-hydroxy-2-hydroxymethyl-γ-pyrone) (LXVII) has been known for a long time. It was isolated (102) originally from the metabolic products formed by a small number of the Aspergillus family in yields of 16-18% of the sugar used. The production of kojic acid was shown to be characteristic of species of the <u>A. flavus-oryzoe</u> group, and the ferric chloride reaction proved a useful diagnostic test for species of Aspergillus belonging to this group. Kojic acid is produced by these fungi from a large number of carbon compounds- including not only glucose, sucrose, polysaccharides, but also five and three carbon compounds such as xylose and glycerol.



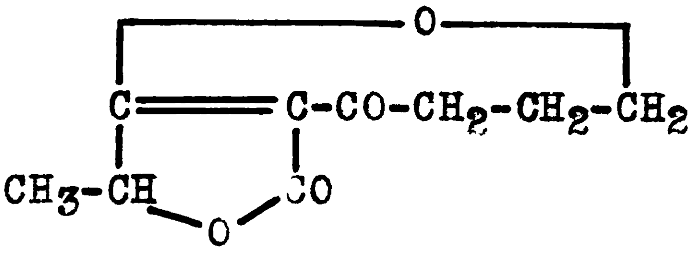
(LXVII)



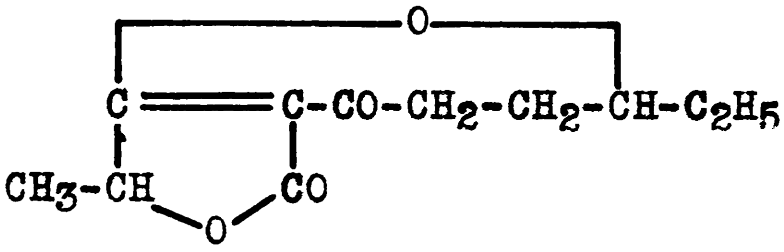
(LXVIII)

Another γ-pyrone (LXVIII) called citromycetin is formed by the action of various species of citromycetes (102) on glucose or glycerol. From the study of the properties of its derivatives and degradation products, the formula (LXVIII) was assigned to it. The nature of the side chain is unknown and the position of the seventh oxygen has not been determined.

Closely related to kojic acid are carolic (LXIX) and terrestric acids (LXX). The latter is produced by *Penicillium terrestic Jensen* (103) when grown on the Raulin-Thom medium which is a 5% solution of glucose containing mineral salts including tartrates. The two acids have very similar properties.



(LXIX)



(LXX)

Both form monohydrates and on acid hydrolysis yield carbon dioxide, acetoin, and a lactone.

Simpler compounds in addition to those already mentioned, synthesized by different strains of molds are 6-hydroxy-2 methyl-benzoic acid (104) and 2,5-dihydroxybenzoic acid (105). Many other metabolic products have been isolated, but their complicated structures have not yet been established. The chlorine of the mineral salts in the Czapek-Dox medium is metabolized by the species of a few "mold" genera into chlorine-containing metabolic products, such as the three compounds griseofulvin (106) ($C_{17}H_{17}O_6$ Cl), geodin ($C_{15}H_6O_5Cl_2$) ($(OCH_3)_2$) and erdin (107) ($C_{15}H_7O_6Cl_2(OCH_3)_2$). In each case the chlorine atoms are believed to be attached to an aromatic nucleus. The two latter compounds are believed to be derivatives of benzophenone. The action of Byssochlamys fulva Olliver and Smith (108) on glucose gives high yields of mannitol (30%) as well as a yellowish acid-insoluble substance, Byssochlamic acid ($C_{16}H_{20}O_6$) in small yields (0.5%). The latter substance is toxic to mice. It is a tetrabasic acid and forms silver, iron and barium salts readily.

The production of anthraquinones from glucose by the genera Helminthosporum has already been described. In addition to these, more recently (109) two other compounds have been isolated from H. leersi Atkinson to which the names luteoleersin ($C_{26}H_{38}O_7$) and alboleersin ($C_{26}H_{40}O_7$) have been given. The structures of these compounds have not yet been determined, but they appear to be of great importance. The biochemical significance of luteoleersin and alboleersin lies in their relationship to each other. They are very readily interconvertible in vitro and while both substances have been isolated from all cultures of H. leersi so far examined, their relative proportions varied in different preparations. Luteoleersin may be readily reduced to alboleersin by catalytic reduction with palladium-charcoal-hydrogen or by heating with phenylhydrazine. Alboleersin may be smoothly and almost quantitatively oxidized to luteoleersin by treatment with a cold ferric chloride solution. Luteoleersin behaves as a quinone or semiquinone and alboleersin as the corresponding phenol. It is believed that these two substances play a role as an oxidation-reduction system in the life process of the mold H. leersi. Several other similar products have been isolated and their structures are under investigation by Raistrick and co-workers. The probable relationship of these metabolic products and their origin to analogous lignin units isolated in the ethanolysis of wood is reviewed in the "Discussion of Experimental Results".

Part C. Properties of Hydroxy-ketones and their Relationship to the Corresponding Enediols, Diketones, and Monoketones.

Hydroxy-ketones, diketones, and also monoketones of the desoxybenzoin type are all found among the ethanolysis products from wood. α-Hydroxypropiovanillone is obtained from softwoods (41a), and a mixture of α-hydroxypropiovanillone and α-hydroxypropiosyringone (41b) from hard woods. The isolation of the 1,2-diketones namely vanilloyl methyl ketone (43a) and syringoyl methyl ketone (110) (43b) and α-hydroxypropiovanillone and syringone from the ethanolysis products from maple wood, has raised a question of considerable biochemical importance. Hibbert (111) (4) has discussed the probable function of the aromatic enediol -1,2-diketones as oxidation-reduction systems, and compared them with the similar Szent Györgyi, catechol, ascorbic acid and dioxymaleic acid systems (76) (page 24).

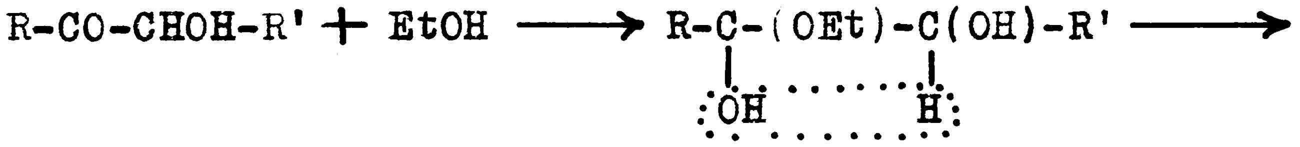
A more complete understanding of the relationship of α-hydroxy-ketones, 1,2-diketones, enediols and monoketones to the mechanism of plant synthesis and their probable role as building units from which lignin is derived, can be obtained by a consideration of the general properties of these compounds.

(1) Etherification and Polymerization Reactions of Hydroxy-ketones

One of the most interesting properties of α-hydroxy-ketones, considered in relation to the structure of ethanol- and methanol-lignins is their behavior towards alcoholic solutions of hydrogen chloride. Although the hydroxyl and carbonyl groups are attached to adjacent carbon atoms, the former still retains many of the properties of carboxylic hydroxyls. E. Fisher (112) discovered that the hydroxyl group in benzoin may be etherified by the same process as used for ordinary acid etherification, i.e. treatment with ethanol and hydrochloric acid. Furthermore α-acetoxypropioveratrone (41a) and related acetoxy compounds behave more like acid anhydrides than ordinary esters. For example when the acetate is refluxed with ethanol and hydrochloric acid, ethyl acetate and the ethyl ether of the benzoin are formed.

While studying these etherification reactions Irvine and McNicol (113) noted curious anomalies which served to demonstrate the marked influence of substituents in the benzene ring on the functional groups in the side chain. Thus, although benzoin is readily methylated or ethylated, anisoin can be ethylated but not methylated by alcoholic hydrogen chloride treatment. Another case showing the influence of substituents in the benzene ring is to be found in the behavior of symmetrical o-dimethoxybenzoin. This compound can be methylated only under most carefully controlled conditions i.e. using 15% methanolic hydrogen chloride at 10°C.. On the other hand furoin appears to ethylate almost instantaneously in the presence of traces of hydrogen chloride, but the resulting compound is so active that it immediately undergoes a further intermolecular condensation so that the simple methyl ether cannot be isolated. α-Hydroxypropiovanillone (41a), -syringone, and -veratrone can be ethylated by using 0.5% solution of hydrogen chloride in ethanol, but also here, the parent substances are so reactive that polymerization occurs and only a small amount of the ethylated product can be isolated.

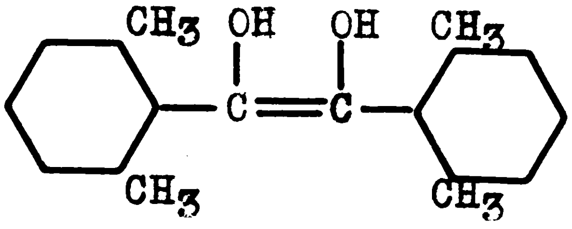
Nothing definite is known regarding the mechanism of etherification, but one possibility would seem to be the formation of a primary addition product, followed by loss of water and migration of hydrogen.



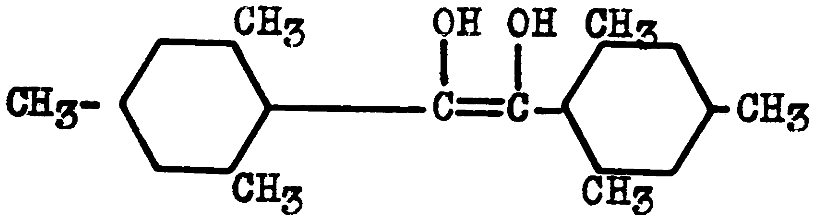
$$\text{R-CO-CHOH-R}' + \text{EtOH} \longrightarrow \text{R-}\underset{\text{OH}}{\text{C}}\text{-(OEt)-}\underset{\text{H}}{\text{C}}\text{(OH)-R}' \longrightarrow$$

$$\text{R-C(OEt)}=\text{C(OH)-R}' \longrightarrow \text{R-C(HOEt)-CO-R}'$$

Curiously enough, in the same manner in which the substituents on the benzene rings of the benzoin influence etherification so those on the benzene rings of the enediols influence their stability. Thus although the enediol of benzoin is very unstable and has never been prepared, several enediols of substituted benzoins have been isolated. Their stability is determined by the size, position and kind of substituents. Symmetrical tetramethylstilbenediol (LXXI) (115), hexamethylstilbenediol (LXXII) (114) (116) (117), hexaethylstilbenediol (118) and hexaisopropylstilbenediol (119), show increasing stability towards oxidation or isomerization.

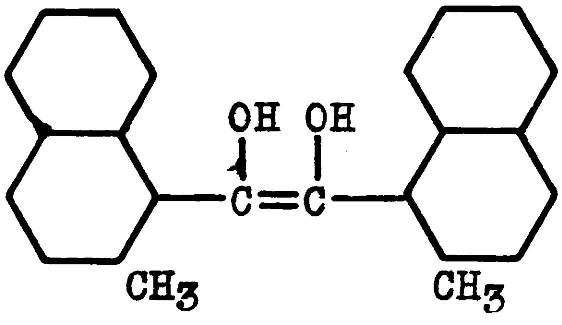


(LXXI)

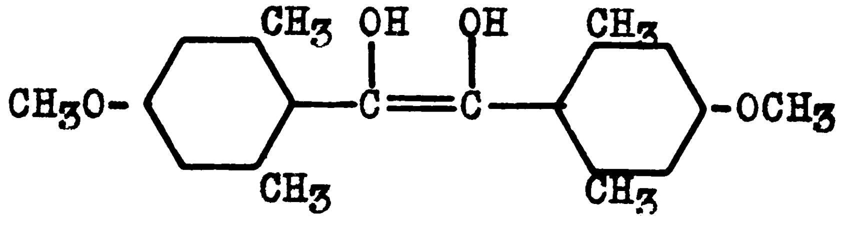


(LXXII)

Enediols in the naphthalene series (LXXIII) (120) are also stable but methoxystilbenediols are unstable, especially p-dimethoxytetramethylstilbenediol (LXXIV) (121).



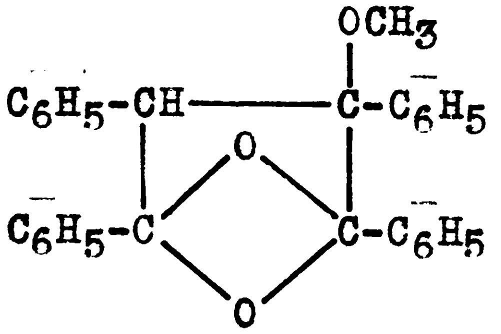
(LXXIII)

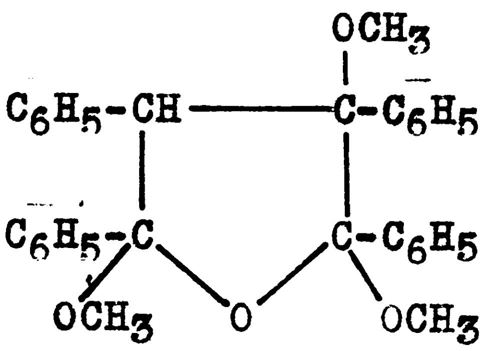


(LXXIV)

The stability of enediols is also determined by their stereoconfiguration. Thus the trans modification is much more stable towards oxidation and isomerization than the cis modification.

Another interesting property of α-hydroxy-ketones is their tendency to polymerize in the presence of acids. When α-hydroxypropiovanillone (41a) is treated with 5% sulfuric acid, 5% methanolic hydrogen chloride or 95% formic acid, amorphous lignin-like polymers are obtained. Benzoin, when subjected to treatment with methanol and hydrogen chloride, in addition to undergoing methylation, is partially converted into condensation polymers (113). To two of these, the structures (LXXV) and (LXXVI) have been assigned; their confirmation by direct synthesis is still lacking. Anisoin and furoin give products analogous to those from benzoin.


(LXXV)

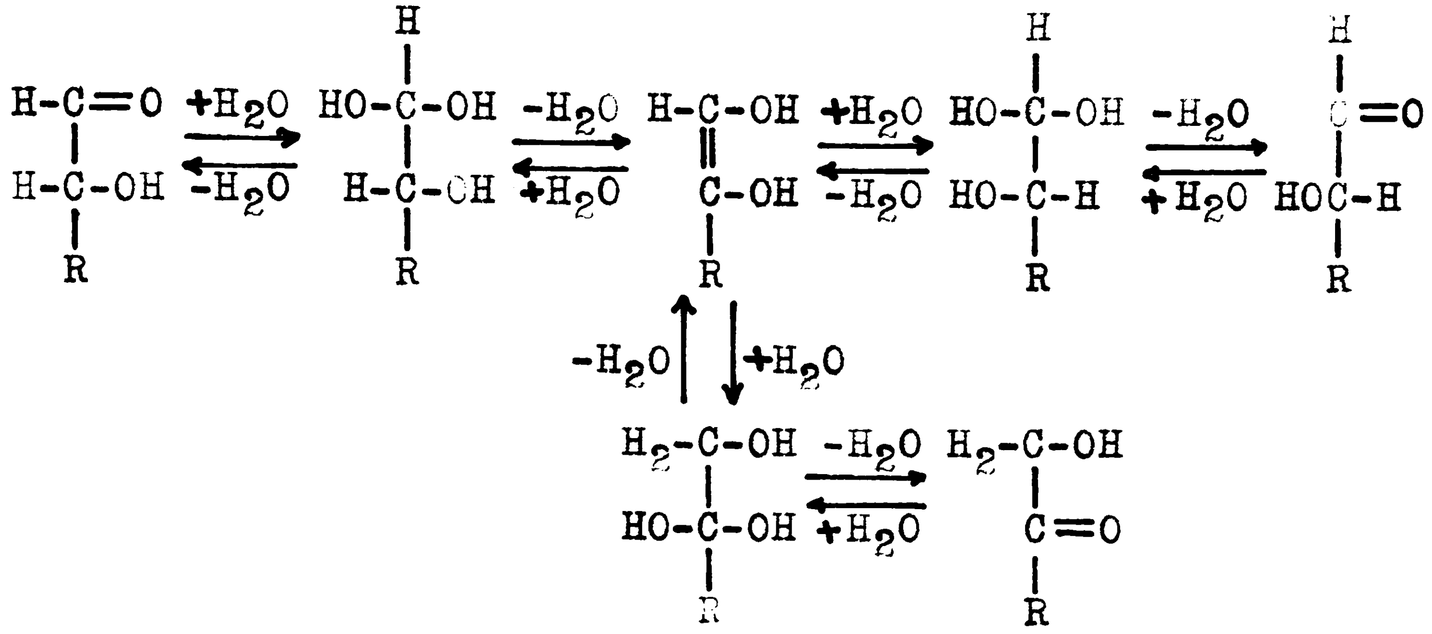

(LXXVI)

(2) Dismutation Reactions of Hydroxy-ketones

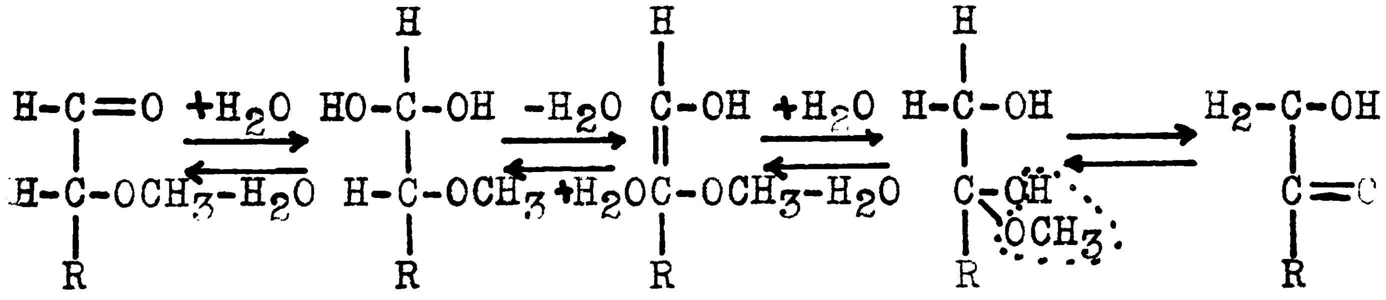
One of the most interesting properties of hydroxy-ketones is their marked tendency to undergo intramolecular dismutation transformations, such as typified below:

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

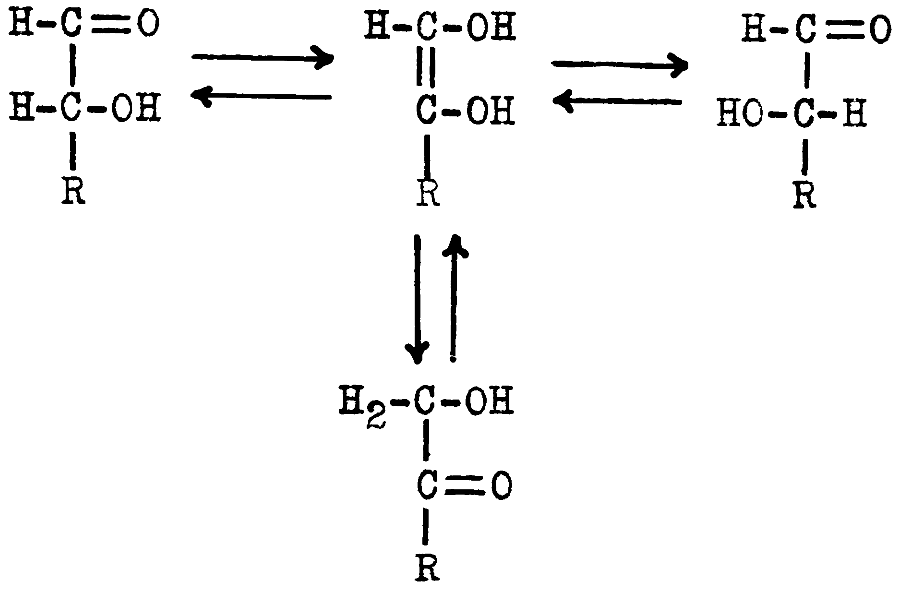
The best known examples of such intramolecular dismutation rearrangements are those which occur in the field of carbohydrate chemistry. The well known Lobry de Bruyn transformations, embracing the equilibrium system, glucose, mannose, fructose (122) and that of glyceric aldehyde and dihydroxy acetone (123, 124, 125), both of which occur in weakly alkaline solution, are only two of a very large number of similar examples. Nef (126) explained these phenomena on the basis of selective hydration and dehydration of the sugars involved, and postulated a common enediol intermediate:



Lewis and co-workers (127), however showed that such a mechanism was not tenable - at least in the case of methylated sugars. Thus, for example, on the basis of Nef's hypothesis, tetramethyl glucose should be converted by alkali into methylated fructose by the loss of methanol from the intermediate hemiacetal:



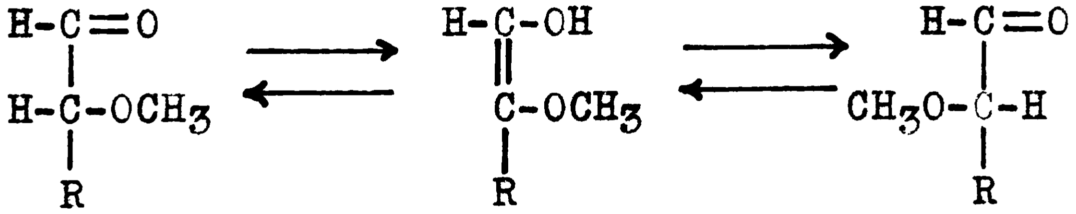
Wolfrom and Lewis (128) showed, however, that no methylated fructose was formed by the action of dilute alkali on tetramethyl glucose. To explain this result, as well as those obtained in the alkaline oxidation studies of methylated sugars, Gustus and Lewis (127) postulated a simple keto-enol shift to the intermediate enediol. Thus in the case of the unsubstituted sugars the labile hydrogen could migrate in the following manner:



$$\begin{array}{c}\mathrm{H-C=O}\\ |\\ \mathrm{H-C-OH}\\ |\\ \mathrm{R}\end{array} \rightleftarrows \begin{array}{c}\mathrm{H-C-OH}\\ \|\\ \mathrm{C-OH}\\ |\\ \mathrm{R}\end{array} \rightleftarrows \begin{array}{c}\mathrm{H-C=O}\\ |\\ \mathrm{HO-C-H}\\ |\\ \mathrm{R}\end{array}$$

$$\begin{array}{c}\mathrm{H-C-OH}\\ \|\\ \mathrm{C-OH}\\ |\\ \mathrm{R}\end{array} \rightleftarrows \begin{array}{c}\mathrm{H_2-C-OH}\\ |\\ \mathrm{C=O}\\ |\\ \mathrm{R}\end{array}$$

whereas in the case of the methylated sugars, the methyl group of the methoxyl presumably could not shift in this way and the changes would be limited to the following:



$$\begin{array}{c}\mathrm{H-C=O}\\ |\\ \mathrm{H-C-OCH_3}\\ |\\ \mathrm{R}\end{array} \rightleftarrows \begin{array}{c}\mathrm{H-C-OH}\\ \|\\ \mathrm{C-OCH_3}\\ |\\ \mathrm{R}\end{array} \rightleftarrows \begin{array}{c}\mathrm{H-C=O}\\ |\\ \mathrm{CH_3O-C-H}\\ |\\ \mathrm{R}\end{array}$$

Such dismutations are also encountered with many other types of compounds. Of special interest in the field of lignin chemistry is the marked ease of rearrangement of compounds of the type C_6H_5-CO-CHOH-R where R may be either aliphatic or aromatic. The nature of the R group and that of the substituents in the benzene ring have a marked influence on the tendency towards intramolecular dismutation changes. While a thorough study of the effect of these substituent groups has not been made as yet, sufficient work has been done to indicate a few basic principles, and these are of considerable importance in view of their bearing on some of the postulated lignin units, e.g. α-hydroxypropiovanillone.

Thus Henze (129) effected the isomerization of benzoyl acetonyl carbinol (LXXVII) into its isomer mandelyl acetone (LXXVIII) by treatment with a cold alcoholic solution of sodium ethoxide.

$$C_6H_5CO\text{-}CHOH\text{-}CH_2\text{-}CO\text{-}CH_3 \xrightarrow[\text{Alc}]{\text{NaOEt}} C_6H_5\text{-}CHOH\text{-}CO\text{-}CH_2\text{-}CO\text{-}CH_3$$

(LXXVII) (LXXVIII)

Considerable work has been done on rearrangements of the two isomeric ketols, phenyl acetyl carbinol (LXXIX) and benzoyl methyl carbinol (LXXX).

$C_6H_5\text{-}CHOH\text{-}CO\text{-}CH_3$ (LXXIX)

$C_6H_5\text{-}CO\text{-}CHOH\text{-}CH_3$ (LXXX)

Favorskii (130) heated (LXXX) with a few drops of concentrated sulfuric acid in a sealed tube at 120-130° and obtained the isomer (LXXIX). This isomerization has since been carried out by Temnikova and co-workers (131,132) and by Auwers and co-workers (133,134) under a variety of conditions.

Temnikova and Favorskii (131) showed that each of the ketols (LXXIX) and (LXXX) was stable in the absence of catalysts (acids, bases, yeast), but that on treatment with simicarbazide, phenyl isocyanate, the Grignard reagent or benzoyl chloride, they behaved as tautomeric mixtures. Auwers and co-workers found that the conversion of methyl benzoyl carbinol into phenyl acetyl carbinol could be effected by refluxing with aqueous barium carbonate for 20 hours (133); by the action of dilute sodium ethoxide at 0° for 24 hours (133), or by heating with 2% methanolic hydrogen chloride (134). Temnikova came to the conclusion that benzoyl methyl carbinol (LXXX) is readily converted into its stable isomer, phenyl acetyl carbinol (LXXIX) by the action of either acid or alkali but that the reverse isomerization does not take place.

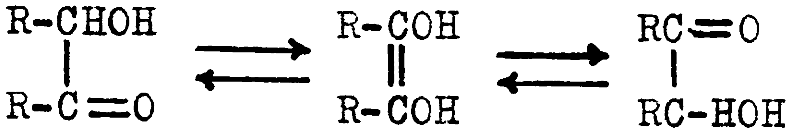
Recently she (135,136) has extended this work to a study of the two isomeric ketols, ethyl benzoyl carbinol (LXXXI) and phenyl propionyl carbinol (LXXXII).

$C_6H_5CO\text{-}CHOH\text{-}CH_2\text{-}CH_3$ (LXXXI)

$C_6H_5CHOH\text{-}CO\text{-}CH_2\text{-}CH_3$ (LXXXII)

When (LXXXII) is heated in a sealed tube with a little sulfuric acid in ethanol an equilibrium mixture results containing 60-65% (LXXXI) and 40-35% (LXXXII). The same equilibrium mixture is obtained when either (LXXXI) or (LXXXII) is allowed to stand at room temperature with a little ethanolic potassium hydroxide.

McKenzie and co-workers (137) assumed that benzoin transformations represent equilibrium reactions proceeding through the enediol stage:



$$\begin{array}{c}\text{R-CHOH}\\ |\\ \text{R-C=O}\end{array} \rightleftarrows \begin{array}{c}\text{R-COH}\\ \|\\ \text{R-COH}\end{array} \rightleftarrows \begin{array}{c}\text{RC=O}\\ |\\ \text{RC-HOH}\end{array}$$

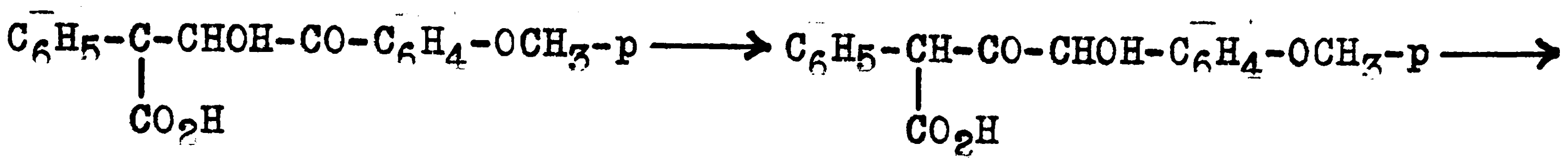
Kohler and Kimball (138) found that when the β-lactone of α-phenyl-β-hydroxy-β-benzoyl propionic acid was shaken for an hour with 5% aqueous sodium hydroxide, it lost carbon dioxide with the formation of a mixture of the isomeric hydroxyketones (LXXXIII) and (LXXXIV).

$C_6H_5\text{-}CH_2\text{-}CO\text{-}CHOH\text{-}C_6H_5$
(LXXXIII)

$C_6H_5\text{-}CH_2\text{-}CHOH\text{-}CO\text{-}C_6H_5$
(LXXXIV)

They believed that (LXXXIII) was the primary reaction product and that this was converted secondarily to its isomer (LXXXIV).

Kohler and Leers (139) investigated α-phenyl-β-hydroxy-β-anisoyl propionic acid (LXXXV) in which one of the phenyl groups is "tagged" by a methoxyl group. The behavior of this compound as outlined below confirmed their previous views. At ordinary temperatures in the presence of cold dilute alkali, the only product of the reaction is (LXXXVI), but when the latter is heated with dilute alkali, it passes completely into a low melting isomeric ketol (LXXXVII).



$$C_6H_5\text{-}\underset{\displaystyle CO_2H}{\underset{|}{C}}\text{-}CHOH\text{-}CO\text{-}C_6H_4\text{-}OCH_3\text{-}p \longrightarrow C_6H_5\text{-}\underset{\displaystyle CO_2H}{\underset{|}{CH}}\text{-}CO\text{-}CHOH\text{-}C_6H_4\text{-}OCH_3\text{-}p \longrightarrow$$

(LXXXV)

$$C_6H_5\text{-}CH_2\text{-}CO\text{-}CHOH\text{-}C_6H_4\text{-}OCH_3\text{-}p \longrightarrow C_6H_5\text{-}CH_2\text{-}CHOH\text{-}CO\text{-}C_6H_4\text{-}OCH_3\text{-}p$$

(LXXXVI) (LXXXVII)

Kohler and co-workers believed that these isomeric hydroxy-ketones were in equilibrium, but they cite no experimental evidence and were unable to demonstrate the reverse shift $\text{-}CH_2\text{-}CHOH\text{-}CO\text{-} \longrightarrow \text{-}CH_2\text{-}CO\text{-}CHOH\text{-}$.

Stevens (140), however, working with the para-chlor analog of (LXXXVI) succeeded in doing so and provided definite evidence for this equilibrium.

$$C_6H_5\text{-}CH_2\text{-}CHOH\text{-}CO\text{-}C_6H_4\text{-}Cl\text{-}p \rightleftharpoons C_6H_5\text{-}CH_2\text{-}CO\text{-}CHOH\text{-}C_6H_4\text{-}Cl\text{-}p$$

(LXXXVIII) (LXXXIX)

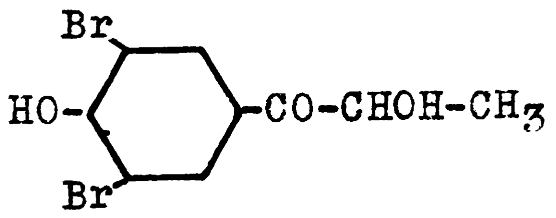
Starting with either isomer he was able to show that under the influence of aqueous alcoholic sodium carbonate both isomers were present in the reaction mixture. He concluded that probably all such 1,2-ketols represent, in solution, equilibrium mixtures of dismutation isomers, and that the failure to observe this in some cases may be due either to the position of the equilibrium being almost entirely on one side or the other, or to the peculiar properties of one of the two isomers in question, which render the isolation difficult.

The position of the equilibrium, as well as the stability of the isomeric ketols, is markedly affected by the presence of substituents in the benzene ring. Thus Auwers and co-workers (141) have shown that, whereas in the parent ketols, phenyl acetyl carbinol (LXXIX) and benzoyl methyl carbinol (LXXX), (LXXIX) is the stable

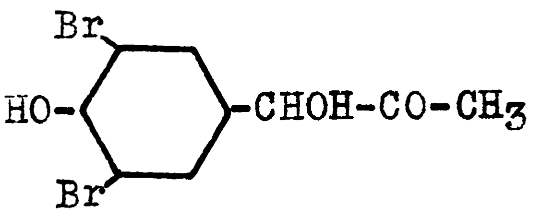
C_6H_5-CHOH-CO-CH_3
(LXXIX)

C_6H_5-CO-CHOH-CH_3
(LXXX)

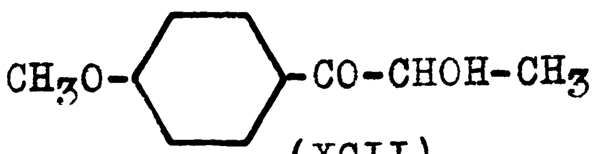
form, this stability is reversed by introducing substituents in the benzene ring. Thus (XC) and (XCII) are more stable



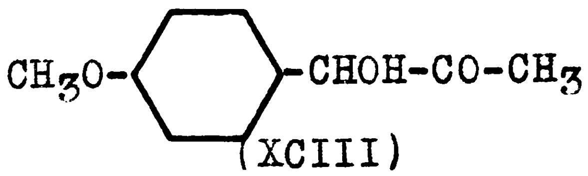
(XC)



(XCI)



(XCII)



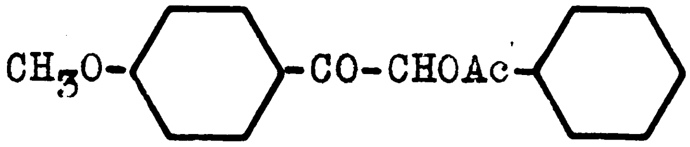
(XCIII)

than their isomers (XCI) and (XCIII) respectively.

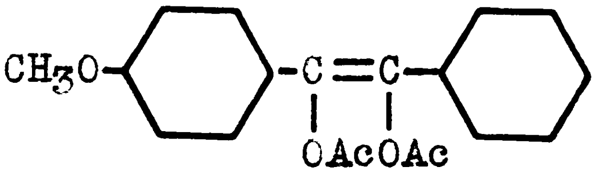
This inversion of stability due to the presence of a para-methoxyl group is also evident in the work of Kohler and co-workers (138, 139). Thus while C_6H_5-CHOH-CO-CH_2-C_6H_5 appears to be the stable form of the unsubstituted benzoin,

$C_6H_5-CO-CHOH-CH_2-C_6H_5$, in the case of the para-methoxyl substituted compounds the stable form is $C_6H_5-CH_2-CHOH-CO-C_6H_4-OCH_3$-p.

Barnes and Tulane (142) have studied this same effect and obtained similar results. They acetylated anisbenzoin ($p-CH_3O-C_6H_4-CHOH-CO-C_6H_5$), its dismutation isomer, and the bromo derivative, anisoyl phenyl bromomethane and in each case obtained a mixture of the monoacetate (XCIV) and the diacetate (XCV). When an alcoholic solution of the diacetate was treated

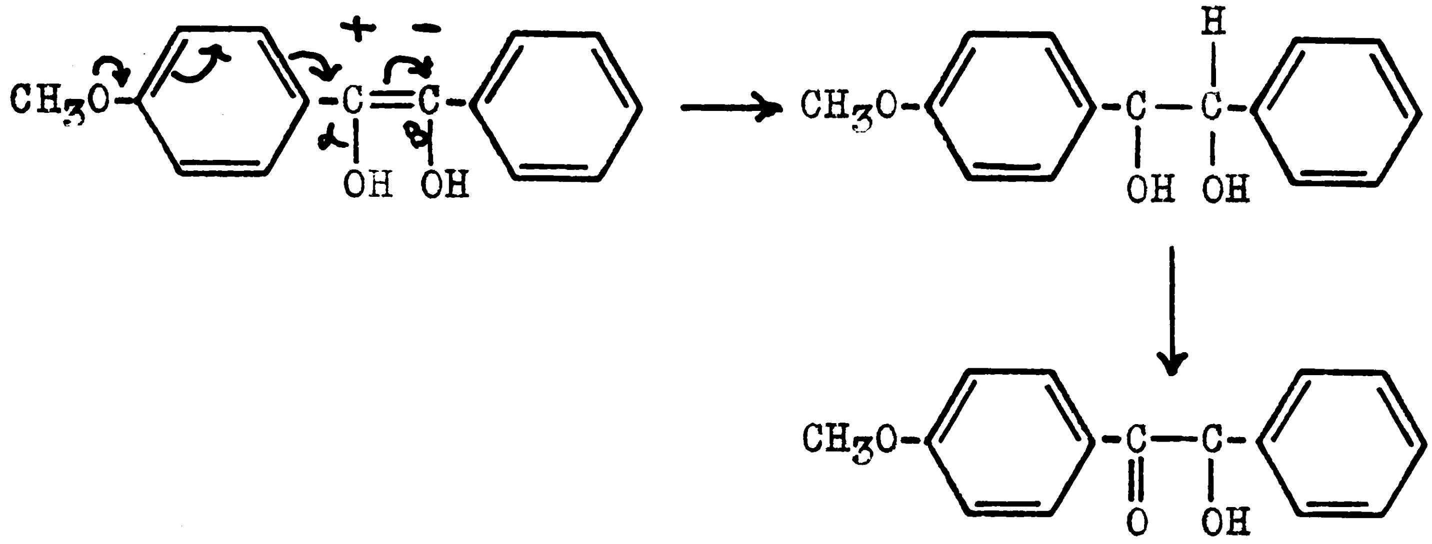


(XCIV)

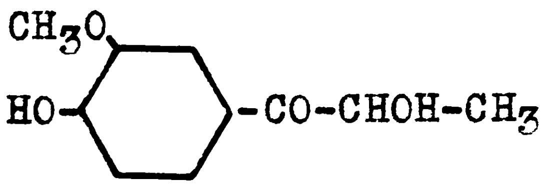


(XCV)

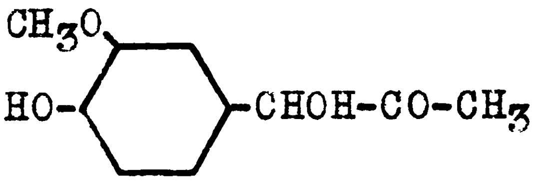
with acid, benzanisoin ($p-CH_3O-C_6H_4-CO-CHOH-C_6H_5$) (XCVI) resulted. The authors believe that the diacetate on acid hydrolysis is converted into the unstable enediol which then passes into the more stable high melting benzanisoin (XCVI). They believe also that the directing influence of the methoxyl is manifest in the intermediate enediol and explain this effect in the following manner: The methoxyl group has a strong tendency towards electron release thereby giving rise to an electronic strain in the aromatic nucleus. The para-carbon will be negatively charged and the two intermediate carbon atoms (alpha and beta) charged positively and negatively, respectively. A proton is therefore added at the beta carbon followed by ejection of a proton from the alpha-hydroxyl, the stable benzanisoin resulting:



One of the ethanolysis products from maple wood is α-hydroxypropiovanillone (XXVI), and its dismutation isomer (XCVII) has not yet been isolated.



(XXVI)



(XCVII)

In view of the foregoing discussion this is not surprising since the influence of the para-hydroxyl should make (XXVI) the more stable isomer. (The meta-methoxyl group should have no influence).

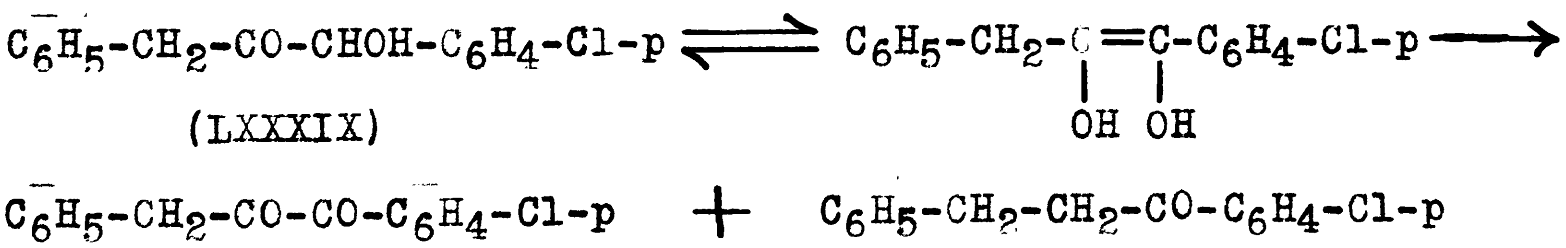
(3) Oxidation and Reduction Reactions of Keto-alcohols

α-Keto-alcohols are very susceptible to oxidation; the following oxidizing agents convert them to the corresponding diketones: nitric acid, copper sulfate and pyridine (43a) (43b), Fehling's solution (143) (144), alkaline iodine solution (145), lead tetra-acetate (146), and ammonium nitrate (147). The mechanism of the reaction is not quite clear as yet, but it is quite likely that the oxidation goes through the enediol stage. Thus the reaction of benzoin with iodine and sodium methylate is believed to compriseof the formation of the enediol-disodium salt, followed by the removal of the two sodium atoms by two atoms of iodine. Lead tetra-acetate is a more drastic reagent than iodine, and in the presence of hydroxyl-forming solvents such as water, alcohols or hydrocyanic acid, oxidative cleavage of benzoin to one mole of an acid and one of an aldehyde or a ketone occurs. However, in the absence of hydroxyl-forming solvents the keto-alcohol is oxidized slowly to the diketone. Ammonium nitrate is a good reagent for oxidizing benzoins to benzils, although, in the case of certain substituted benzoins, the tendency towards nitration is greater than that towards oxidation.

In addition to a marked susceptibility to oxidation, α-hydroxy-ketones are also characterized by their tendency to undergo intramolecular oxidation and reduction. The commonest examples are found in the carbohydrate field. As is well known, glucose, fructose, and mannose readily undergo oxidation in alkaline solution in the presence of atmospheric oxygen. Kohler and co-workers (138) encountered this phenomenon in their work on hydroxy-ketones and hydroxy-ketonic acids. Thus in alkaline solution (5% sodium hydroxide) 1,3-diphenyl-2-hydroxy-propanone-1 undergoes a simultaneous oxidation and reduction even in the absence of atmospheric oxygen.

$$C_6H_5\text{-}CH_2\text{-}CHOH\text{-}CO\text{-}C_6H_5 \longrightarrow C_6H_5\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}C_6H_5 + C_6H_5\text{-}CH_2\text{-}CO\text{-}CO\text{-}C_6H_5$$

The sensitive character of α-hydroxy-ketones in the presence of alkalis was also observed by Stevens (140) in his work on the dismutation of α-hydroxy-p-chlorobenzyl benzyl ketone (LXXXIX). Apparently this reaction involved formation of the intermediate enediol followed by simultaneous reduction and oxidation.



$$\underset{\text{(LXXXIX)}}{C_6H_5\text{-}CH_2\text{-}CO\text{-}CHOH\text{-}C_6H_4\text{-}Cl\text{-}p} \rightleftharpoons C_6H_5\text{-}CH_2\text{-}\underset{OH}{C}=\underset{OH}{C}\text{-}C_6H_4\text{-}Cl\text{-}p \longrightarrow C_6H_5\text{-}CH_2\text{-}CO\text{-}CO\text{-}C_6H_4\text{-}Cl\text{-}p + C_6H_5\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}C_6H_4\text{-}Cl\text{-}p$$

In acid media similar results are obtainable. Thus anisoin in methanol or ethanol hydrogen chloride (113) at room temperature gives anisil among other products. Benzoin in the presence of methanolic hydrogen chloride, (148) is converted to its ether and two other compounds which readily hydrolyse to benzoin and desoxybenzoin.

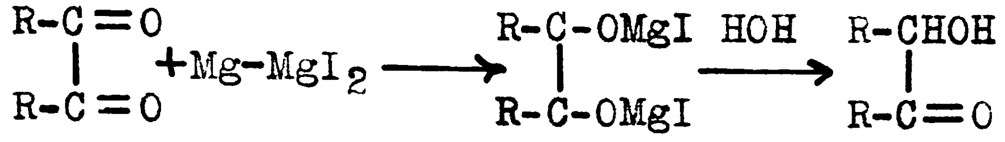
(4) Properties of 1,2-Diketones

1,2-Diketones are nearly always colored substances, for example, diacetyl is greenish yellow, benzil and vanilloyl methyl ketone are deep yellow, while syringoyl methyl ketone is light orange. They are quite stable substances, especially the aromatic derivatives. Benzil, when heated with concentrated hydrochloric acid, remains unchanged (149) whereas diacetyl polymerizes in the cold to the trimolecular form (150). Syringoyl methyl ketone is stable to boiling 2% ethanolic-hydrogen chloride. Diketones are cleaved when exposed to sunlight. Thus benzil dissolved in alcohol and irradiated gives rise to benzoin,benzaldehyde, benzoic acid as well as to a considerable amount of resinous material (151).

1,2-Diketones are easily reduced catalytically, by a combination of a metal and an acid or an alkali, or phytochemically. Aryl diketones (152) may be reduced almost quantitatively to the corresponding benzoins or hydrobenzoins by use of low pressure hydrogen and platinum black as catalyst. Hindered diketones such as dimesityl diketone or veratril are more difficult to reduce and require more drastic treatment.

Gomberg and co-workers (153) (154) have developed a mild method of hydrogenating diketones. A series of benzils, some of which were sterically hindered, were treated with the binary system magnesium-magnesium iodide with excellent results.

The mechanism of the reaction has been proven beyond doubt to consist of the formation of the magnesium glycolate (XCVIII) followed by hydrolysis and rearrangement.

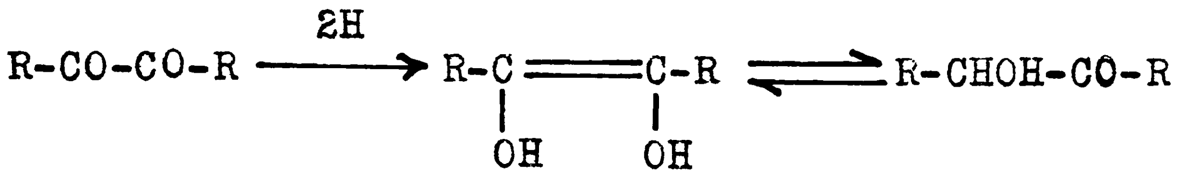


$$\begin{array}{c} R\text{-}C{=}O \\ | \\ R\text{-}C{=}O \end{array} + Mg\text{-}MgI_2 \longrightarrow \begin{array}{c} R\text{-}C\text{-}OMgI \\ | \\ R\text{-}C\text{-}OMgI \end{array} \xrightarrow{HOH} \begin{array}{c} R\text{-}CHOH \\ | \\ R\text{-}C{=}O \end{array}$$

(XCVIII)

Combinations of a metal and an acid produce stepwise reactions, the extent of reduction depending upon the concentration, and reaction time. Thus in the presence of tin and hydrochloric acid or zinc and acetic acid, mesityl phenyl diketone (155) is reduced to a mixture of 2,4,6,-trimethylbenzoin, and 2',4',6'-trimethylbenzoin, whereas a prolonged treatment yields 2,4,6-trimethylbenzyl phenyl ketone only. Amalgamated tin and hydrochloric acid (156) and zinc with dilute sulfuric acid (100) have been used successfully to reduce benzoins to the corresponding desoxybenzoins. Pearl and Dehn (157) observed that the extent of the reduction of benzil to benzoin or desoxybenzoin by means of amalgamated tin, zinc, aluminum or magnesium and hydrochloric acid, depended upon the temperature, solvent and concentration. With properly controlled conditions these workers were able to prepare each of the reduction products in almost quantitative yield.

The mechanism of reduction of 1,2-diketones has been established by Gomberg (153) (154) and by Thompson (114). It consists of a 1,4 addition of hydrogen to the dicarbonyl compound, and rearrangement of the resulting enediol to the more stable keto-alcohol.



$$R\text{-}CO\text{-}CO\text{-}R \xrightarrow{2H} R\text{-}\underset{OH}{C}=\underset{OH}{C}\text{-}R \rightleftharpoons R\text{-}CHOH\text{-}CO\text{-}R$$

Gomberg isolated the magnesium glycolate (XCVIII) of the intermediate enediol. This glycolate reacted with acyl halides to produce diacylates; it added two atoms of a halogen and the halogenated addition product on hydrolysis yielded the benzil quantitatively. Thompson observed that 1,2-diketones underwent catalytic hydrogenation in the presence of an acetylating agent (acetic anhydride) to yield the diacetate of the unstable enediol. He also prepared the stable enediol of dimesityl diketone. Recently, it has been found that substituents on the benzene rings of an aromatic enediol have a stabilizing influence, and now several stable enediols are known (115, 116, 117, 118, 119, 120, 121).

Yeast, in a fermenting solution of sugar reduces aliphatic diketones to glycols (158) and aromatic diketones to keto-alcohols (159).

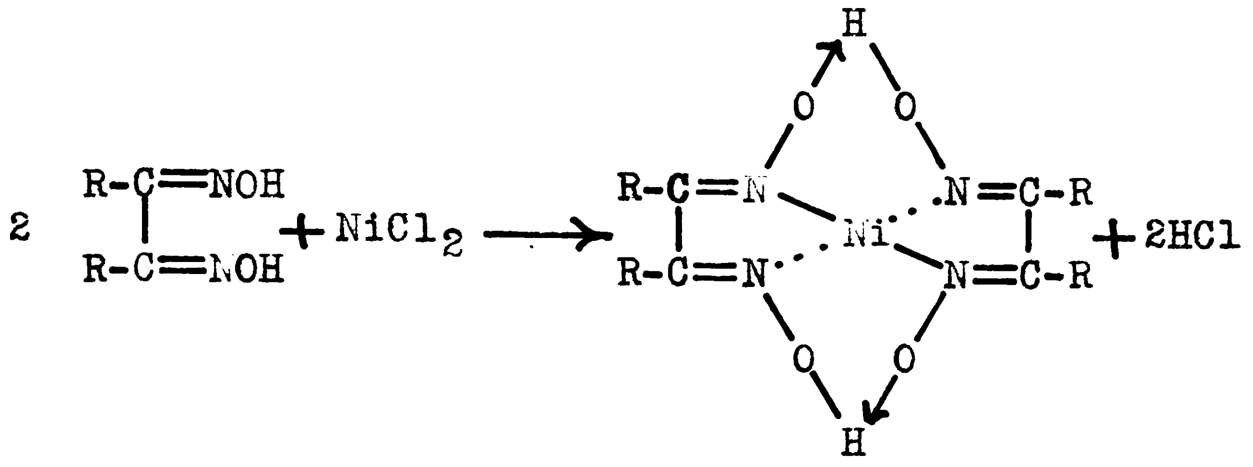
The ease of reduction of 1,2-diketones is of great significance for it indicates a close relationship among the ethanolysis products of wood, e.g. between vanilloyl methyl ketone, α-hydroxypropiovanillone, and (4-hydroxy-3-methoxyphenyl)-

propanone-2 on the one hand, and between syringoyl methyl ketone, α-hydroxypropiosyringone, and (4-hydroxy-3,5-dimethoxyphenyl)-propanone-2, on the other.

One of the characteristic tests for 1,2-diketones is cleavage by alkaline hydrogen peroxide; this results in the formation of two moles of an acid (160) (161). Cyclic diketones are cleaved to one mole of a dibasic acid (162) (163).

The sensitivity of dicarbonyl compounds to alkalis has been known for a long time. Thus benzils are readily converted to benzilic acids by alkali treatment. The extent of rearrangment is dependent on the temperature, the concentration of alkali and the type of dicarbonyl compound. Most benzils rearrange in the presence of potassium ethylate at room temperature (164). Mesityl glyoxal in the presence of warm dilute calcium hydroxide rearranges completely to mesityl glycollic acid (165). Sterically hindered diketones such as dimesityl diketones (166) are not altered, even by concentrated sodium hydroxide at high temperatures.

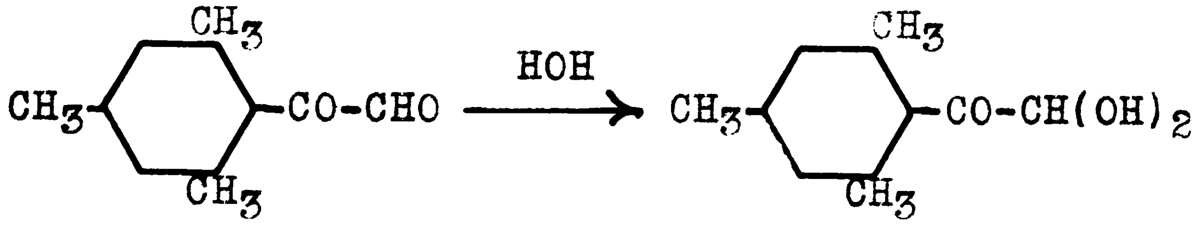
A very important property of 1,2-diketones is their ability to form dioximes in acid or alkali media, which in the presence of nickel, cobalt or iron salts produce insoluble glyoximates (XCIX). This property has been made use of in the separation of 1,2-diketones from monocarbonyl and other compounds. The diketones may be regenerated by treatment of the glyoximates with strong acids. Thus dioximes find application in analytical chemistry (167) (168) (169).



(XCIX)

The action of hydroxylamine on aromatic diketones is slow, and substituents in the ortho positions of benzene nuclei seem to retard oximation, as in the case of the inert dimesityl diketone. In the case of mixed aryl-alkyl diketones, two structurally isomeric monoximes are possible, the β-monoxime (aryl-CO-CNOH-alkyl) and the α-monoxime (aryl-CNOH-CO-alkyl). It has been found (170) that the latter are more difficult to form and less difficult to decompose than the corresponding β-monoximes; also the α-monoximes when refluxed with 2% sulfuric acid, rearrange completely to the β-monoximes. The apparent rearrangement is probably a matter of relative stability.

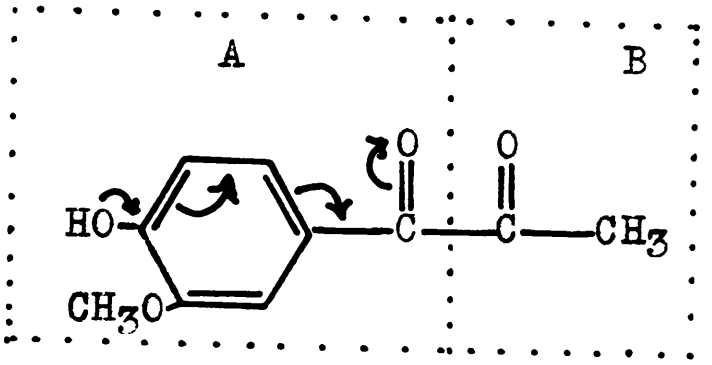
The two carbonyl groups of symmetrical 1,2-diketones are of equal reactivity, the symmetrical aliphatic diketones being more reactive than the aromatic. Unsymmetrical diketones such as the aryl-alkyl compounds, contain one highly reactive carbonyl, and one comparatively unreactive. It is an accepted fact that substituted aryl radicles tend to deactivate neighboring carbonyl groups, while the most activating grouping is a second carbonyl, as in compounds containing twinned carbonyl groups. The reason for the greater reactivity of the β-carbonyl relative to the α-carbonyl in aryl-alkyl diketones is thus apparent. That mesityl glyoxal (165) (C) contains a highly activated carbonyl group is demonstrated by the fact that it readily forms a hydrate (CI).



(C) (CI)

Aryl-alkyl diketones also undergo condensation reactions with the common reagents such as substituted hydrazines and semicarbazides to form mono-hydrazones and monosemicarbazones. This again shows the presence of an activated and a less reactive carbonyl group. Conversion of an aryl-alkyl diketone to a dihydrazone or a disemicarbazone (43) requires drastic treatments. The relative degree of stability and the relative ease of formation of α- and β-monoximes of aryl-alkyl diketones is further evidence of a difference in the reactivity of the two carbonyl groups.

The difference in the reactivity of the two carbonyl groups in vanilloyl methyl ketone and related substituted diketones, may be due to electronic displacements as shown below:

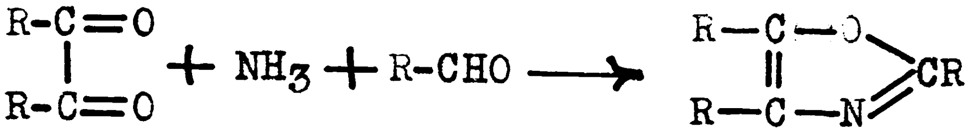


The hydroxyl group has a strong tendency towards electron release, giving rise to an electronic strain in the aromatic nucleus. The para-carbonyl together with the phenolic-hydroxyl form a neutralized system (fragment A) similar to that in a carboxyl group. The reactivity of the para-carbonyl is thus reduced in the same way as that of a carboxyl group. No such neutralization of the carbonyl in fragment B can take place.

The separation of syringyl from guaiacyl dicarbonyl derivatives by the precipitation from ethanol of the former, as the ammonium phenolate salts has been standardized in this laboratory (43b). It is interesting and important to note the possible effect of ammonia on other functional groups present. When benzil, p-anisil or p-tolil is heated with concentrated ammonium hydroxide in a sealed tube at 120°, triphenyl-, tri-p-tolyl and tri-p-anisil oxazoles are formed respectively (171).

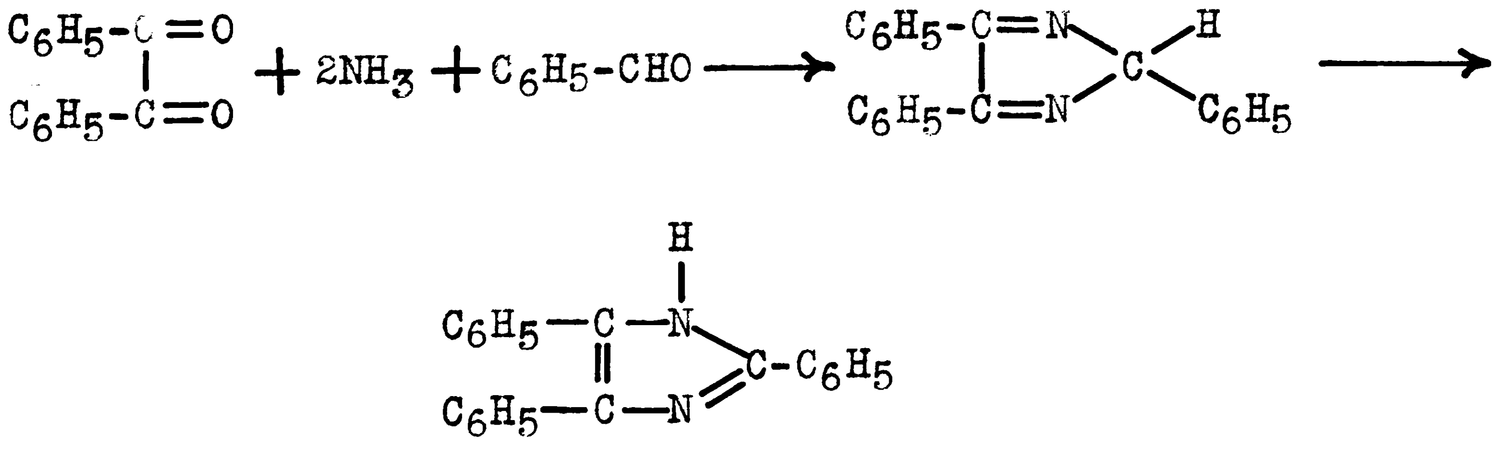
The course of the reaction is indicated by the following equations:

$$R\text{-}CO\text{-}CO\text{-}R \xrightarrow{HOH} R\text{-}COOH + R\text{-}CHO$$



More recently (172) the effect of an alcoholic solution of ammonia on benzil at 40° has been studied. Along with the oxazole, two other condensation products were obtained -- benzilimide and imabenzil.

In the presence of aldehydes and ammonia or ammonium salts, 1,2-diketones react to form glyoxalines. Thus, when benzil, benzaldehyde and ammonium acetate in acetic acid are heated, lopine (CII) is produced quantitatively.



(CII)

Thus in using ammonia for the separation of phenolic salts of 1,2-diketones, conditions must be so controlled as to prevent carbonyl condensation products.

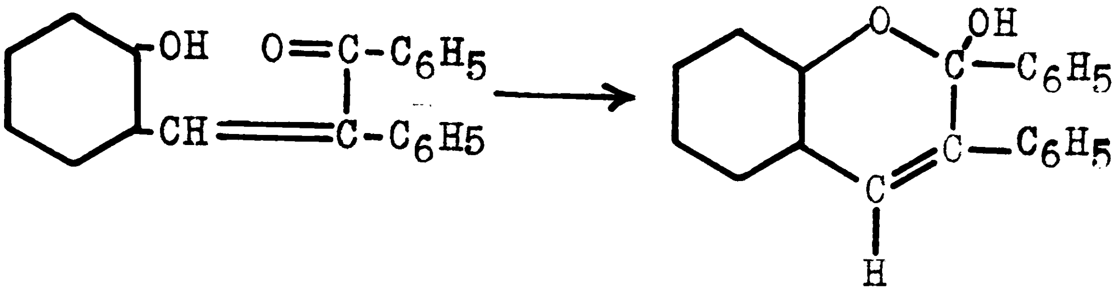
(5) Properties of Desoxybenzoins

Closely related to keto-alcohols and diketones are compounds of the desoxybenzoin type (CIII). A study of the properties of such compounds is also helpful to an understanding of their relationship to plant synthesis and their probable role as lignin building units.

$$R\text{-}CH_2\text{-}CO\text{-}R' \quad (CIII)$$

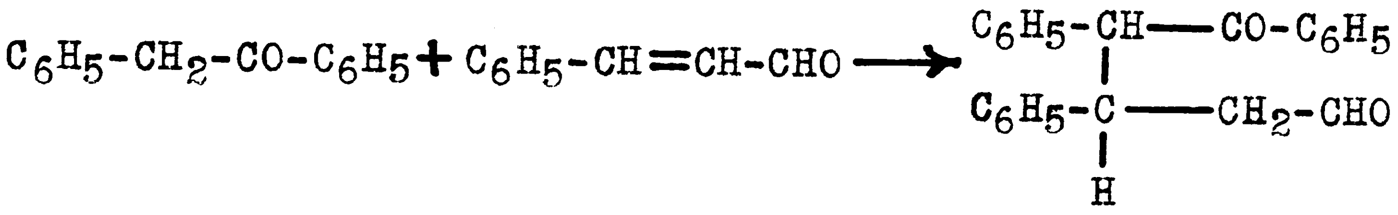
The methylenic group is an active functional group and undergoes the characteristic oxidation and condensation reactions. Specific oxidizing agents such as selenium dioxide (173, 174), nitrous acid (175) and nitrogen tetroxide (176) convert desoxybenzoins to 1,2-diketones. With nitrous acid and nitrogen tetroxide, the monoketone forms a monoxime which readily hydrolyzes to the diketone.

The condensation of desoxybenzoins with aromatic aldehydes, with the elimination of a molecule of water, and the formation of an arylidene-desoxybenzoin have been known for a long time (177). Even such an unreactive aldehyde as vanillin forms vanillylidene-desoxybenzoin under comparatively mild conditions. The arylidene desoxybenzoins are not very stable and may undergo further reactions, especially if the aldehyde portions contain reactive substituents. Thus salicylidene desoxybenzoin (CIV) is very unstable and undergoes immediately an internal ketone-alcohol condensation (178).



(CIV)

Desoxybenzoins may also add to α,β-unsaturated aldehydes (179). Thus cinnamaldehyde reacts with desoxybenzoin in the presence of a small amount of sodium methylate at 5° to give the condensation product (CV).



$$C_6H_5\text{-}CH_2\text{-}CO\text{-}C_6H_5 + C_6H_5\text{-}CH{=}CH\text{-}CHO \longrightarrow \begin{array}{l} C_6H_5\text{-}CH\text{---}CO\text{-}C_6H_5 \\ \qquad\quad | \\ C_6H_5\text{-}C\text{---}CH_2\text{-}CHO \\ \qquad\quad | \\ \qquad\quad H \end{array}$$

(CV)

Although the desoxybenzoins undergo oxidation and condensation reactions, the activity of the methylenic group is not as pronounced as in compounds such as malonic or aceto-acetic acids (180). While substances containing two carbonyl groups separated by a methylenic group are able to form condensation products with aldehydes under the influence of basic catalysts such as piperidine, desoxybenzoins do not react under the same conditions. However, under the influence of gaseous hydrogen chloride or alcoholic alkali, condensation can be brought about.

The relative ease of oxidation of desoxybenzoins and related compounds, and their ability to condense with aromatic aldehydes and α,β-unsaturated carbonyl compounds, is highly important in view of the fact that vanillin, syringaldehyde, (4-hydroxy-3-methoxyphenyl)-propanone-2 and its syringyl analog are found in the ethanolysis products of maple wood. In addition, although α,β—unsaturated carbonyl compounds, such as coniferyl aldehyde, have not been isolated from wood ethanolysis products, they may be present in the wood, since derivatives of coniferyl alcohol have been found.

Discussion of Experimental Results

In the Historical Review the significance of the ethanolysis products from wood has been stressed from the point of view of lignin structure. It has been indicated that the actual progenitors of the "lignin building units" have not been isolated as yet and may be present in part, at least, in the unidentified ethanolysis oils. Also certain of the ethanolysis products were considered to represent "stabilized end products" of other more labile active forms, the latter representing the true lignin building units. The problem assigned the writer was, firstly, that of improving the technique of ethanolysis with a view to easier identification and improvement of yields of the products already isolated, and secondly, the identification of the unknown constituents.

1. Ethanolysis of Wood

(a) Status of Wood Ethanolysis Prior to the Writer's Investigation.- The ethanolysis procedure (181) consisted of the following steps:

(i) Removal of the fats, waxes, resins and tannins from the wood meal by extraction with organic solvents.

(ii) Refluxing the dried wood meal with 2% ethanol-hydrogen chloride in an inert atmosphere.

(iii) Separation of the "ethanolysis oils" from the ethanol lignin by precipitation of the concentrated alcoholic liquor into a large volume of water, filtration from the precipitated ethanol lignin and extraction of the water-soluble ethanolysis products from the concentrated aqueous solution.

(lV) Fractionation of the "ethanolysis oils" into bisulfite-soluble, bicarbonate-soluble, alkali-soluble and the neutral fractions.

The bisulfite-soluble fraction of maple wood had been investigated previously (42) and found to contain in addition to syringaldehyde (VII) two other substances later identified as syringoyl methyl ketone (XI) (182) and vanilloyl methyl ketone (X), isolated as semicarbazones, but not as the free carbonyl compounds. The presence of vanillin in this fraction was noted in only one or two instances and this was also true in a similar examination of the bisulfite fraction of spruce (43a).

Specifically, it was desired to develop a method for the isolation of the 1,2-diketones as such, and to isolate and identify the remaining ethanolysis components of the bisulfite fraction. Therefore efforts were first directed to improving the yields and purity of the water-soluble ethanolysis products.

(b) Modifications of the Ethanolysis Procedure. - A separation of the water-insoluble ethanol lignin from adsorbed oils was customarily effected by extraction with benzene (181). The lignin tars are however partially soluble in this solvent, so that the separation was incomplete. In the modified procedure, used by the writer (Flow Sheet I) a complete separation was ensured by dissolving the ethanol lignin in acetone and reprecipitating into water (183).

The previous method of extracting the water-soluble ethanolysis oils from the combined aqueous filtrates, i.e. by benzene extraction of the *concentrated* water-ethanol precipitation solutions, was substituted by extraction in the *unconcentrated* state in a twenty liter continuous extractor. In this way the tendency towards polymerization of the labile components during the concentration, as well as loss due to volatility in water vapor, were avoided. In spite of these precautions, it was found that the ethanolysis oils so obtained, still contained a considerable amount of resinous material. This was removed by application of the petroleum ether fractionation technique (183).

A modification introduced to afford a more efficient fractionation was that of back-extraction with benzene of each of the bisulfite-soluble, bicarbonate-soluble, and alkali-soluble fractions. This was found necessary in view of the fact that the ethanolysis oils are water-soluble, and in consequence there was always a tendency for a small amount of the true alkali-soluble phenol fraction to remain in the bisulfite and bicarbonate solutions respectively.

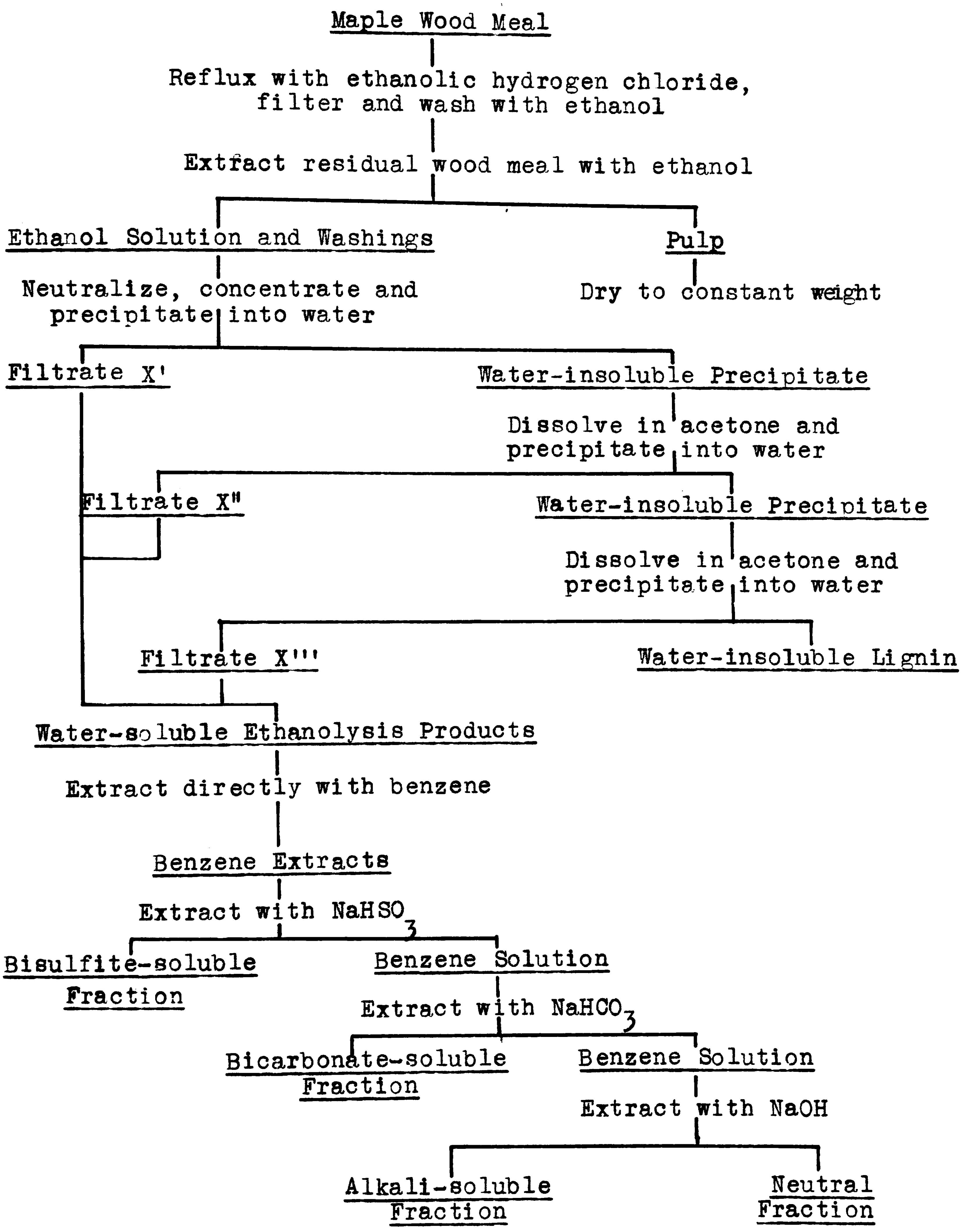
The primary aim of the modified ethanolysis technique was the elimination of the resinous material present in the water-soluble ethanolysis oils, with a view to the isolation of the monomolecular water-soluble ethanolysis products in a purer state and higher yield. The latter object was not attained, but it was found possible not only to isolate the various constituents of the bisulfite fraction in a far purer state than heretofore, but also to separate therefrom and identify two previously unknown constituents, namely, (4-hydroxy-3-methoxyphenyl)-propanone-2 and (4-hydroxy-3,5-dimethoxyphenyl)-propanone-2.

2. Separation of the Bisulfite-soluble Components

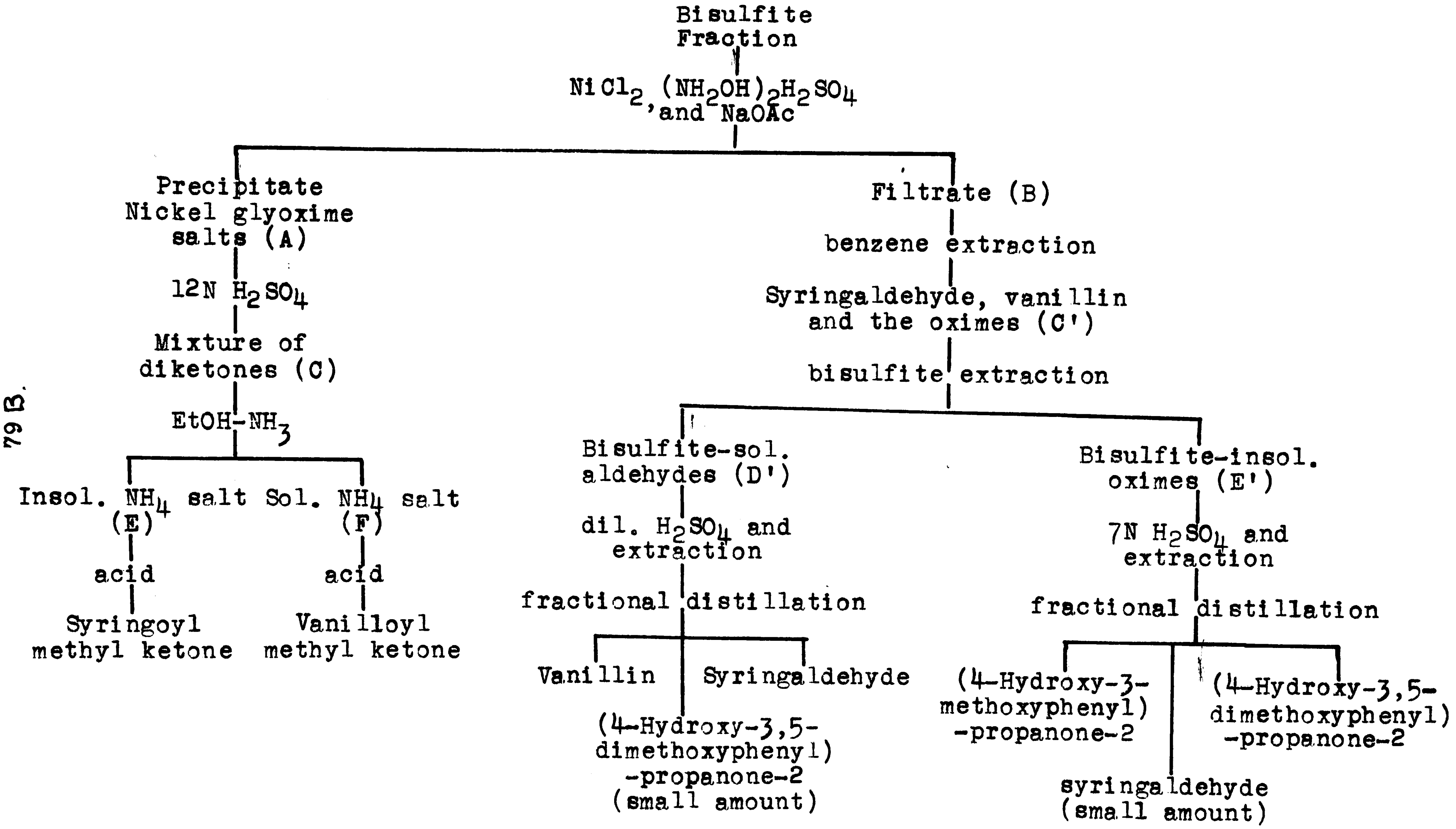
Method (a)

The bisulfite fraction of maple wood,as already pointed out, was known to consist of dicarbonyl compounds, aromatic aldehydes and unknown carbonyl derivatives. The problem which confronted the writer was to separate these components, in the free state, if possible, and to identify the unknown carbonyl derivatives. Several attempts such as sublimation and mercury salt precipitation were unsuccessful. The final method of separation (outlined in the flow sheet page 79B) was only developed after an extensive investigation of the properties of the diketones and aldehydes considered in relation to those of the unknown products, the latter possessing the properties of monoketones.

I. Flow Sheet of the Ethanolysis of Maple Wood Meal
(Flow Sheet I)



II. Flow Sheet of Analysis of the Bisulfite-soluble Fraction (Flow Sheet II)



Advantage was taken of the well-known tendency of 1,2-diketones to form dioximes which could be precipitated quantitatively as the nickel salts. Under the conditions used, it was found that vanillin and syringaldehyde did not form oximes, while the unknown carbonyl derivatives reacted readily with hydroxylamine, so that after removal of the insoluble nickel salts, and extraction of the filtrate with benzene, the two aldehydes could be removed from the benzene solution by shaking with sodium bisulfite solution. Thus the components of the bisulfite fraction were separated into three different classes of compounds: the dicarbonyl compounds isolated in the form of nickel glyoxime salts, the aromatic aldehydes in the free form and the unknown compounds as oximes.

Recovery of the Diketones (Flow Sheet II)

The next problem was to recover the free compounds from their derivatives i.e. the diketones from their nickel salts and the unknown compounds from their oximes, and to separate the syringyl from the guaiacyl components present in each of the three classes of compounds.

The nickel salts of the diketones were subjected to treatments with different acids, varying the concentration, temperature and time, and at the same time control runs with the free diketones were made to ensure their stability under the conditions used. It was found that 12N sulfuric acid at room temperature was the best hydrolytic reagent and that 80-85% of the diketones could be recovered from the hydrolytic mixture.

The determination of the solubility products of the ammonium salts of the two diketones, namely vanilloyl methyl ketone and syringoyl methyl ketone, showed that the mixture of these dicarbonyl derivatives, isolated from the above hydrolytic solution by extraction, could be separated by precipitation of the ammonium salt of syringoyl methyl ketone from anhydrous ethanol solution, leaving the ammonium salt of vanilloyl methyl ketone in solution. The free carbonyl derivatives were easily obtained from the ammonium salts by acidification. The total dicarbonyl content of the ethanolysis oils of maple wood was found to be 2 to 3% of the Klason lignin.

Recovery of the Aldehydes (Flow Sheet II)

It was found that the two aldehydes, namely, syringaldehyde and vanillin (D' Flow Sheet II), could also be separated by precipitation of the ammonium salt of the former from anhydrous ethanol solution, but a simpler method was that of fractional distillation (Flow Sheet II). The total aromatic aldehyde content of the ethanolysis oils of maple wood was found to be 0.4 to 0.8% of the Klason lignin.

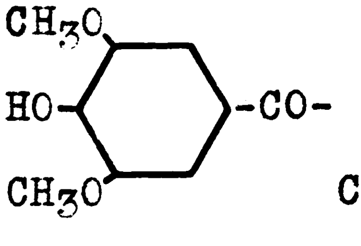
Recovery of the Unknown Phenol Products

The oximes (E' Flow Sheet II) of the unknown carbonyl derivatives, insoluble in the bisulfite solution, were decomposed with 7N sulfuric acid, recovered by solvent extraction and finally submitted to a careful fractionation in a new Cooke-Bower type of micro-fractionating column developed in these laboratories. This apparatus is vacuum-jacketed and contains a new type of packing so that a column some thirteen centimeters in length has an efficiency of around seven plates. In this way it was possible to separate two new products each containing a ketone grouping.

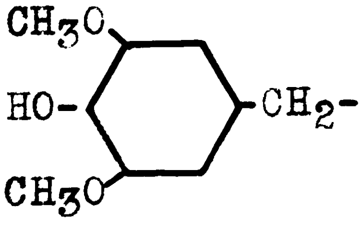
Method (b)

Another method of separating the components of the bisulfite fraction was that of removal of the diketones as nickel salts and treatment of the mixture of aldehydes and oximes, dissolved in anhydrous ethanol, with ammonia in ethanol solution. This latter operation presumably precipitated the syringyl components, leaving the guaiacyl components in solution. However, on acidification of the ammonium salt solutions, extraction of the free phenolic derivatives and fractional distillation, it was found that the ethanol-ammonia-soluble fraction, presumably containing only guaiacyl components, yielded a mixture of both the guaiacyl and the syringyl derivatives. This method of separation was incomplete, and was therefore abandoned.

That the ammonium salt of the unknown syringyl derivative is soluble in ethanol is not surprising since, as found later, it has no carbonyl para to the phenolic hydroxyl. Recently it has been shown (198) that salts of phenols of the type



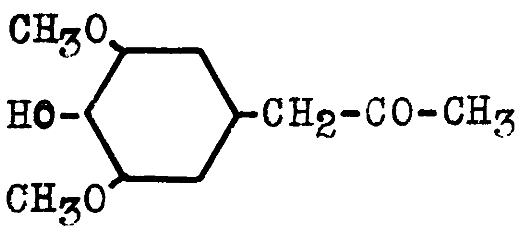
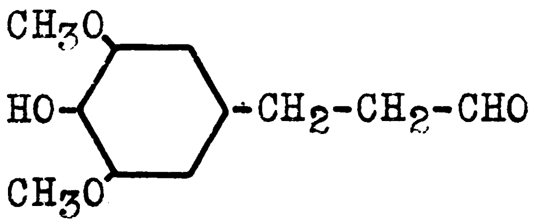
are insoluble in ethanol while salts of phenols of the type



are much more soluble. Thus, while the ammonium and potassium salts of α-ethoxypropiosyringone (IX), α-hydroxypropiosyringone, propiosyringone, and syringaldehyde are precipitated from ethanol by ammonia and potassium acetate respectively, the ammonium and potassium salts of 4-hydroxy-3,5-dimethoxyphenyl-propane and pyrogallol 1,3-dimethyl ether are not. Apparently, the carbonyl para to the phenolic hydroxyl confers the property of insolubility in ethanol on salts of syringyl derivatives.

3. Identification of the two Unknown Ethanolysis Products

These were separated by fractional distillation (Flow Sheet II) and Table 4, page 111. The molecular weight determination of the higher boiling product, its analysis and that of its semicarbazone, established the empirical formula of the former as $C_{11}H_{14}O_4$ and also proved the presence of one carbonyl and two methoxyl groups. Oxidation of the diazomethane methylated compound with potassium permanganate yielded 3,4,5-trimethoxybenzoic acid, thus establishing definitely the presence of the syringyl nucleus. It was therefore possible to assign to the unknown substance one of the two following structures:

A.  or B. 

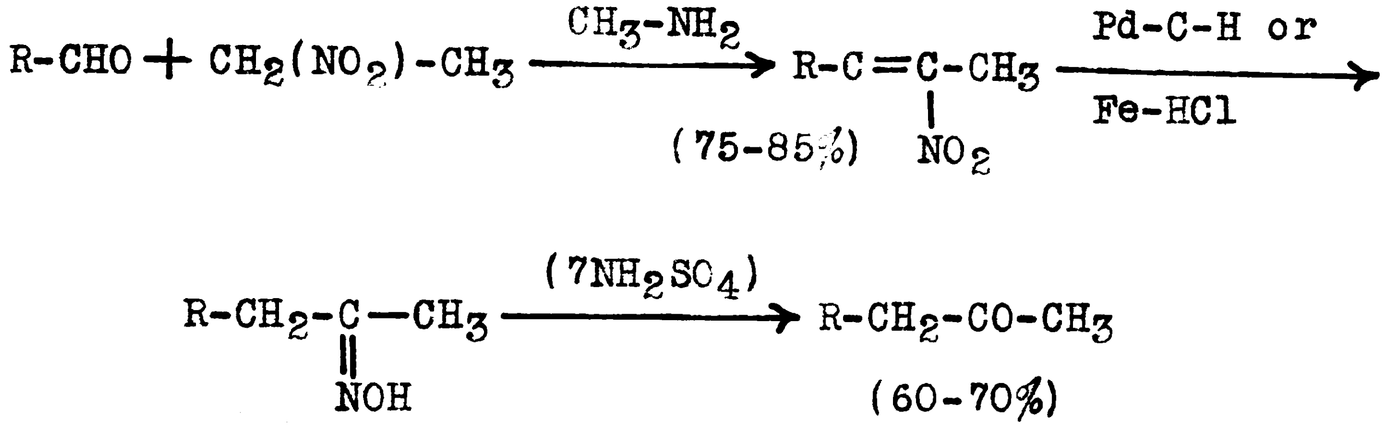
Since the product yielded iodoform on treatment with alkaline iodine it could not have the structure B and must therefore correspond to A. Direct synthesis and comparison of the pure crystalline derivatives provided final confirmation of A as (4-hydroxy-3,5-dimethoxyphenyl)-propanone-2. The identity of the second lower boiling unknown product was established in a similar manner as (4-hydroxy-3-methoxyphenyl)-propanone-2.

4. Synthesis of (4-Hydroxy-3,5-dimethoxyphenyl)-propanone-2 and its Guaiacyl Analog

An attempt to synthesize these new compounds by the reduction of the corresponding diketones, namely, syringoyl and vanilloyl methyl ketones, was unsuccessful for the reason that a mixture of the reduction products was formed and could not be separated due to limited amount of material. The synthesis was finally accomplished in the following manner: Syringaldehyde (VII) was condensed with nitroethane and the nitroalkene so formed, was reduced to the oxime, which gave on hydrolysis, (4-hydroxy-3,5-dimethoxyphenyl)-propanone-2. The guaiacyl analog, (4-hydroxy-3-methoxyphenyl)-propanone-2, was synthesized similarly using vanillin (VI) as the starting material instead of (VII).

Catalytic reduction of the nitroalkenes using Raney nickel or palladiumized charcoal as catalyst was attempted first. The former catalyst brought about too drastic a reaction, in that hydrogenolysis took place and the nitrogen of the nitroalkene was liberated as ammonia, so that only small amounts (10-25%) of the new compounds could be isolated from the reaction mixtures. The application of palladiumized charcoal as catalyst to the reduction of the nitroalkenes in pyridine as solvent, proved successful and yields of 50-60% of the carbonyl compounds could be obtained. The method of reduction finally employed, namely, use of iron powder, ferric chloride and hydrochloric acid, was that developed and patented by the Purdue Research Institute (191). The yields obtained were higher (60-70%) and the technique much simpler than when palladiumized charcoal and hydrogen were used.

The method of synthesis is illustrated in the following equations:



$$\text{R-CHO} + \text{CH}_2(\text{NO}_2)\text{-CH}_3 \xrightarrow{\text{CH}_3\text{-NH}_2} \underset{(75\text{-}85\%)}{} \text{R-C}{=}\underset{\substack{|\\ \text{NO}_2}}{\text{C}}\text{-CH}_3 \xrightarrow[\text{Fe-HCl}]{\text{Pd-C-H or}}$$

$$\text{R-CH}_2\text{-}\underset{\substack{\|\\ \text{NOH}}}{\text{C}}\text{-CH}_3 \xrightarrow{(7\text{NH}_2\text{SO}_4)} \underset{(60\text{-}70\%)}{\text{R-CH}_2\text{-CO-CH}_3}$$

R = guaiacyl or syringyl radical.

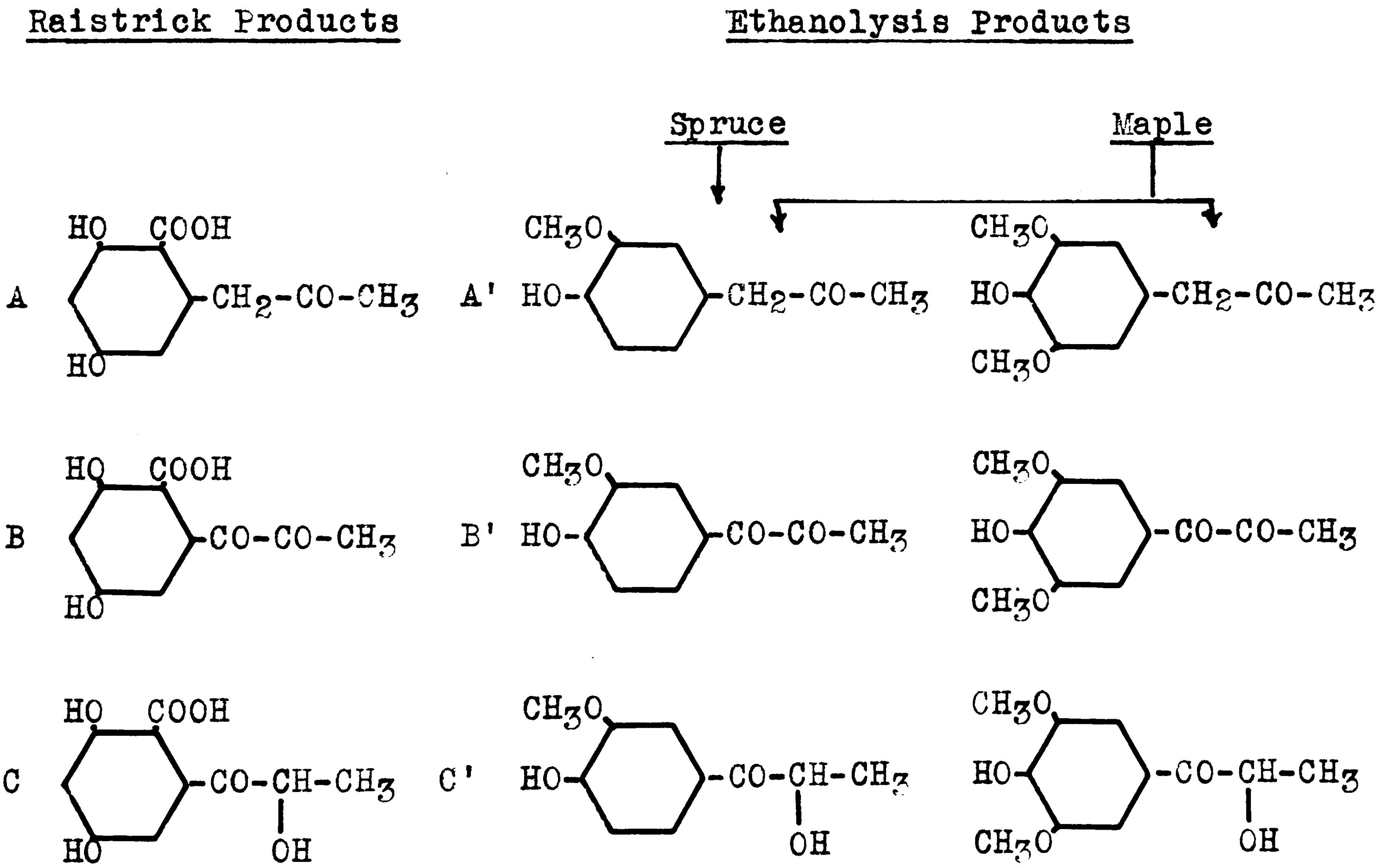
The reaction represented in the first equation is that of Knövenagel and has been used previously for the synthesis of various nitroalkenes. For example, benzaldehyde (184, 185), veratrylaldehyde (186), p-methoxybenzaldehyde (184), vanillin (184), and others (187), have been condensed with both nitromethane, and (with the exception of vanillin) with nitroethane, to yield the corresponding nitroalkenes.

The second phase is also a well known reaction. Nitrostilbenes (188, 189) as well as nitrostyrenes (190) have been reduced catalytically using palladium on carbon with very good results. Recently the reduction of nitrostilbenes to the oximes of desoxybenzoins using iron, ferric chloride and hydrochloric acid in dilute ethanol has proved very successful (191). The hydrolysis of the oximes to the corresponding ketones was accomplished readily by using 7N sulfuric acid at a low temperature.

5. Possible Relation of the Isolated Units to Lignin Structure

It is of interest to discuss briefly the possible bearing of these new ethanolysis products on the structure of lignin. In the section dealing with the "Present Status of the Lignin Problem" (page 19), it was pointed out that lignin probably originates from a series of plant respiratory catalysts of the type $R-C-C-CH_2OH$ where R is the guaiacyl or the syringyl radical. It was emphasized that there appears to be a remarkable parallelism between these units and those of the C_4 dicarboxylic acid system of Szent-Györgyi. Although no actual proof of their function as respiratory catalysts has yet been obtained, work on this phase is now in progress in these laboratories. In spite of this gap in our knowledge, interesting and valuable support is to be found for the theory of Hibbert in the parallelism between the author's newly discovered desoxybenzoins ((4-hydroxy-3-methoxyphenyl)-propanone-2 and the corresponding syringyl derivative) and the recently-discovered 3,5-dihydroxy-2-carboxybenzyl methyl ketone, found by Raistrick and co-workers (100) among the water-soluble metabolic products, formed by the action of a large number of different molds of the Penicillium family on glucose (as the sole organic substrate) in aqueous solution.

They were able to isolate in each case the three aromatic substances (A), (B) and (C), the side chains of (B) and (C) being identical with those of the two diketones (B') and of the two α-hydroxy derivatives (C') respectively isolated from the ethanolysis products from maple wood (C' as the ethyl ether). It can be seen that the author's newly discovered ketones (A') (desoxybenzoins) fit perfectly into the picture.



It is thus of considerable importance that ethanolysis products, derived from higher forms of plant life, namely, gymnosperms and angiosperms, should show such a remarkable similarity with the Raistrick products formed by lower forms of plant life, molds, from glucose in slightly acid aqueous solution.

According to Hibbert (4) the ethanolysis lignin units represent stabilized end products derived from more labile C_6-C-C-C units, the latter, in turn being analogs of the Szent-Györgyi animal cell respiratory system. In Hibbert's theory of plant respiration, the first member of the Szent-Györgyi system of animal respiration catalysts, oxalacetic acid ($HOOC-CH_2-CO-COOH$), is replaced by 1-guaiacyl-3-hydroxypropanone-2 (XIII) which in its enol form is oxyconiferyl alcohol. Thus far it has not been possible to isolate this (XIII), as such, from the ethanolysis products from wood and the explanation of this, as suggested earlier by Hibbert (4) is to be found in the very remarkable ease with which it has been shown to undergo an allylic shift (70), thus yielding as a stabilized end product,

$$\begin{tikzcd}
R-CH_2-CO-CH_2-OH \arrow[r] & R-CH=C(OH)-CH_2-OH \arrow[r] & R-CHOH-C(OH)=CH_2 \arrow[d] \\
 & R-CO-CHOH-CH_3 & R-CHOH-CO-CH_3 \arrow[l]
\end{tikzcd}$$

α-hydroxypropiovanillone as its ethyl ether.

With regard to the 1,2-diketones (vanilloyl and syringoyl methyl ketones), found among the ethanolysis products, the opinion of Hibbert (Private Communication) is that these also represent stabilized end products. Their progenitors are probably lignin respiratory catalysts of the oxyconiferyl or dioxyconiferyl alcohol types:

$$\begin{tikzcd} R\text{-}CH{=}C(OH)\text{-}CH_2OH \arrow[d, shift right] & R\text{-}C(OH){=}C(OH)\text{-}CH_2OH \arrow[d, shift right] \\ R\text{-}CH_2\text{-}CO\text{-}CH_2OH \arrow[u, shift right] & R\text{-}CHOH\text{-}CO\text{-}CH_2OH \arrow[u, shift right] \end{tikzcd}$$

With the former a primary conversion into the isomeric aldehyde can be assumed:

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

This, under the influence of acids could then yield a mixture of a benzoin ($R\text{-}CHOH\text{-}CO\text{-}CH_3$) and a diketone ($R\text{-}CO\text{-}CO\text{-}CH_3$), a reaction similar to that undergone by benzyl glycollic aldehyde (XXVIII) (69).(see Historical Review page 18). In the latter case, the dioxy coniferyl alcohol might undergo an intramolecular shift (allyl shift type) to yield the diketones.

$$\begin{tikzcd} R\text{-}CHOH\text{-}CO\text{-}CH_2OH \arrow[r] & R\text{-}C(OH){=}C(OH)\text{-}CH_2OH \arrow[d] \\ R\text{-}CO\text{-}CO\text{-}CH_3 & R\text{-}C(OH)_2\text{-}C(OH){=}CH_2 \arrow[l] \end{tikzcd}$$

Nothing definite can be stated at the moment regarding the origin of the desoxybenzoins (4-hydroxy-3-methoxyphenyl)-propanone-2 and its syringyl derivative and their relation to lignin structure, although it is very probable that they also represent stabilized end products derived from more reactive lignin progenitors. The following speculations may perhaps be permissible in this connection.

The ease with which benzoins, in the presence of acids, undergo simultaneous intramolecular oxidation-reduction reactions to yield the corresponding benzil and desoxybenzoin, has already been discussed (page 63).

$$R\text{-}CO\text{-}CHOH\text{-}R \xrightarrow{\text{acids}} R\text{-}CO\text{-}CO\text{-}R + R\text{-}CH_2\text{-}CO\text{-}R$$

It is possible that the two newly discovered compounds are degradation products derived from the corresponding benzoins namely, α-hydroxypropiovanillone and its syringyl analog. While this hypothesis could account for the presence of the newly-discovered desoxybenzoins, it would not suffice to explain the much larger quantity of the diketone present in the ethanolysis mixture. However, as indicated above in all probability there are two additional sources from which this could originate.

Another possible source of the author's desoxybenzoins may be 1-guaiacyl-2,3-dihydroxypropane (CVI) suggested by Hibbert as the second member of the new plant respiratory catalyst system, corresponding to malic acid in the Szent-Györgyi system. Thus analogously constituted products, such as 1-anisyl,-1,2-dihydroxypropane (CVII) under the influence of dilute sulfuric acid are converted into ketones, in this case anisic acetone (192).

$$CH_3O\text{-}C_6H_4\text{-}CHOH\text{-}CHOH\text{-}CH_3 \xrightarrow{\text{dil. } H_2SO_4} CH_3O\text{-}C_6H_4\text{-}CH_2\text{-}CO\text{-}CH_3$$

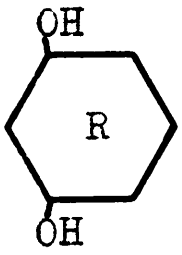
(CVII)

A similar reaction might be expected with (CVI).

$$R\text{-}CH_2\text{-}CHOH\text{-}CH_2OH \xrightarrow{H^+} R\text{-}CH_2\text{-}CO\text{-}CH_3 + H_2O$$

CVI.

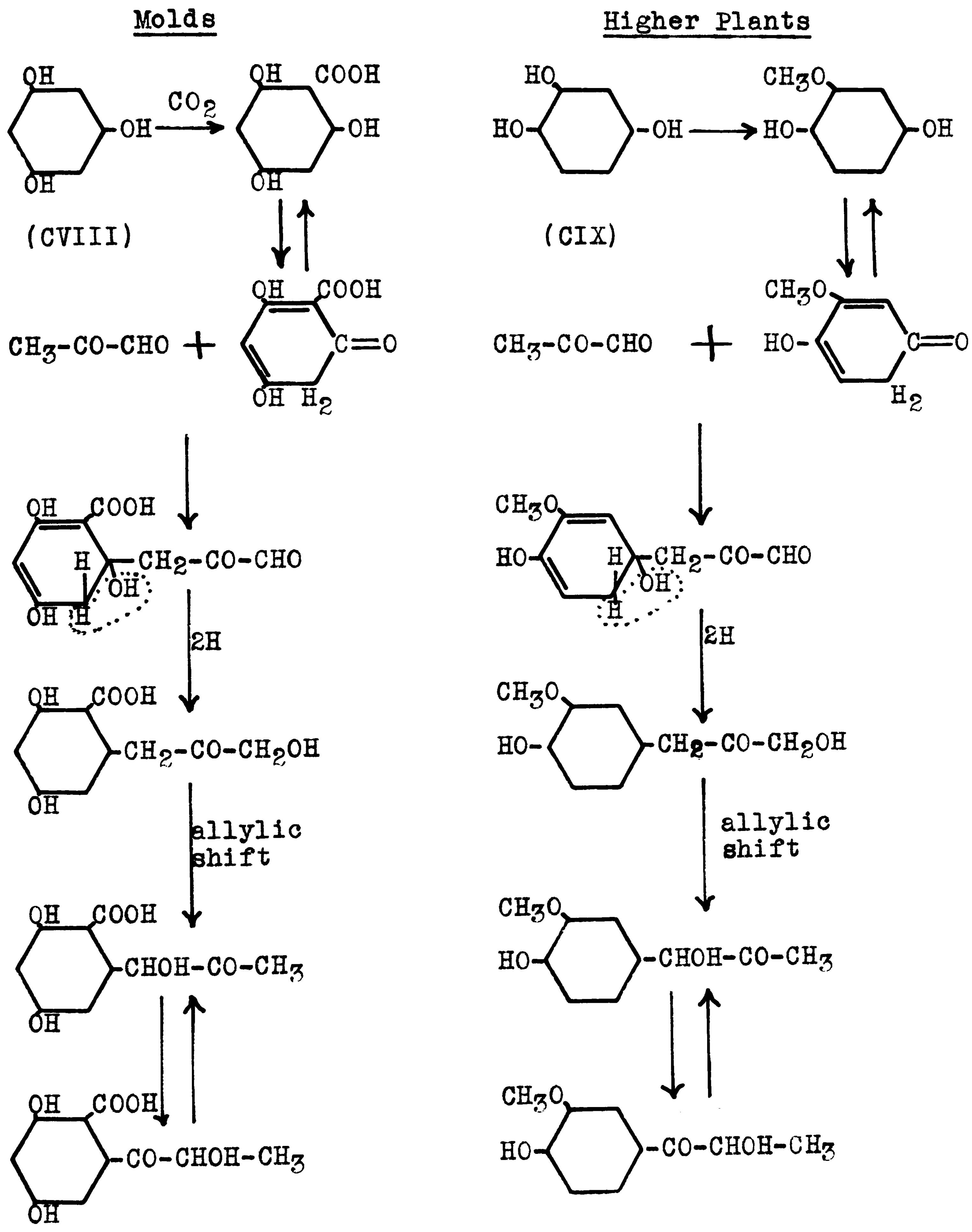
Inasmuch as the above changes are predicated on transformations in an acidic medium, already shown applicable for example to the formation of α-hydroxypropiovanillone and syringone, it seems highly probable that the metabolic products isolated by Raistrick (100) by evaporation of slightly acid solutions, also represent final stabilized end products originating from analogous progenitors functioning as mold respiratory catalysts in a similar type of new plant respiratory system, but in which the guaiacyl and syringyl radicles are replaced by R.



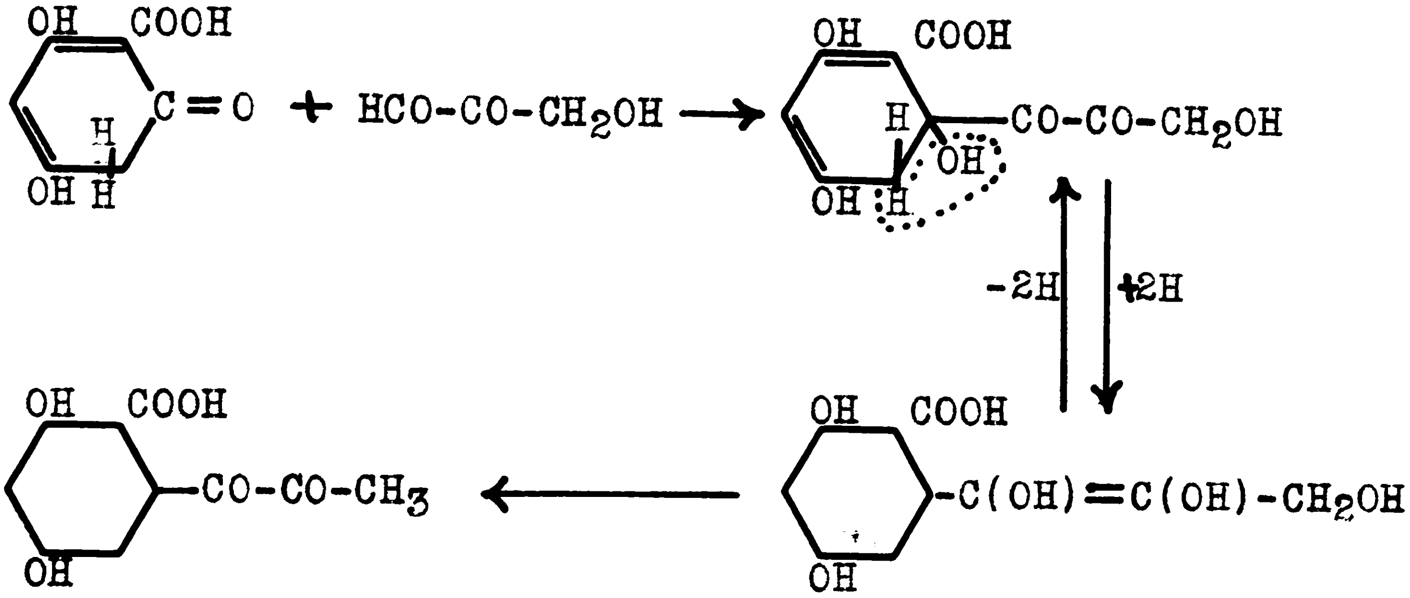
The presence of the carboxyl group, presumably formed at a later stage in the respiratory process, is not surprising in view of the ease with which carboxylation takes place with phenols in aqueous solutions of bicarbonates at low temperatures (50-80°) (195, 196).

Since the mold respires exclusively on glucose, it seems reasonable to assume that the phenolic substances originate also in this case from the methyl glyoxal formed as an intermediate carbohydrate product of metabolism. A comparison of the isolated phenolic products and their probable forerunners, with those obtained from the higher plants indicates that the former could be derived from condensation reactions between methyl or hydroxymethyl glyoxal and the keto form of 1,3,5-trihydroxybenzene (CVIII) while in the latter the condensation takes place with the keto form of 1,2,4-trihydroxybenzene (CIX) as illustrated in the following diagram:

Suggested Mechanism for Plant Synthesis of Respiratory Catalysts



Furthermore a similar type of condensation, e.g. of the above phenols (CVIII) and (CIX) with hydroxymethyl glyoxal instead of methylglyoxal, would yield R-CO-CO-CH_2OH, convertible on reduction into R-CHOH-CO-CH_2OH, the previously suggested progenitor of the 1,2-diketones. (see page 20)



Inasmuch as it is difficult to avoid the conclusion that the Raistrick products are formed from respiratory catalysts of lower plant forms arising by degradation (respiration) of a single monomeric substrate, namely, glucose, the remarkable analogy between them and the ethanolysis products from wood, seems to provide strong indirect evidence for Hibbert's theory of the origin of lignin as originating from an analogous series of higher plant respiratory catalysts.

There remains only the question of the possible origin of the aromatic aldehydes present in the ethanolysis reaction mixture. It is certain that these are not derived from either α-ethoxypropiovanillone (70, 72) or the corresponding syringyl analog. Neither are they degradation products of the diketones.

It seems possible they may originate from either an enol or an aldol type of condensation polymer, or from an unsaturated ethylene union, $R\text{-}CHOH\text{-}\overset{|}{\underset{|}{C}}\text{-}\overset{|}{\underset{|}{C}}$ or $R\text{-}CH{=}\overset{|}{C}\text{-}\overset{|}{\underset{|}{C}}$ respectively, R being the guaiacyl or syringyl radical. The fact that by the action on wood of mild oxidizing agents such as nitrobenzene, cuprammonium hydroxide etc., high aldehyde yields are obtainable (27) may be cited in support of the above view. Much further investigation is necessary to elucidate this point.

Experimental

1. Modified Procedure for the Ethanolysis of Wood(Flow SheetI)

(a) Extraction of the Wood - Thirty-five to forty-five year old maple wood, ground to a meal (40 mesh) was extracted with a 50-50 mixture of ethanol-benzene for 48 hours, followed by 24 hours extraction with ethanol. Hot water (50-60°) was then allowed to run for 12 hours through the extracted wood meal contained in a cotton bag. The wax- and resin-free wood meal was air-dried (3 days) and vacuum-oven dried (50°, 20 mm. 24 hours); moisture content, 2.4%; Klason lignin, 20.3%.

The resulting dry wood meal (1000 g.) was transferred to a 12-liter flask, dry ice was added to sweep out the air, then ethanol (8.5 liters) and more dry ice. A stirrer operating through a short condenser, and a long condenser were fitted to the flask and the mixture was stirred for one half hour to expel any remaining air. Then a solution (18.5%) of hydrogen chloride in ethanol (1030cc.) was added through the long condenser. The reaction mixture was refluxed for 48 hours in an inert atmosphere (carbon dioxide) on a water bath (80-85°), cooled, and filtered through a cloth on a Büchner funnel. The residual wood meal was extracted with ethanol exhaustively (e.g. until no more colored material was removed, about 12 hours) in a Soxhlet apparatus, air-dried and vacuum-dried at 50°; weight, 496 g.. The alcoholic extract was added to the main alcoholic filtrate, and the combined solution was neutralized (pH, 6) by adding sodium bicarbonate (175 g.) and water (50 cc.) and stirring overnight. The resulting sodium chloride was filtered off, and the filtrate concentrated to about 2 litres at 45-50° under reduced pressure (20 mm.), in an inert atmosphere.

(b) Separation of the Ethanol Lignin from the Water-soluble Ethanolysis Oils - The concentrated alcohol solution was precipitated in a fine stream into distilled water (15 litres) with vigorous mechanical stirring, and the mixture then allowed to agglomerate and settle overnight. As much as possible of the clear aqueous liquor was siphoned off, the remaining "sludge" filtered by suction and sucked dry on the Büchner funnel. The lignin was dissolved in acetone (1200 cc.) and precipitated in a fine stream into distilled water (12 litres) as described above. The resulting colloid was coagulated by stirring with a few cc. of a concentrated sodium chloride solution and the lignin separated as before. The reprecipitation was repeated once more to ensure a complete separation. The resulting ethanol lignin was air-dried and vacuum-dried at 25°; weight 116.5 g.

The combined aqueous liquors containing the ethanolysis oils were extracted with benzene in a 20-liter continuous extractor, similar to that designed by Hossfield (197). The extraction was carried out in an atmosphere of carbon dioxide, and the benzene was renewed at intervals of about 10 hours. After 100 hours the extraction was essentially complete.

The combined benzene extracts were dried with sodium sulfate and the solvent removed under reduced pressure (20 mm.).

(c) Separation of the Resinous Material in the Crude Water-soluble Ethanolysis Oils - The crude ethanolysis oils (76.0 g.) were dissolved in acetone (750 cc.) and the solution introduced as a fine stream into petroleum ether (b.p. 30-50°) (15 liters) with vigorous mechanical stirring. The petroleum ether solution was siphoned off, from the dark oil which had settled in the vessel and the oil dissolved in acetone (150 cc.) and reprecipitated into petroleum ether (3 liters). The reprecipitation was repeated, and the solution again siphoned off. The residual petroleum ether-insoluble viscous resinous material weighed 25.3 g. The solvent from the combined petroleum ether solutions was distilled off on a water bath (50-60°) and the last traces removed under reduced pressure; weight of the residual light-colored petroleum ether-soluble ethanolysis oils, 43.3 g..

(d) Solvent Group Fractionation of the Petroleum Ether-soluble Ethanolysis Oils - By this procedure the crude ethanolysis oils were separated into four fractions, soluble in (1) Bisulfite (2) Bicarbonate and (3) Alkali together with a final "neutral fraction". The oils (43.3 g.) were dissolved in benzene (300 cc.) and the solution extracted successively with (a) eight-30 cc. portions of 20% sodium bisulfite (the bisulfite extract was back-extracted with two-30 cc. portions of benzene and the back-extracts were combined with the main benzene solution); (b) three-

30 cc. portions of 8% sodium bicarbonate (back-extraction as in (a)); and (c) six-35 cc. portions of 5% sodium hydroxide (back-extraction as in (a)). The residual oil remaining in the benzene solution was the "neutral fraction".

Each of the extracted fractions (a), (b) and (c) was neutralized individually with dilute sulfuric acid (the sulfur dioxide removed from the neutralized solution (a) by bubbling in carbon dioxide under reduced pressure), and each then extracted with benzene. The benzene solutions including that containing the neutral fraction were dried with sodium sulfate and the solvent removed, yielding a bisulfite-soluble fraction (10.3 g.); bicarbonate-soluble fraction (0.4 g.); alkali-soluble or phenol fraction (24.7 g.); and neutral fraction (5.4 g.).

2. Preliminary Experiments on the Separation of Mono- and Dicarbonyl Compounds in the Bisulfite Fraction

(a) Solubilities of the Ammonium Salts of Syringoyl Methyl Ketone, Vanilloyl Methyl Ketone, Syringaldehyde and Vanillin. - The relative solubilities of the ammonium salts of the two diketones were determined in the following manner: Each of the dicarbonyl compounds (0.10 g.) was dissolved in absolute ethanol at 0° (10 cc.) and to each of the solutioms a 7% solution of ammonia in anhydrous ethanol was added dropwise with stirring until a saturated solution was obtained, i.e. the ammonium salt just began to precipitate. A few drops

(0.04 cc.) of the ethanolic ammonia solution were sufficient to cause the precipitation of the ammonium salt of syringoyl methyl ketone, while the precipitation of the ammonium salt of vanilloyl methyl ketone required 6.0 cc.. Therefore the approximate solubility products were calculated as 7.3×10^{-4} and 5.0×10^{-2} respectively.

The solubility products of the ammonium salts of syringaldehyde and vanillin were determined in the same way by R.H.J. Creighton in these laboratories, and found to be 1.7×10^{-2} and 5.5×10^{-2} respectively.

(b) Action of a Mixture of Hydroxylamine and Nickel Chloride on Vanillin, Syringaldehyde, and 1,2-Dicarbonyl Compounds. A mixture of vanillin (0.120 g.) and syringaldehyde (0.067 g.) was dissolved in water (75 cc.) and hydroxylamine-hydrochloride (0.2 g.), 5% nickel chloride solution (2 cc.) and sodium acetate (5 g.) added. The resulting solution was refluxed for 100 hours in an inert atmosphere, cooled and extracted with benzene. The benzene solution was extracted with 20% sodium bisulfite, the bisulfite extract neutralized with dilute sulfuric acid, the sulfur dioxide removed by bubbling in carbon dioxide under reduced pressure, and the resulting solution extracted with benzene. The benzene extract was dried and removal of the solvent left in large yield a mixture of vanillin and syringaldehyde, indicating that the treatment had had little effect on the aldehydes. Their separation was effected by addition of anhydrous ammonia in an anhydrous ethanol solution, whereon the ammonium salt of syringaldehyde was precipitated (see section 2c below). About 80% of syringaldehyde (0.061 g. ammonium salt) and 70% of vanillin (0.170 g. as m-nitrobenzoyl-hydrazone) were recovered. A similar treatment of syringoyl methyl ketone or vanilloyl methyl ketone or a mixture of the two with hydroxylamine and nickel chloride resulted in an almost quantitative precipitation (90-95%) of the nickel glyoxime salts (see section 2c).

(c) Investigation on the Separation of a Synthetic Mixture of Vanillin, Syringaldehyde, Syringoyl Methyl Ketone and Vanilloyl Methyl Ketone.

The first step in the separation was that of precipitation of the diketones as their nickel glyoxime salts, followed by their hydrolysis. It was therefore necessary to determine the conditions for a maximum recovery of the diketones.

Hydrolysis of the Nickel Glyoxime Salts under Various Conditions

The optimum conditions for the hydrolysis of the nickel glyoxime salts were determined from a series of studies on the behavior of the nickel salt of vanilloyl methyl ketone with different acids using 20 cc. of the acid to 50 mg. of the nickel salt- at varying temperatures for varying periods of time. The resulting acid solution was cooled, extracted with ether , the ether extract neutralized with sodium bicarbonate and the solvent removed by distillation. The residue was dissolved in water and precipitated and estimated as the quinoxaline (see section 4Ab). The following table shows the results obtained:

Table I.

Hydrolysis of the Nickel Glyoxime Salt

Hydrolytic Reagent Used	Wt. of Ni Salt g.	Reaction Time, hrs.	Reaction Temp. °C	Weight of Quinoxaline found, g.	% Diketone Recovered
15% H_2SO_4	0.050	0.25	reflux	0.033	65
" "	0.049	1	"	0.026	54
" "	0.051	2	"	0.018	36
15% H_2SO_4 + 0.1g. $Fe_2(SO_4)_3$	0.040	0.25	"	0.026	63
5% H_2SO_4 + 0.03g. C_6H_5CHO	0.036	2	"	0.015	40
5% H_2SO_4 + 0.5cc. H_2CO	0.046	2	"	0.016	35
5% H_2SO_4 + 0.1g. $Fe_2(SO_4)_3$	0.041	2	"	0.016	40
12N HOAc + 0.5cc. H_2CO	0.043	5	"	0.026	55
12N HCl + 0.05g. $Fe_2(SO_4)_3$	0.026	0.01	60	0.000	--
12N H_2SO_4	0.033	5	90	0.015	46
" "	0.032	16 2	20 + 60	0.027	80
" "	0.031	12	60	0.018	55
" "	0.035	12	40	0.030	79
" "	0.031	48	20	0.024	75
" "	0.033	120	20	0.025	74
" "	0.036	16 3	20 + 40	0.032	84

Thus the best conditions found were those using 12N sulfuric acid at room temperature for 16 hours, followed by 3 hours at 40°.

Separation of the Mixture. - This contained the following components; syringoyl methyl ketone (0.158 g.), vanilloyl methyl ketone (0.237 g.), vanillin (0.160 g.) syringaldehyde (0.144 g.) and water (400 cc.). It was treated with hydroxylamine hydrochloride, 5% nickel chloride solution and sodium acetate (see section 3A). The combined nickel glyoxime salts weighed 0.4184 g., and were hydrolysed using the conditions described in section 3A below. The acid solution was extracted with benzene and the resulting mixture of the diketones was separated by precipitation of the ammonium salt of syringoyl methyl ketone (0.131 g.) from an ethanol solution (see section 3A below); OCH_3, 25.6%; Calcd. 25.8%. The vanilloyl methyl ketone remaining in the ethanol-ammonia solution was estimated as the quinoxaline (0.225 g.); m.p. 159-160°. Thus the two diketones were recovered in yields of 76% and 70% respectively.

Separation of the Vanillin-Syringaldehyde Components - The aqueous filtrate containing the two aldehydes, was extracted with benzene, the extract dried with sodium sulfate and the solvent removed. The residue was dissolved in absolute ethanol (15 cc.), a 7% solution of ammonia in ethanol (2 cc.) added and the mixture allowed to stand at 0° for several hours. The precipitated ammonium salt of syringaldehyde was filtered off and reprecipitated, after dissolving in ethanol (10 cc.), by adding 7% ethanol-ammonia solution (1.5 cc.) as before. The ammonium salt was filtered off, dissolved in water, acidified and converted to the m-nitro-benzoyl hydrazone (20) (0.123 g.); m.p. 190-192°; 69% recovery of syringaldehyde.

The filtrate from the first precipitation of the ammonium salt of syringaldehyde, was neutralized with dilute sulfuric acid, concentrated to a small volume under reduced pressure at room temperature, diluted with water and the solution treated with m-nitrobenzoyl hydrazine (20); weight of hydrazone, 0.226 g.; recrystallized from dilute ethanol, m.p. 207-208°; 70% recovery of vanillin.

3. Investigation of the Bisulfite Fraction of Water-soluble Ethanolysis Products from Maple Wood.

A. 1,2-Dicarbonyl Components of the Bisulfite Fraction

(a) Isolation of the Diketones. - The bisulfite fractions from three different ethanolysis runs made by Dr. W.L. Hawkins using the usual procedure (181), were analyzed for their dicarbonyl contents, by the following procedure: An aliquot portion of the bisulfite-soluble fraction (3.253 g.) was dissolved in water (900 cc.), and hydroxylamine sulfate (2 g.), 5% nickel chloride solution (12 cc.) and crystalline sodium acetate (15 g.) added. The reaction mixture was refluxed gently in an atmosphere of carbon dioxide for twenty-four hours. The precipitated nickel glyoxime salts were removed by filtration through a sintered glass filter, more hydroxylamine sulfate (0.3 g.) and nickel chloride solution (3 cc.) added to the filtrate, and the refluxing continued for an additional twenty-four hours. This process was continued until there was no further precipitation (four to five days). The combined precipitated salts were washed with cold water and dried at 100°; yield 1.732 g.. They were now mixed with 12N sulfuric acid (400 cc.), the solution allowed to stand at room temperature for twenty-four hours and then maintained at 40° for four hours. After cooling it was extracted with benzene, the benzene extract dried over sodium sulfate and sodium bicarbonate to neutralize any sulfuric acid, and the benzene removed at atmospheric pressure leaving a mixture of the crude diketones (C). (see Flow Sheet II page 79B)

(b) Separation of the Diketones. - The mixture (C)* (1.2 g.) was dissolved in absolute ethanol (40 cc.), cooled to 0° and 4 cc. of a cold (0°) 9% solution of ammonia in absolute ethanol added. After standing at 0° for a few hours, the precipitated ammonium salt of syringoyl methyl ketone (E) was filtered, and washed with a cold (0°) 1% solution of ammonia in ethanol (10 cc.). The filtrate (F) was neutralized immediately with dilute sulfuric

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* The letters refer to those used in Flow Sheet II.

acid and extracted with benzene. On removal of the solvent, vanilloyl methyl ketone separated out as a yellow oil which crystallized on standing; yield, 0.21 g. The crude product, after one crystallization from water, melted at 68-69° and gave no depression when mixed with an authentic sample of vanilloyl methyl ketone. The quinoxaline prepared from this product melted at 160-161° and showed no depression when mixed with a pure synthetic product.

The ammonium salt of syringoyl methyl ketone (E) was purified by dissolving in hot ethanol, cooling and adding an excess of a 9% solution of ammonia in ethanol. The reprecipitated ammonium salt (0.911 g.) was filtered, washed as before, dried (vacuum), dissolved in hot water, and an equivalent amount of dilute sulfuric acid added. On standing, syringoyl methyl ketone separated out in the form of yellow needles (0.7 g.); it was recrystallized first from water and then from petroleum ether (b.p. 100-110°); m.p. 80-81°; no depression when mixed with a pure synthetic syringoyl methyl ketone. Anal. Calcd. for $C_{11}H_{12}O_5$: C, 58.9; H, 5.4; OCH_3, 27.7. Found: C, 59.1; H, 5.7; OCH_3, 27.6.

Monosemicarbazone of Syringoyl Methyl Ketone. - Fine, white, needle-like crystals; m.p. 210-211° (with decomposition); no mixed melting point depression. Anal. Calcd. for $C_{12}H_{15}O_5N_3$; C, 51.2; H, 5.4; N, 14.9; OCH_3, 22.1. Found: C, 51.0; H, 5.6; N, 15.0; OCH_3, 21.9.

<u>Quinoxaline of Syringoyl Methyl Ketone.</u> - Light yellow needles; m.p. 160.5-161.5°; no mixed melting point depression. Anal. Calcd. for $C_{17}H_{16}O_3N_2$: C, 68.9; H, 5.5; N, 9.5; OCH_3, 20.9. Found: C, 69.1; H, 6.0; N, 9.5; OCH_3, 20.8. The complete results from the above separations are shown in Table 2.

Table 2.

Analysis of Bisulfite-soluble Fractions

Amount of dry maple wood taken for each experiment was 1000 g. containing 220 g. of Klason lignin

	Ethanolysis experiments			
	1		2	3
	Duplicate runs			
Total bisulfite-sol. oils, g.	13.0		17.5	9.0
Total recovered distilled oils, g.	10.6		10.0	7.3
Bisulfite-sol. fraction used, g.	1.030	1.730	5.258	3.253
Nickel glyoxime salts found, g.	0.425	0.691	3.544	1.732
Ammonia salt of syringoyl methyl ketone, g.	0.193	0.280	1.627	0.911
Syringoyl methyl ketone in distilled fraction				
(a) Found, %	17	15	29	26
(b) Corrected, % *	22	20	38	34
Quinoxaline of vanilloyl methyl ketone, g.	0.062	0.079	0.659	0.172
Vanilloyl methyl ketone in distilled fraction				
(a) Found, %	4	4	9	4
(b) Corrected, % *	6	5	13	6
Total diketone in distilled fraction				
(a) Found, %	21	19	38	30
(b) Corrected, % *	28	25	51	40

In experiment I the results are given of two duplicate experiments carried out with the bisulfite fraction of the ethanolysis oils obtained by use of an ethanolysis procedure varying from that customarily employed. In this case ethyl orthoformate was added to the ethanol-hydrochloric acid extraction medium with the object of "fixing" free, possibly very reactive, carbonyl groups. The ethanolysis yields obtained, however, were considerably lower than in the case where the normal procedure was used (Expts. 2 and 3); no explanation for this can be given. The average value found for the total isolated diketones (Expts. 2 and 3), based on the Klason lignin content of the original wood, was 2%, and that of the corresponding "corrected" values 2.5%.

B. Isolation of New Components from Bisulfite Fraction of Maple Wood Obtained by use of the New Ethanolysis Procedure

In this investigation the bisulfite fraction was obtained from the ethanolysis mixture using the new modified ethanolysis procedure (Flow Sheet I page 79A). The final method of analysis of this fraction is illustrated in Flow Sheet II page 79B.

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* Control runs showed that 76% of syringoyl methyl ketone and 70% of vanilloyl methyl ketone could be recovered from a mixture of the synthetic diketones (section 2(c)). The "correction factors" are thus 1.3 and 1.4, respectively.

(a) Removal of the Diketones. - The bisulfite-soluble fraction (10.79 g.) was dissolved in water, and the solution treated with hydroxylamine sulfate, nickel chloride and sodium acetate as previously described (section 3A). The nickel glyoxime salts (6.20 g.) were filtered off, the filtrate (B) (flow sheet, page 79B) extracted with benzene, and the benzene extract dried over sodium sulfate; on removal of the solvent, a mixture of the aldehydes and oximes (C') remained. The presence of the oximes was indicated by nitrogen in the residue (sodium fusion test), and by the absence of nitrogen on hydrolysis with 7N sulfuric acid (see section 3Bc).

(b) Isolation of Vanillin and Syringaldehyde. - The residue (C') (4.72 g.) was dissolved in benzene (70 cc.) and extracted with seven - 17 cc. portions of 20% sodium bisulfite solution.* The bisulfite solution was back-extracted with benzene (20 cc.) and this extract combined with the main benzene solution containing the bisulfite-insoluble oximes. The bisulfite solution (D') containing the aldehydes was neutralized with dilute sulfuric acid, the sulfur dioxide removed by bubbling in carbon dioxide under reduced pressure, and the resulting solution extracted with benzene. The benzene solution was dried and on remeval of the solvent, a mixture of the two aldehydes remained (1.68 g.).

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* During the extraction, a non-distillable tar (0.55 g.) separated at the interface of the benzene-bisulfite solutions; it was readily separable from the aqueous solutions.

This was fractionally distilled in the Cooke-Bower column and the results are shown in Table 3.

Table 3

Fractional Distillation of the Mixture of Vanillin and Syringaldehyde. (Pressure - 0.25 mm.)(Flow Sheet II)

Fraction number	Bath temp.	Col. temp.	Weight crude, g.	Weight** purified	Identified as
1	168°	94°	0.19	0.14	vanillin
2	179°	102°	0.06	0.03	syringaldehyde
3	189°	106°	0.54	0.54	syringaldehyde
4	195°	110°	0.10	0.05	syringaldehyde
5	200°	117°	0.13	0.05	(4-hydroxy-3,5-dimethoxyphenyl)-propanone-2
6	200°	117°	0.06	____	

** Fraction I was recrystallized from water; m.p. 80-81°; no melting point depression when mixed with vanillin. Fractions 2, 3 and 4 were each recrystallized from water; m.p. 111-112°; no melting point depression when mixed with syringaldehyde.

(c) Isolation of (4-Hydroxy-3,5-dimethoxyphenyl)-propanone-2 and (4-Hydroxy-3-methoxyphenyl)-propanone-2 (Flow Sheet II) - The benzene solution containing the oximes (E') was filtered to remove the tar, then dried and the solvent removed. The residue (2.30 g.) was treated with 7N sulfuric acid (150 cc.) at room temperature for 24 hours followed by 4 hours at 40°.

The acid solution was extracted with benzene, the benzene extract dried over sodium sulfate and sodium bicarbonate, and the solvent removed by distillation. The residue (1.56g.) was distilled from a Späth bulb and the distillate (1.23g.) was fractionally distilled. The results are shown in Table 4.

Table 4

Fractional Distillation of the Mixture of (4-Hydroxy-3,5-dimethoxyphenyl)-propanone-2 and its Guaiacyl Analog.

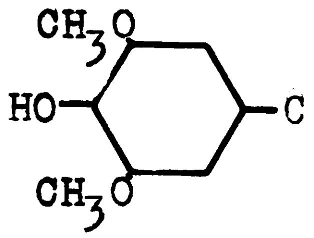
(pressure 0.25 mm.)

Fraction number	Bath Temp.°	Column Temp.°	Weight Crude, g.	Weight purified, g.	Identified as
1	165	89	0.08	0.03	(4-hydroxy-3-methoxyphenyl)-propanone-2
2	175	99	0.09	0.06	"
3	185	107	0.05	0.02	"
4	192	114	0.08	0.05	syringaldehyde
5	198	124	0.13	0.06	(4-hydroxy-3,5-dimethoxyphenyl)-propanone-2
6	200	130	0.27	0.27	"
7	202	130	0.05	0.01	"
8	202	130	0.06	____	

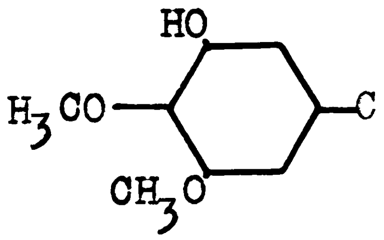
(i) Investigation of the Higher-boiling Component (Fractions 5, 6 and 7. - These three fraction had the same structure shown by recrystallizing each from a mixture of petroleum ether (b.p. 30-50°) and ether; m.p. in each case,68-69°; no melting point depressionwhen mixed with the synthetic product. They were united and samples taken for analysis. Anal. Calcd. for $C_{11}H_{14}O_4$; C, 62.8; H, 6.7; OCH_3, 29.5; Mol. wt., 210.1. Found: C, 62.9; H, 6.7; OCH_3,29.5; Mol. wt. (Rast), 218.

Proof of Structure

(1) The presence of a dimethoxy phenol group having the structure of either (A) or (B) was proven by methylation



(A)



(B)

followed by oxidation to 3,4,5-trimethoxybenzoic acid.

Method

The product (0.15g.) was methylated with diazomethane, and the methylated product oxidized with a 3% solution of potassium permanganate (25cc.) at 100° for two hours. The reaction mixture was extracted with ether, acidified and extracted again. The second extract contained 3,4,5-trimethoxybenzoic acid (0.053g.); recrystallized from water; m.p. 165-166°; no melting point depression when mixed with an authentic sample of 3,4,5-trimethoxybenzoic acid.

(2) Proof of the Presence of an end $-CO-CH_3$ Grouping. - A small amount (0.01g.) of the unknown product was dissolved in a solution of 0.1N sodium hydroxide (5cc.) and water (70cc.) and 0.1N iodine solution containing 24g. potassium iodide per liter (35cc.) followed by sodium hydroxide (50cc.), added. After standing at room temperature for two hours the iodoform

was filtered off, washed with water and dried; yield, 0.007g; m. p. 118-120°.

Semicarbazone of Fractions 5,6 and 7 (Table4). - Its semicarbazone, obtained in the form of fine white needle-like crystals from ethanol melted at 155-156° and showed no melting point depression when mixed with that prepared from the synthetic product (page 119). Anal. Calcd. for $C_{12}H_{17}O_4N_3$: C, 53.9; H, 6.4; OCH_3, 23.2; N, 15.7; Found: C, 54.0; H, 6.7; OCH_3, 23.4; N, 15.7.

(ii)
Investigation of the Low-boiling Component (Fractions 1,2, and 3, Table 4). - This was identified by melting points of the semicarbazone and of the thiosemicarbazone and comparison with those obtained from the synthetic product. (page 120)

Semicarbazone. - Fine, white needle-like crystals from ethanol; m.p. 157-158°; no melting point depression when mixed with the synthetic product (page 120). Anal. Calcd. for $C_{11}H_{15}O_3N_3$: C, 55.7; H, 6.3; OCH_3, 13.1; N, 17.7. Found: C, 55.7; H, 6.5; OCH_3, 13.1; N, 17.7.

Thiosemicarbazone. - Microscopic, white, needle-like crystals from ethanol; m.p. 187-188° (rate of heating, 5-6° per minute); no melting point depression when mixed with the synthetic product (page 120). Anal. Calcd. for $C_{11}H_{15}O_2SN_3$: C, 52.2; H, 6.0; OCH_3, 12.3; N, 16.6; Found: C, 52.2; H, 6.4; OCH_3, 12.4; N, 16.6.

(d) Summary of the Analyses of the Bisulfite Fractions. In Table 5 a summary is given of the analytical data obtained from the bisulfite fraction from three different ethanolysis experiments.

Table 5

Analysis of the Bisulfite-soluble Fraction

Amount of dry maple wood meal taken for each experiment was 1000 g. containing 203 g. of Klason lignin.

	Ethanolysis Experiments		
	1	2	3
Total bisulfite-sol. oils, g.	10.30	11.47	10.79
Nickel glyoxime salts found, g.	6.23	6.58	6.20
Mixture of the diketones, calcd., g.	4.90	5.16	4.89
% of Klason lignin	2.42	2.54	2.41
Syringaldehyde (pure), found, g.	0.73	1.30	0.67
% of Klason lignin	0.36	0.64	0.33
Vanillin (pure), found, g.	0.08	0.23	0.14
% of Klason lignin	0.04	0.11	0.07
(4-Hydroxy-3,5-dimethoxyphenyl)-propanone-2 (pure), found, g.	0.47	0.26	0.39
% of Klason lignin	0.23	0.13	0.19
(4-Hydroxy-3-methoxyphenyl)-propanone-2 (pure), found, g.	0.12	0.08	0.11
% of Klason lignin	0.06	0.04	0.05

(e) Attempted Separation of the Monocarbonyl Components of the Bisulfite Fraction into Ethanol-ammonia-soluble and -insoluble Fractions. - It was believed that the separation of the monocarbonyl components of the bisulfite fraction could be simplified by use of a preliminary separation of the syringyl components from the guaiacyl involving precipitation of the former as ammonium salts from ethanol solution, followed by fractionation of each component. The details of the attempted separation follow: The mixture of the aldehydes and the oximes (C') (4.62 g.) (Flow Sheet II and Ethanolysis Experiment 1, Table 5) was dissolved in ethanol (75cc.), a 9% solution of ammonia in ethanol (25cc.) was added, and the reaction mixture allowed to stand at 0° for several hours. The precipitated ammonium salts were filtered, washed with a cold 1% solution of ammonia in ethanol (15cc.) and treated with 7N sulfuric acid to remove the oximino groups from the syringyl components. (section 3Bc).

The combined filtrate and washings were neutralized with dilute sulfuric acid, concentrated to about 25cc., water added (50cc.) and the resulting solution extracted with benzene. The benzene was removed by distillation and the residue was treated with 7N sulfuric acid (see section 3Bc).

Each of the acidified solutions,, presumably containing the guaiacyl and syringyl components respectively, were extracted with benzene. Removal of the solvent left an ethanol-ammonia-soluble fraction (1.35g.) and an ethanol-ammonia-insoluble fraction (1.71g.) Fractional distillation of these showed that each contained a mixture of both guaiacyl and syringyl components, namely, vanillin, syringaldehyde and the two new monoketones. The separation of syringyl from the guaiacyl components was thus incomplete and the method was therefore abandoned.

4. Syntheses

A. Synthesis of Syringoyl Methyl Ketone

(a) Oxidation of α-Hydroxypropiosyringone. - α-Hydroxypropiosyringone (5.0 g.) was dissolved in a hot solution of copper sulfate (14.4 g.), pyridine (24 cc.) and water (12 cc.), the mixture heated for two hours on a steam bath, and, after cooling, poured into a slight excess of dilute hydrochloric acid. The aqueous solution was extracted with ether, and the latter removed, leaving 3.5 g. of an oily residue distilling at 147° (0.04 mm.). This distillate (2.9 g.) was recrystallized, first from hot water and then from petroleum ether (b.p. 100-110°); light orange-colored needle-shaped crystals; m.p. 80-81°. Anal. Calcd. for $C_{11}H_{12}O_5$: C, 58.9; H, 5.4; OCH_3, 27.7. Found: C, 58.7; H, 5.7; OCH_3, 27.7.

(b) Derivatives of Syringoyl Methyl Ketone

Quinoxaline of Syringoyl Methyl Ketone. - Syringoyl methyl ketone (0.20 g.) was dissolved in hot water (20 cc.) and this solution was added to a hot solution (70°) of o-phenylenediamine (0.12 g.) in water (10 cc.). After standing overnight, the precipitate (0.24 g.) was filtered, washed, and recrystallized first from water and then from aqueous ethanol (1 part ethanol to 6 parts of water); light yellow colored crystals; m.p. 161-161.5°. Anal. Calcd. for $C_{17}H_{16}O_3N_2$: C, 68.9;

H, 5.5; N, 9.5; OCH_3, 20.9. Found: C, 69.0; H, 5.6; N, 9.4; OCH_3, 20.9.

Semicarbazone of Syringoyl Methyl Ketone. - Syringoyl methyl ketone (0.25 g.) was dissolved in hot water (15 cc.) and the solution added to semicarbazide hydrochloride (0.15 g.) and potassium acetate (0.18 g.) in water (10 cc.). After standing for several hours, the precipitate (0.20 g.) was filtered, washed and recrystallized first from water and then from ethanol; fine, white, needle-like crystals; m.p. 210-211° (with decomposition). Anal. Calcd. for $C_{12}H_{15}O_5N_3$: C, 51.2; H, 5.4; N, 14.9; OCH_3, 22.1. Found: C, 51.4; H, 5.7; N, 14.7; OCH_3, 22.0.

B. Synthesis of (4-Hydroxy-3,5-dimethoxyphenyl)-propanone-2

(a) 1-(4-Hydroxy-3,5-dimethoxyphenyl)-2-nitropropene-1. - Syringaldehyde (3.0 g.) was dissolved in ethanol (12 cc.) and to the solution, nitroethane (1.5 cc.), methylamine hydrochloride (0.1 g.) and sodium carbonate (0.08 g.) were added. After standing for 14 days at room temperature in the dark, the reaction mixture was cooled to 0°, the orange needle-like crystals were filtered off, washed with dilute sulfuric acid and with water, then dried; yield, 3.4 g. m.p. 101-102°; recrystallized from ethanol and from ether; m.p. 103-104°.

Anal. Calcd. for $C_{11}H_{13}O_5N$: C, 55.2; H, 5.4; OCH_3, 26.0; N, 5.9. Found: C, 55.1; H, 5.6; OCH_3, 26.1; N, 5.8.

(b) (4-Hydroxy-3,5-dimethoxyphenyl)-propanone-2. - The nitroalkene (1.1 g.) was dissolved in hot ethanol (10 cc.) and hot water (25 cc.) was added. To this solution iron dust (2.0 g.), ferric chloride (0.08 g.) and concentrated hydrochloric acid (1 cc.) were added. The reaction mixture was refluxed for 5 hours, concentrated to about half the original volume, then filtered, and the black iron oxide washed with hot water. The combined filtrate and washings were extracted with benzene, and the benzene extract was extracted with 20% sodium bisulfite solution. The bisulfite solution was acidified, the sulfur dioxide removed, and the resulting solution extracted with ether. The ether extract was dried, and the solvent removed; the residual oil (0.50 g.) soon crystallized. The benzene solution which contained a small amount of the oxime, was evaporated to dryness and the residue treated with 7N sulfuric acid (20 cc.) for 24 hours followed by 4 hours at 40°. By extraction of the acid solution with benzene, an additional 0.12 g. of the ketone was obtained; total yield, (0.62 g.), 87% distillable (0.05 mm.); fine, white, needle-like crystals from a mixture of petroleum ether and ether; m.p. 68-69°. Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.8; H, 6.7; OCH_3, 29.5. Found: C, 62.8; H, 6.7; OCH_3, 29.4.

(c) Semicarbazone of (4-Hydroxy-3,5-dimethoxyphenyl)-propanone-2. - (4-Hydroxy-3,5-dimethoxyphenyl)-propanone-2 (0.20 g.) in water (10 cc.) was added to a solution of semicarbazide-hydrochloride (0.18 g.) and potassium acetate (0.18 g.) in water (5 cc.), and the resulting solution was heated at 60-70° for two hours. The mixture was cooled, the white needle-like crystals (0.23 g.) were filtered off, washed and dried at 100° in vacuum; m.p. 153-154°; recrystallized first from benzene and then from ethanol; m.p. 155-156°. Anal. Calcd. for $C_{12}H_{17}O_4N_3$: C, 53.9; H, 6.4; OCH_3, 23.2; N, 15.7. Found: C, 54.0; H, 6.5; OCH_3, 23.1; N, 15.5.

C. Synthesis of (4-Hydroxy-3-methoxyphenyl)-propanone-2

(a) 1-(4-Hydroxy-3-methoxyphenyl)-2-nitropropene-1. - Vanillin was dissolved in a minimum amount of warm ethanol and condensed with nitroethane, the technique being the same as that used for the preparation of 1-(4-hydroxy-3,5-dimethoxyphenyl)-2-nitropropene-1; yield, 75%; m.p. 99-100°; recrystallized from ethanol and from ether; m.p. 101-102°; yellow plates. Anal. Calcd. for $C_{10}H_{11}O_4N$: C, 57.4; H, 5.3; OCH_3, 14.8; N, 6.7. Found: C, 57.5; H, 5.4; OCH_3, 14.7; N, 6.6.

(b) (4-Hydroxy-3-methoxyphenyl)-propanone-2. - The nitroalkene was reduced with iron and hydrochloric acid as described in the preparation of (4-hydroxy-3,5-dimethoxyphenyl)-propanone-2;

Total yield, 70%. The yellow oil was 94% distillable; b.p. 115° (0.15 mm.), $n_D^{25} = 1.5444$. Anal. Calcd. for $C_{10}H_{12}O_3$: C, 66.6; H, 6.7; OCH_3, 17.2. Found: C, 66.5; H, 6.7; OCH_3, 17.1.

(c) Semicarbazone of (4-Hydroxy-3-methoxyphenyl)-propanone-2. - Fine, white needle-like crystals from ethanol; m.p. 157-158°. Anal. Calcd. for $C_{11}H_{15}O_3N_3$; C, 55.7; H, 6.3; OCH_3, 13.1; N, 17.7. Found: C, 55.8; H, 6.5; OCH_3, 13.1; N, 17.4. (For details of the preparation see section 4B(c)).

(d) Thiosemicarbazone of (4-Hydroxy-3-methoxyphenyl)-propanone-2. - The ketone was treated with thiosemicarbazide in the same manner as described in section 4B(c); yield 93%; micro white needle-like crystals from ethanol; m.p. 187-188° (rate of heating, 5 to 6° per minute). This melting point varies with the rate of heating, apparently due to decomposition at temperatures slightly below the melting point. Anal. Calcd. for $C_{11}H_{15}O_2SN_3$: C, 52.2; H, 6.0; OCH_3, 12.3; N, 16.6. Found: C, 52.1; H, 6.2; OCH_3, 12.4; N, 16.7.

D. Catalytic Reduction of 1-(4-Hydroxy-3-methoxyphenyl)-2-nitropropene-1

(a) Using Palladium-carbon as Catalyst. - Animal Charcoal was heated on a steam bath with 20% hydrochloric acid to which a few cc. of concentrated nitric acid was added, until the supernatant solution no longer contained iron. The charcoal was filtered off, washed with water and dried at 100° in vacuum (15 mm.) for 8 hours. This purified charcoal was activated by heating in an evacuated test tube (20 mm.) with the luminous flame of a Bunsen burner for fifteen minutes. The charcoal (3.0 g.) was suspended in a solution of palladium chloride (0.5 g.) in concentrated hydrochloric acid (0.5 cc.) and water (25 cc.) and cooled to 5°. While the suspension was stirred vigorously, 40% formaldehyde (10 cc.) was added, followed by a drop by drop addition of a 50% solution of potassium hydroxide (10 cc.) and the temperature was kept below 5°. The temperature of the mixture was finally raised to 60° and maintained at 60° for a few hours. The catalyst was filtered off, washed with water, dilute acetic acid, and hot water until the filtrate gave no test for chloride, and dried in vacuum.

The palladiumized carbon (0.5 g.) was suspended in a solution of 1-(4-hydroxy-3-methoxyphenyl)-2-nitropropene-1 (2.0 g.) in pyridine (25 cc.), in a reaction vessel fitted with a glass stopcock. The vessel was evacuated, filled with hydrogen (purified by bubbling through successive solutions of dilute sodium bisulfite and alkaline pyrogallol), evacuated, and filled again. This was repeated once more to ensure complete displacement of air from the reaction vessel. The latter was connected to a 500 cc. gas burette containing hydrogen and agitated and heated at a temperature of 60-65° until 2 moles of hydrogen per mole of nitroalkene were absorbed (8 hours). The catalyst was filtered off and washed with pyridine. From the combined filtrate and washings the solvent was removed under reduced pressure (20 mm.) and the residual oxime was hydrolysed with 7N sulfuric acid as described above (section 3B(c)). The ketone (0.94 g.) was obtained by extraction of the acid solution.

The nitroalkene of the syringyl analog was reduced in a similar manner with almost identical results.

(b) Using Raney Nickel as Catalyst. - The reduction of 1-(4-hydroxy-3-methoxyphenyl)-2-nitropropene-1 was repeated as described above, with Raney nickel instead of palladiumized charcoal and at room temperature only. The yields of the ketone obtained varied from 10 to 25% depending on the concentration of the reaction medium. Maximum yield (25%) was obtained by using 100 cc. of pyridine per gram of nitroalkene.

5. Properties of the Compounds Isolated

A. Properties of the 1,2-Diketones

(a) Ethanolysis of Syringoyl Methyl Ketone. - Syringoyl methyl ketone (0.102 g.) was dissolved in a 2% solution of hydrogen chloride in ethanol (50 cc.) and refluxed for 48 hours in an atmosphere of carbon dioxide. The solution was cooled, neutralized with sodium ethylate and taken to dryness at reduced pressure (20 mm.). The residue was dissolved in water and the solution treated with hydroxylamine hydrochloride, nickel chloride and sodium acetate as described in section 3A. The precipitated nickel glyoxime salt was filtered, washed and dried yield, 0.116 g.. The recovery of the diketone was 90%.

(b) Stability of Vanilloyl Methyl Ketone Towards Strong Acids. - Vanilloyl methyl ketone (0.01 g,) was dissolved in the acid (7 cc.) and treated for varying lengths of time at different temperatures. The acid solution was extracted with ether, the solvent was distilled off, the residue dissolved in water and treated with o-phenylenediamine (see section 4A(b)). The quinoxaline was filtered, washed, dried and weighed. The results obtained are summarized in Table 6.

Table 6.

Stability of Vanilloyl Methyl Ketone Towards Acids

Wt. of Diketone	Reaction Temp. °C.	Reaction Time, hrs.	Acid Employed	Quinoxaline Precipitated	% Diketone Recovered
0.033	reflux	4	6N HCl	0.035	80
0.024	60	2	12N H_2SO_4	0.030	93
0.012	60	5	" "	0.014	88
0.012	60	8	" "	0.015	89
0.012	60	12	" "	0.015	89

(c) Reduction of Syringoyl Methyl Ketone. - Syringoyl methyl ketone (1.0 g.) was dissolved in 2N sulfuric acid (75 cc.), heated to 80-90° and zinc dust (4.0 g.) containing a trace of copper sulfate was added in small amounts during the course of one hour. The reaction mixture was then heated on a steam bath for 2 hours, filtered and the excess zinc was washed with hot water. The combined filtrate and washings were extracted with benzene and the benzene extract in turn with sodium bisulfite solution. The bisulfite-soluble material (0.27 g.) formed a semicarbazone; m.p. 127-131°; this derivative could not be purified by recrystallization. It was therefore concluded that bisulfite-soluble material consisted of a number of reduction products.

B. Properties of the Monoketones

(a) Ethanolysis of (4-Hydroxy-3-methoxyphenyl)-propanone-2. The ketone (0.40 g.) was dissolved in a 2% solution of hydrogen chloride in ethanol (50 cc.) and refluxed for 48 hours in an inert atmosphere. The dark alcoholic solution was neutralized with sodium bicarbonate (2.5 g.), filtered, the filtrate concentrated to 5 cc., then poured in a fine stream into water (50 cc.). The lignin-like precipitate (0.125 g.) was allowed to settle overnight, then filtered off, washed and dried at 90°; OCH_3, 12.5%. The filtrate was extracted with benzene, and on removal of the latter, an oil (0.25 g.) remained, 0.20 g. of which was found to be petroleum ether soluble (see section 1d), and identical with the starting material. The other 0.05 g. was a resin-like tar. Thus approximately half of the original ketone polymerized during the ethanolysis reaction.

(b) Klason Lignin Formation from (4-Hydroxy-3-methoxyphenyl)-propanone-2. - The ketone (0.27 g.) was treated with 72% sulfuric acid (5 cc.) for two hours at 20°. The resulting acid solution was poured into water (190 cc.), the dilute solution refluxed for four hours, and the lignin-like precipitate (0.01 g.) filtered off. Thus only 3.7% of the original material was converted into a lignin-like polymer. under these conditions.

SUMMARY

The syringoyl and vanilloyl methyl ketones present in the bisulfite-soluble fraction obtained from the ethanolysis of maple wood have been isolated in the pure crystalline state. Their quantitative separation has been effected by conversion into the insoluble nickel glyoxime salts. The total diketone fraction amounts to 2 to 3% of the Klason lignin in the original maple wood.

Vanillin and Syringaldehyde have been isolated from the bisulfite-soluble fraction obtained from the ethanolysis of maple wood. The total aldehyde fraction amounts to 0.4 to 0.8% of the Klason lignin in the original maple wood.

Two new compounds, namely, (4-hydroxy-3,5-dimethoxyphenyl)-propanone-2 and (4-hydroxy-3-methoxyphenyl)-propanone-2 have been isolated from the bisulfite-soluble fraction obtained from the ethanolysis of maple wood, and identified by direct syntheses. The total monoketone fraction amounts to 0.2 to 0.3% of the Klason lignin in the original maple wood.

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