THE ETHANOLYSIS OF SPRUCE WOOD AND THE STRUCTURE OF LIGNIN

by

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CLAIMS TO ORIGINAL RESEARCH

I. The ethanolysis procedure for spruce wood has been re-investigated and certain improvements introduced:

A. A more complete separation and a larger overall yield of ethanolysis products has resulted from (1) extraction of the residual wood meal with ethanol and (2) the separation of tars ("higher-boiling phenols") from the ethanolysis oils by a new petroleum ether-precipitation technique.

B. It has been shown that a decrease in the time of exposure of the water-soluble oils to alkali in the fraction procedure brings about a reduction in (1) the degradation of one of the constituents to vanillic acid and (2) in the amount of tar formed by the polymerization of the simple units.

II. The presence of vanillin as a definite constituent of the bisulfite-soluble, water-soluble spruce ethanolysis oil has been extablished by a re-investigation of the bisulfite fraction. Vanillpropanone-2, which previously had been found in the ethanolysis products of maple, has been isolated from spruce ethanolysis products for the first time by a modification of the procedure used for maple.

III. A new substance, 1-vanill-1-ethoxypropanone-2, an isomer of α-ethoxypropiovanillone, has been isolated from the alkali-soluble, water-soluble spruce ethanolysis oils.

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FIGURE NO.

Part A. Historical Review

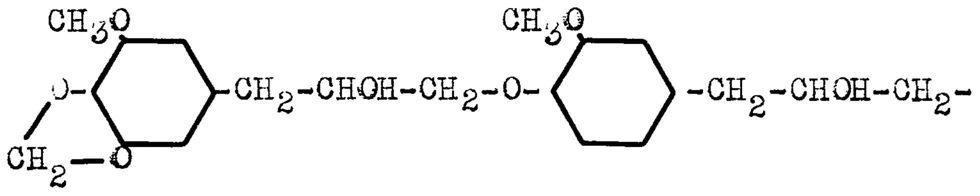
Early Developments in Lignin Chemistry

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.

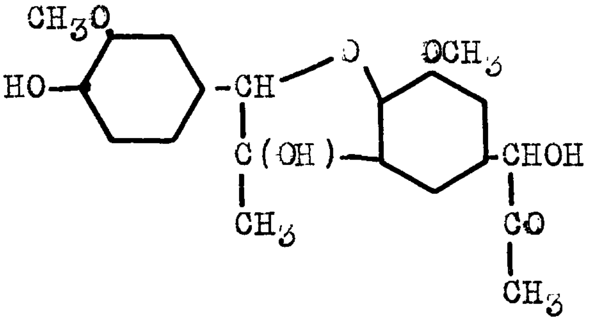
Freudenberg's earlier theory of the structure of lignin (12) was based essentially on the isolation of small amounts of catechol, protocatechuic acid and formaldehyde from spruce hydrochloric acid lignin, and on analyses of this lignin. These results indicated (a) aromatic character and absence of free 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. Such a polymer, however, should readily undergo degradation by hydrolytic action into simple molecular units, but, this not being the case Freudenberg found it difficult to postulate transformation into "secondary lignins"



(I)

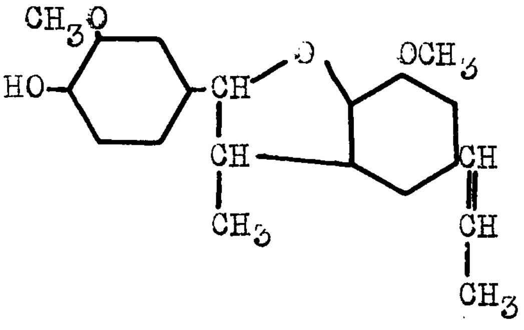
The assumption of the presence of the dioxymethylene group was based on the liberation of a small amount of formaldehyde (up to 1.2 per cent) (13, 14, 15). The side chains visualized by Freudenberg were R-CHOH-CHOH-CH_2OH, R-CH_2-CHOH-CH_2OH, R-CHOH-CH_2-CHO and R-CHOH-CO-CH_3. According to his definition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

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 (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, or in his later theory, centrally disposed aromatic nuclei, to the extent of 25 per cent of the spruce lignin structure. This theory has been seriously criticized (4), especially in 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

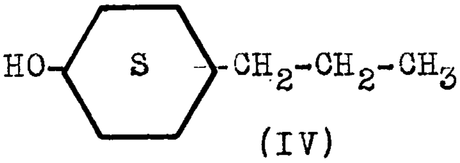
The action of alkali on lignin sulfonic acids has been investigated extensively following earlier observations regarding the presence of vanillin, at least in small quantities, in the reaction mixture. With spruce lignin sulfonic acid a yield of 6-7 per cent of vanillin (20) and small amounts of acetovanillone (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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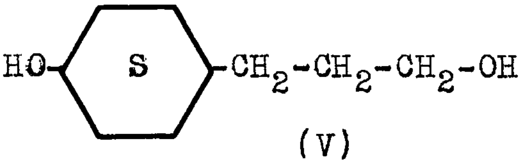


(V)

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.

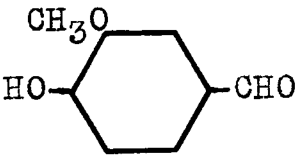
The belief that proto- and extracted lignins exist only as highly polymerized, complex substances led investigators to conclude that the amorphous water-insoluble product obtained by customary extraction methods was the only form in which lignin could be isolated. Precipitation into water from concentrated 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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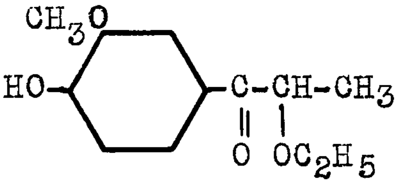

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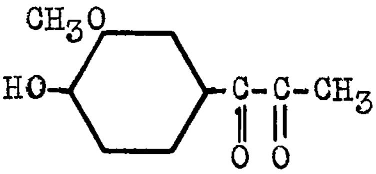
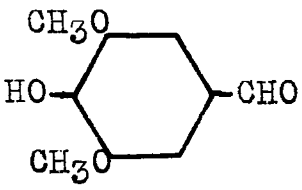


(X)



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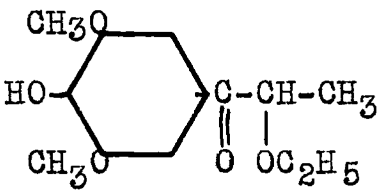
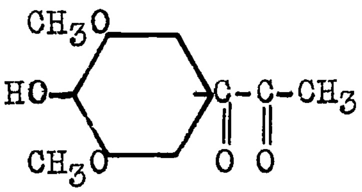


(IX)



(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 behaviour 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 indicated in 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

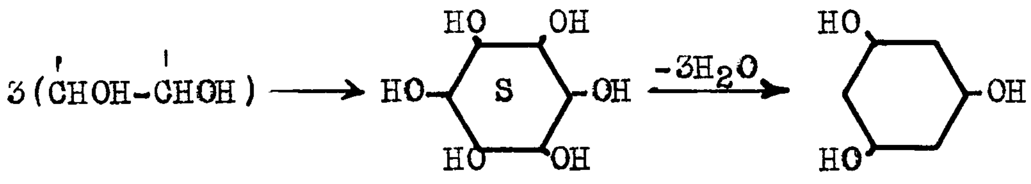
(1) Synthesis of Simple Phenols - Various theories concerning the synthesis of phenolic compounds in plants have been based on assumed transformation of hexoses (61, 62), while a more recent theory (4) considers the phenolic substances as being formed from intermediate photosynthetic and/or plant respiratory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

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

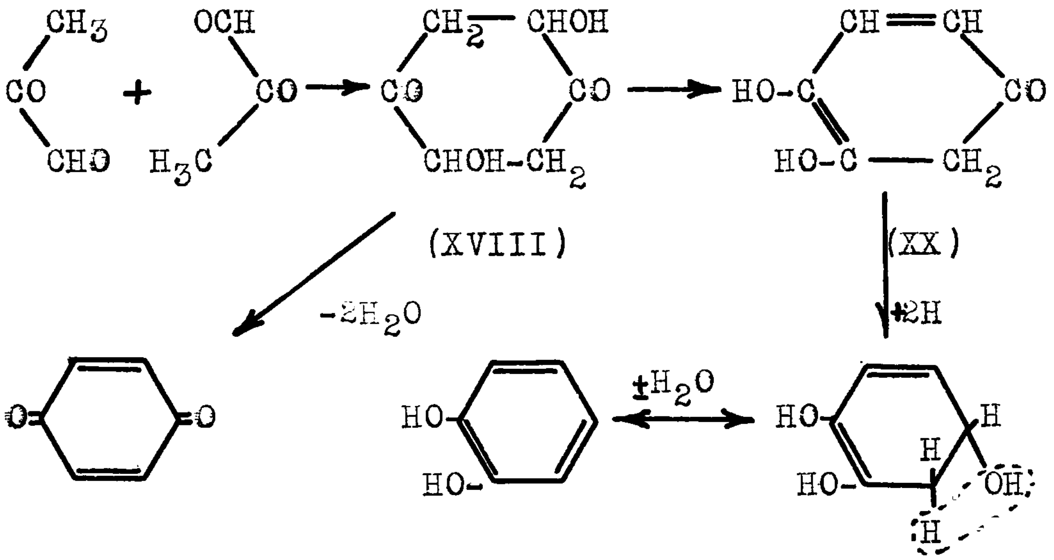
(XIV) (XV) (XVI)

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)

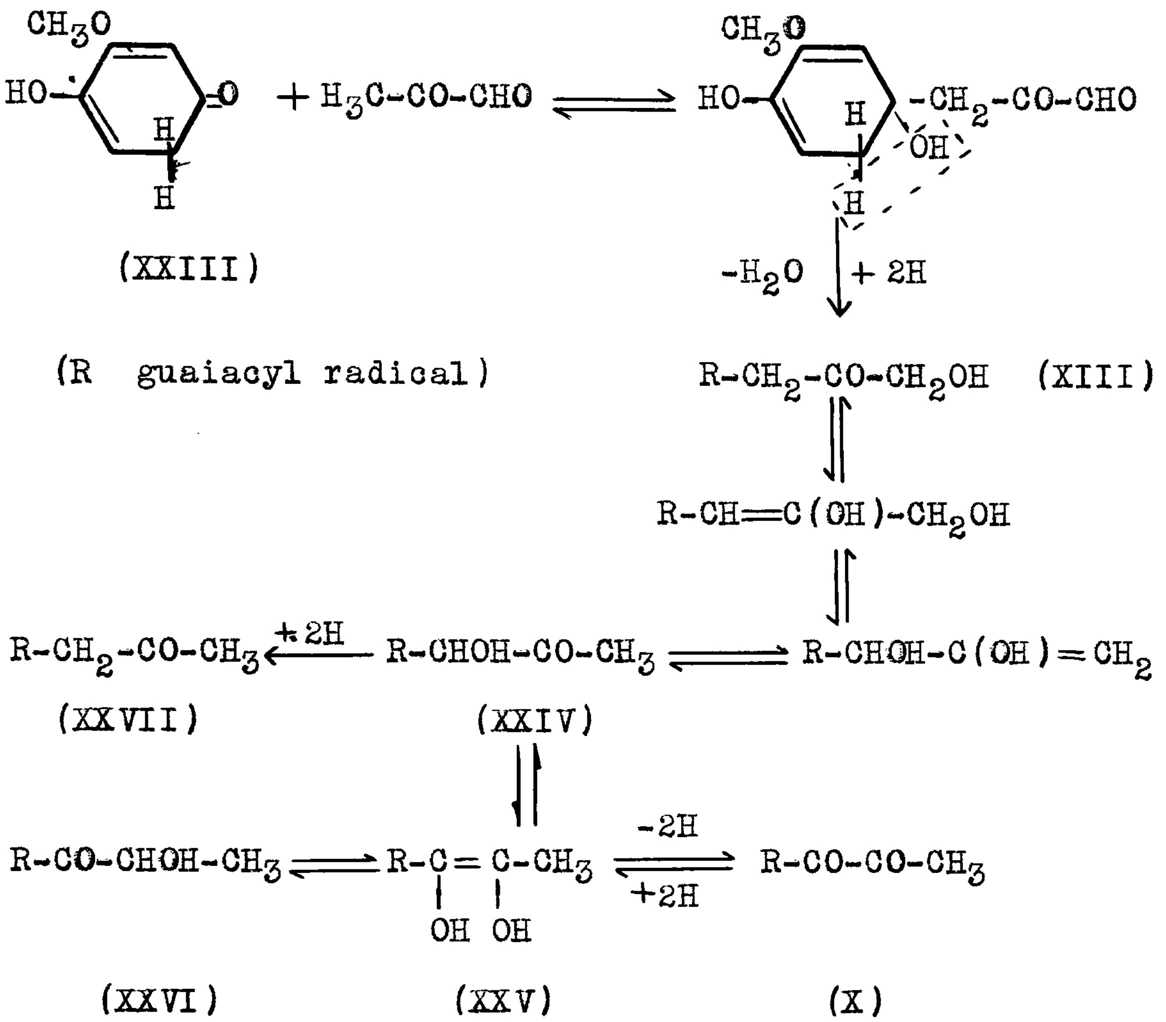
(ii) Methyl Glyoxal Theory of Phenol Formation (64) - Methyl glyoxal, which is a well-recognized intermediate in animal cell respiratory processes, presumably occupies a somewhat analogous position in plant carbohydrate metabolism and has actually been isolated from a number of higher plants (67). Hibbert 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).

(ii) 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 in 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 aldehyde form ($R-CH_2-CO-CH_2OH \rightleftharpoons R-CH_2-CHOH-CHO$), then the reaction undergone by its analog, benzyl glycollic aldehyde, (XXVIII), in the presence of 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 methyl ketone. 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) ⇌ (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 ⇌ mannose ⇌ fructose, and of glyceric aldehyde ⇌ 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).

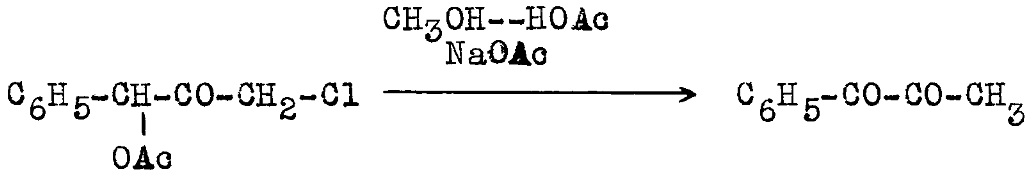
It is therefore of great importance to consider to what extent members of the second type of ethanolysis products isolated, namely, vanilloyl- (X) and syringoyl methyl ketones (XI), represent also stabilized end ethanolysis products derived from more reactive lignin progenitors. A consideration of a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

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

Examination of the enediol (XXXI), shows it differs only from that present in the dismutation system $R\text{-}CO\text{-}CHOH\text{-}CH_3$ $R\text{-}C(OH) = C(OH)\text{-}CH_3 \rightleftharpoons R\text{-}CHOH\text{-}CO\text{-}CH_3$ 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 (196) 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

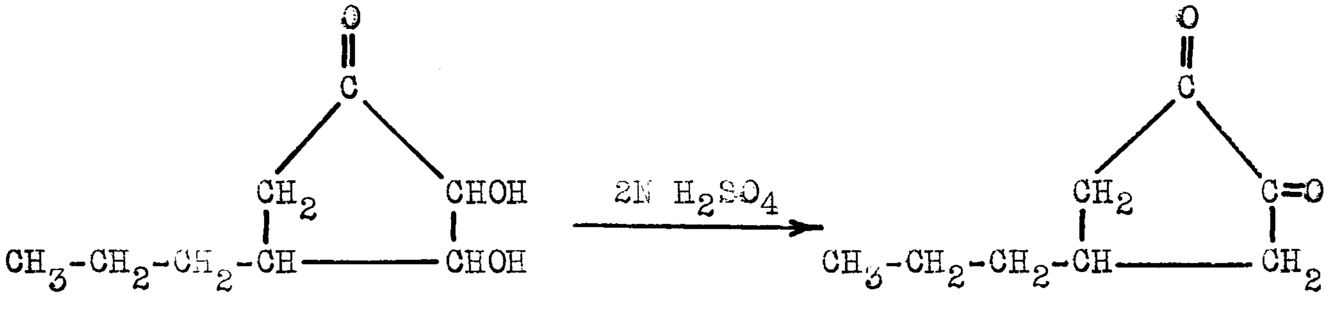


$$C_6H_5\text{-}\underset{\overset{|}{OAc}}{CH}\text{-}CO\text{-}CH_2\text{-}Cl \xrightarrow{\substack{CH_3OH\text{--}HOAc \\ NaOAc}} C_6H_5\text{-}CO\text{-}CO\text{-}CH_3$$

(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 co-workers (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). As tetrahydroterrein contains

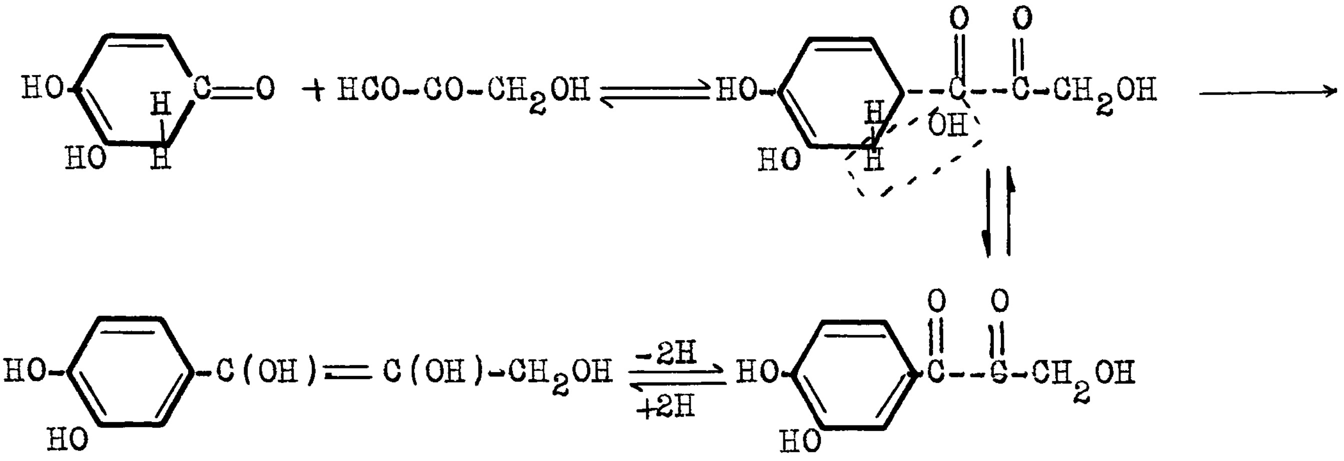


(LXVIb) (LXVIc)

a grouping analogous to that in dioxyconiferyl alcohol, the formation of a diketone during ethanolysis 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.

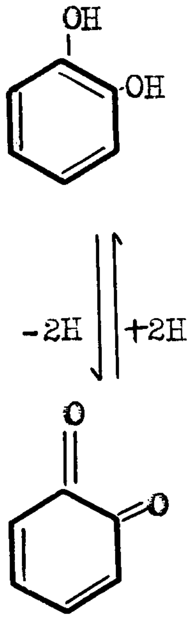
The plant origin of these dioxyconiferyl alcohol derivatives is, as yet, unknown, but it is interesting to note that their plant synthesis can be visualized as consisting of a primary condensation of two moles of methyl glyoxal, with the loss of a mole of water, followed by a further condensation with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



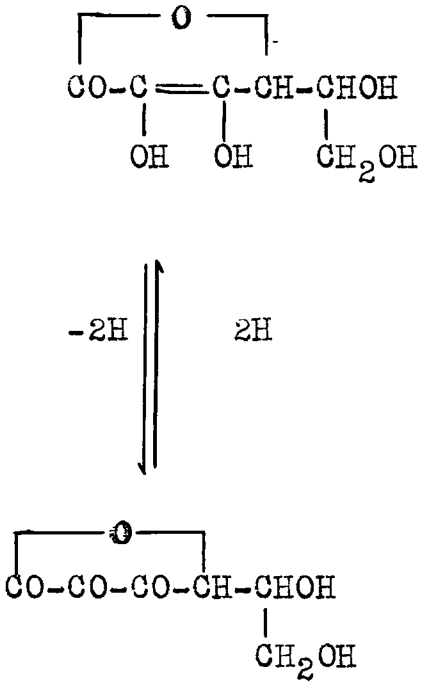
(6) Possible Significance of Lignin Progenitors in Plant Oxidase Systems

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.

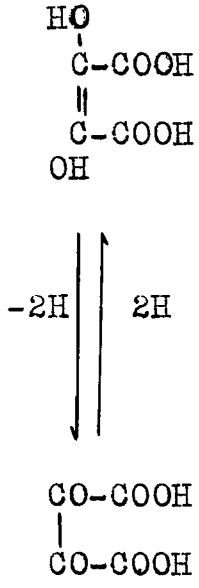
Szent-Györgyi has demonstrated the presence of a number of dehydrogenase-catalyst systems in various forms of plants; such as those of catechol (XXXIV) (73), ascorbic acid (XXXV) (74), and dioxymaleic acid (XXXVI) (75).



(XXXIV)



(XXXV)

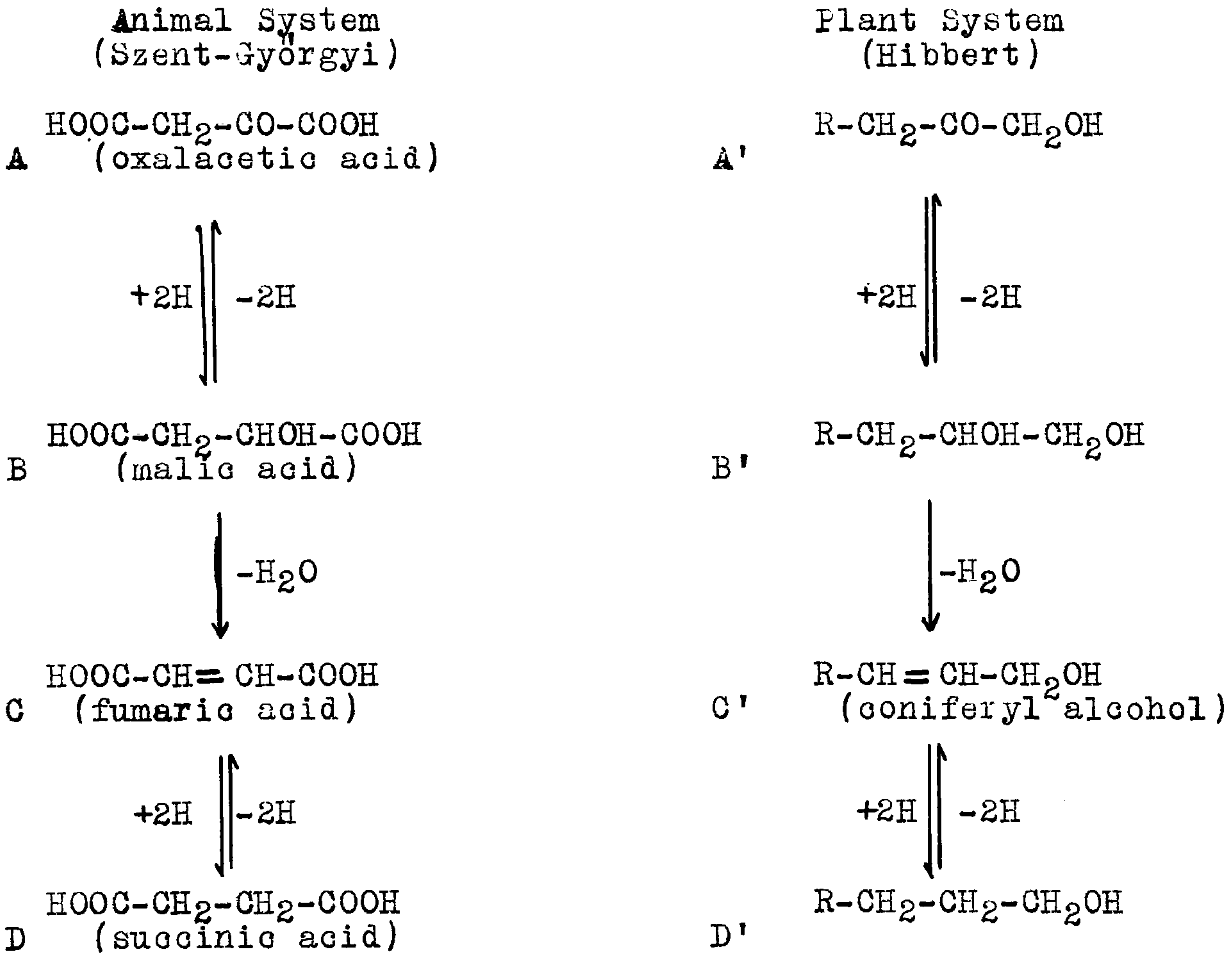


(XXXVI)

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).



R- syringyl or guaiacyl radical

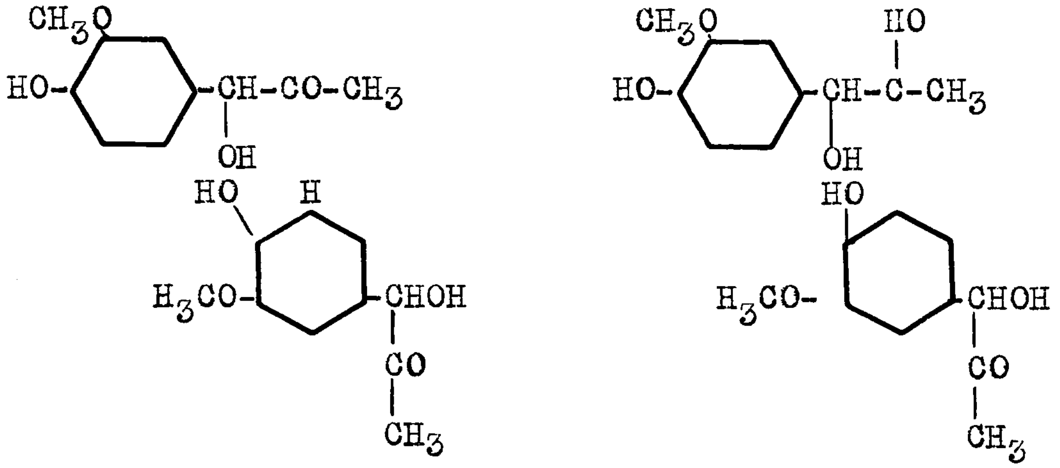
The first member (A') of the plant system, corresponding to oxalacetic acid (A), is the keto-alcohol believed to be obtained from the condensation of three mols of methyl glyoxal (page 17). It is seen that the third member of the new system is coniferyl alcohol corresponding to fumaric acid in the C_4 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 page 17 .

(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 5 of part A).

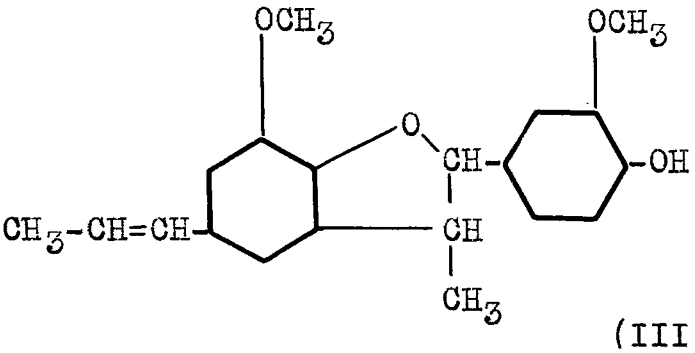
Hibbert (4) points out that the "lignin building units" containing a phenol group together with a carbonyl or an unsaturated linkage in the side chain would have a strong tendency to undergo para- and ortho-nuclear condensations both individually and collectively, under mild conditions, with formation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

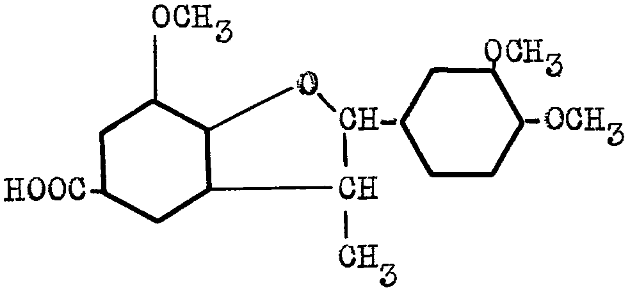
The condensation of the ortho-hydrogen atom of one unit with the carbonyl of another, followed by the elimination of two molecules of water as indicated would result in formation of a benzofurane derivative. Condensation with a third molecule would yield a trimer and so on. It is seen that this mechanism 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 (6) to have the formula (III).



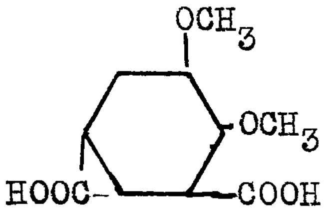
(III)

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.

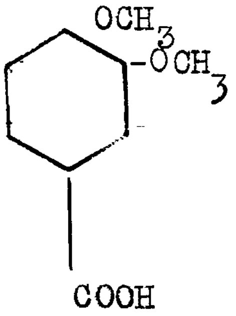


(XXXVIII)

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)

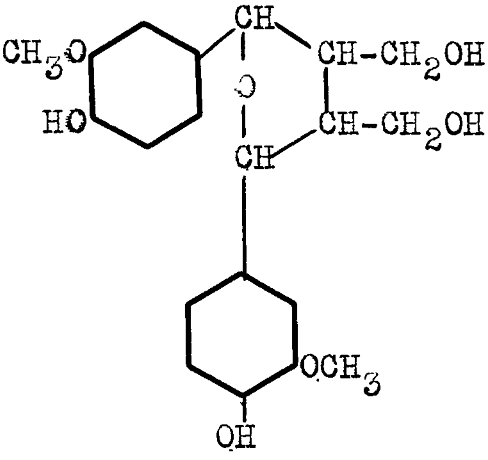


(XL)

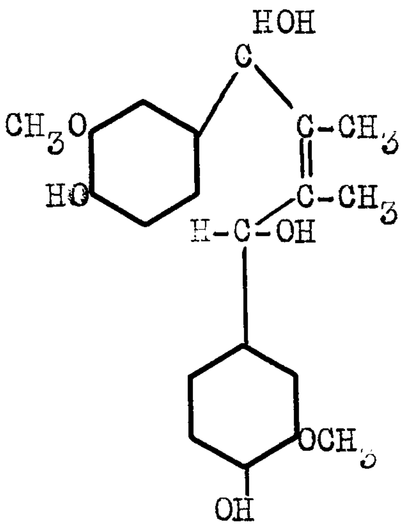
concluded (89) that some of the lignin molecules are of a type capable of yielding isohemipinic acid on alkali degradation, followed by methylation and oxidation. It is apparent that this observation supports the theory of Erdtmann (5) and of Hibbert (78) that protolignin is made up of dimers of the dehydrodiisoeugenol type (III).

Erdtmann in his recent review (5) 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.

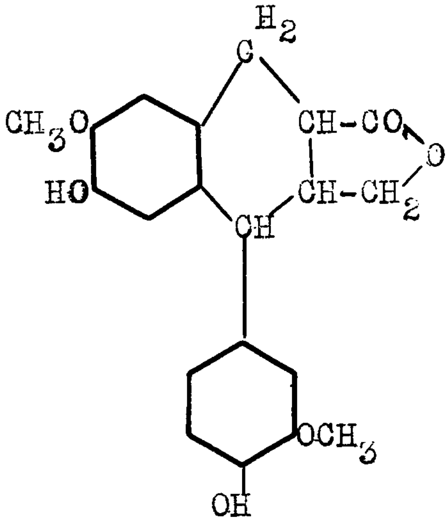
The similarity between these simple natural products and dehydrodiisoeugenol, and the analogies between lignin and dehydrodiisoeugenol have led Erdtmann to the belief that the phenomenon of dehydrogenative coupling of molecules probably plays a great role in the biogenesis of plant products.



(XLI)



(XLII)

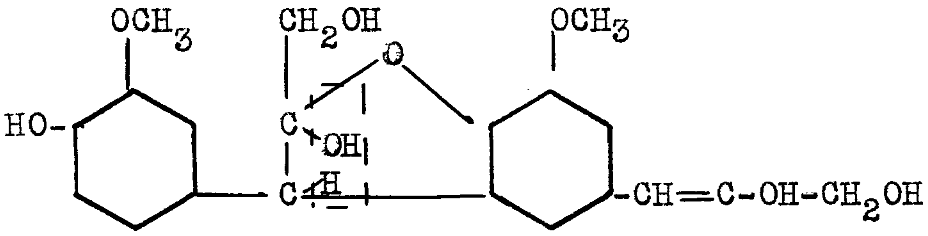


(XLIII)

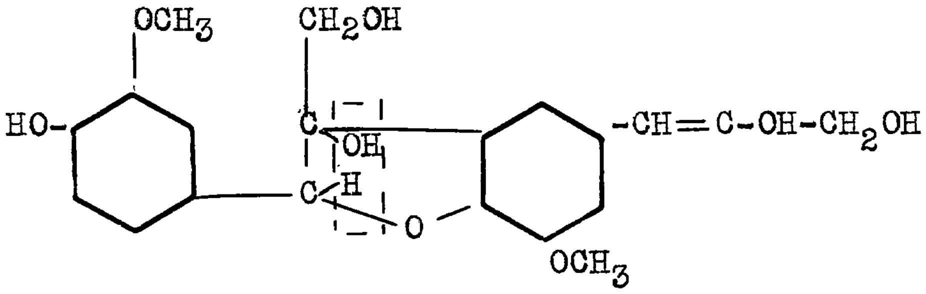
He states that the ethanolysis products α-hydroxypropiovanillone, (XXVI) and vanilloyl methyl ketone (X) and their syringyl analogues are not necessarily true lignin building units, since they may represent stabilized end products originating from dimeric types similar to dehydrodiisoeugenol. That such 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 $R\text{-}C(OH)=C(OH)\text{-}CH_3$ (XXV); $R\text{-}CH=C(OH)\text{-}CH_3$ (XXVII); $R\text{-}CH=C(OH)\text{-}CH_2OH$ (XIII); and the side chains of isoeugenol $R\text{-}CH=CH\text{-}CH_3$ and coniferyl alcohol $R\text{-}CH=CH\text{-}CH_2OH$. 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).

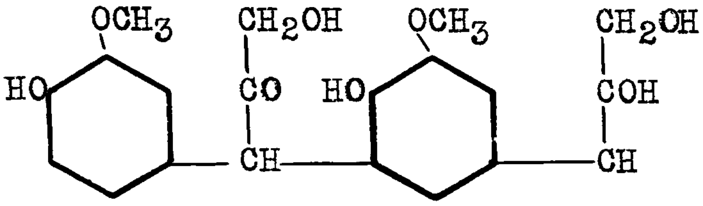


(XLIV)

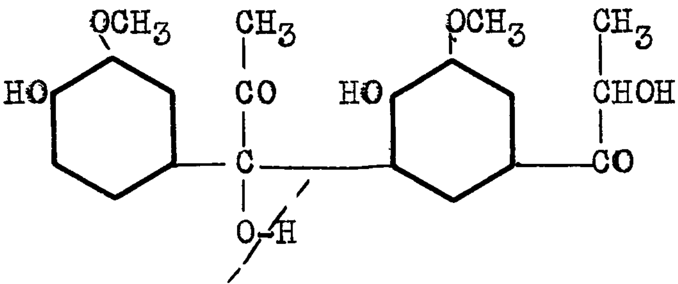


(XLV)

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 <u>tertiary</u> butyl alcohol, and, more significantly with benzyl alcohol (87), substituted benzyl alcohols, and their ethers e.g. phenyl dimethyl carbinol (XLVIII), benzohydrol (XLIX), α-ethoxyphenylethane (L) and benzyl ethyl ether (LI).

$$C_6H_5\text{-}C(CH_3)_2\text{-}OH + HS\text{-}CH_2\text{-}COOH \longrightarrow C_6H_5\text{-}C(CH_3)_2\text{-}S\text{-}CH_2\text{-}COOH + H_2O$$

(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)

$C_6H_5CH_2\text{-}OEt$

(LI)

(XLIX) and (LI) react in the same way as (XLVIII) and (L). In general, thioglycollic acid condenses with benzyl alcohols and their ethers, but does not react with primary alcohols such as phenyl propanol, phenyl ethanol (82), or primary butyl alcohol. These generalizations are substantiated by the non-reactivity of thioglycollic acid and certain heterocyclic compounds such as α-methyl coumarane, 2-phenyl coumarone, flavan and flavanone (85).

The fact that lignin condenses with thioglycollic acid very readily, would seem to 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 in all probability benzyl derivatives, so that from the above evidence, Holmberg's work lends indirect 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.

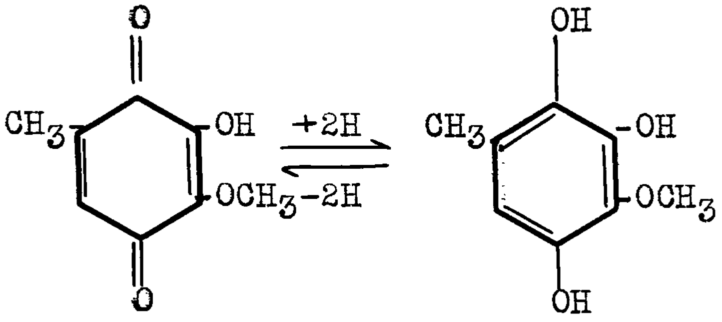
The molds were grown on the Czapek-Dox medium which is a 5% glucose (the sole organic substrate) solution containing mineral salts. The sterilized medium was inoculated with the spores of the mold and incubated. The time of incubation, temperature and pH were varied to suit the particular species or 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.

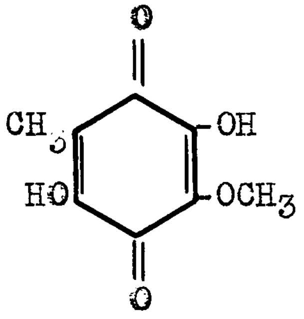
Another example in support of the specificity of micro-organisms is the fact that most of the species of the genus Helminthosporum when grown on the Czapek-Dox medium produce anthraquinones which are formed only by a limited number of species of other genera. It is interesting to note that Hibbert and 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 the guaiacyl analogue (XXVII) of (LXV) in the ethanolysis products from spruce 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).



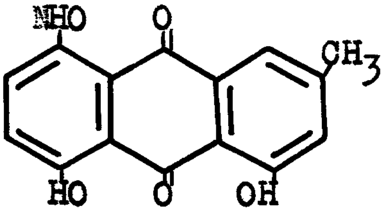
(LII) (LIV)



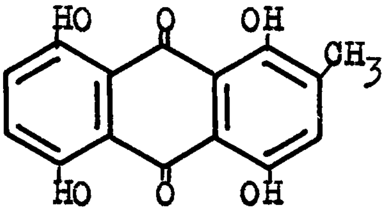
(LIII)

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.

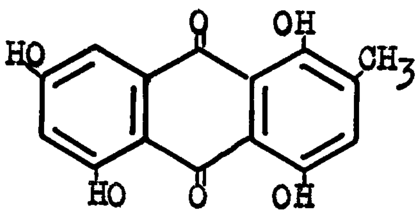
Different species of the genus Helminthosporum and a few of the species of the genera Penicillium and Aspergillus when grown on a modified Czapek-Dox medium produce in the mycelium, a pigment consisting of different polyhydroxyanthraquinones. These were obtained from dried and powdered mycelium by extraction with chloroform. Helminthosporin (4,5,8-trihydroxy-2-methylanthraquinone) (LV) (94), cynodontin (1,4,5,8-tetrahydroxy-2-methylanthraquinone) (LVI) (95), centenarin (1,4,5,6-tetrahydroxy-2-methylanthraquinone) (LVII) (96) and tritisporin (6-(or 7)-hydroxymethyl-1,3,5,8-tetra hydroxyanthraquinone) (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.



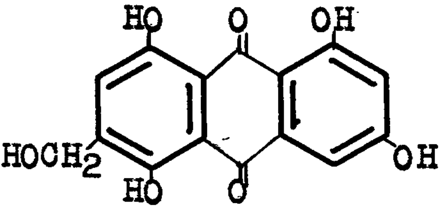
(LV)



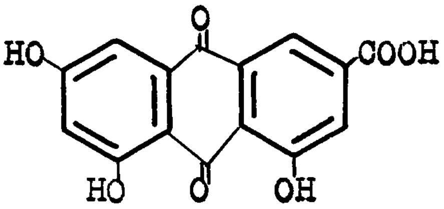
(LVI)



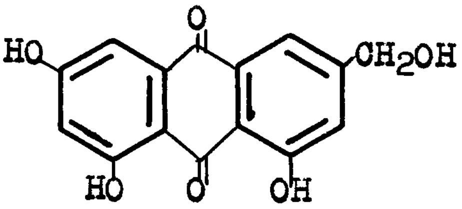
(LVII)



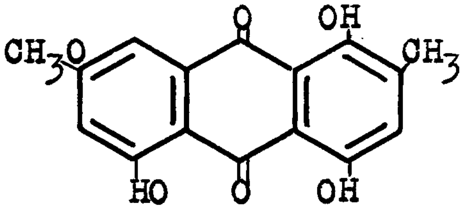
(LVIII)



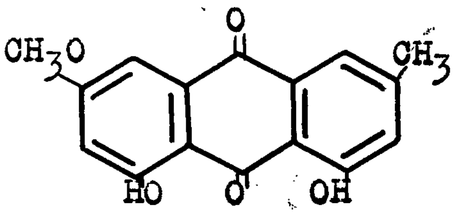
(LIX)



(LX)



(LXI)

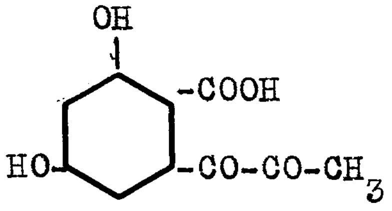


(LXII)

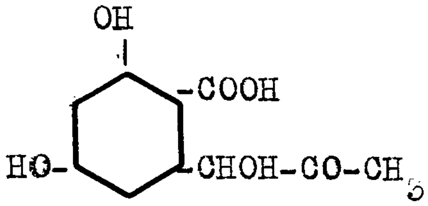
The coloring matters present in the mycelium of a strain of Penicillium cyclopium Westling grown on a glucose solution were also investigated (98). Two anthraquinones, emodic acid (4,5,7-trihydroxyanthraquinone-2-carboxylic acid) (LIX), and ω-hydroxyemodin (4,5,7-trihydroxy-2-(hydroxymethyl)-anthraquinone) (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).

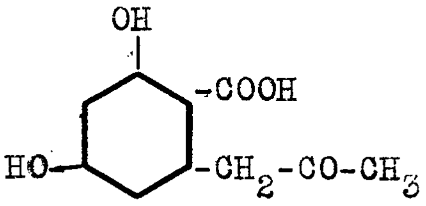
A very interesting series of compounds is that of the phenolic acids formed by the action of Penicillium brevi-compactum Dierckx and related species (100), on 5% glucose solution containing mineral salts. These are 3,5-dihydroxy-2-carboxybenzoylmethyl ketone (LXIII), 3,5-dihydroxy-2-carboxyphenylacetylcarbinol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



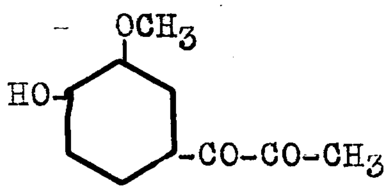
(LXIII)



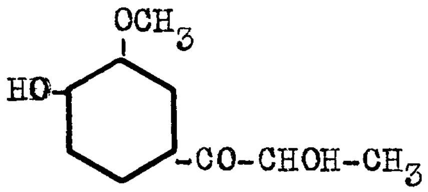
(LXIV)



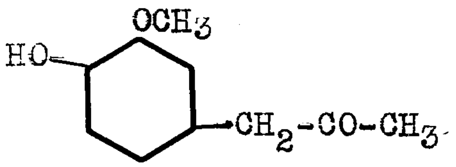
(LXV)



(X)

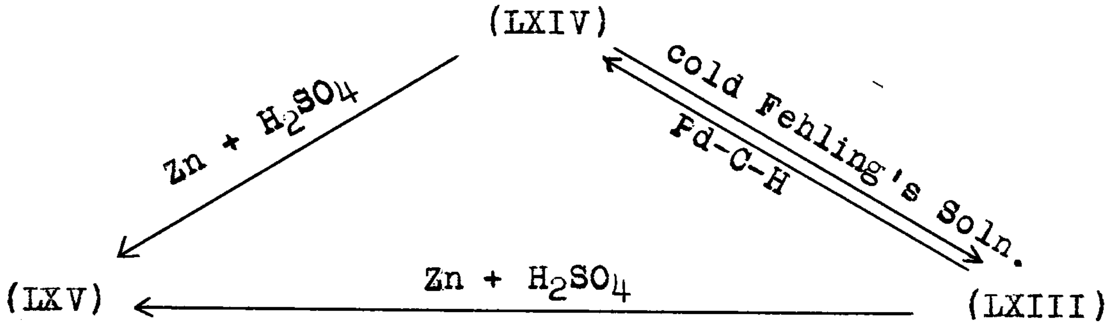


(XXVI)



(XXVII)

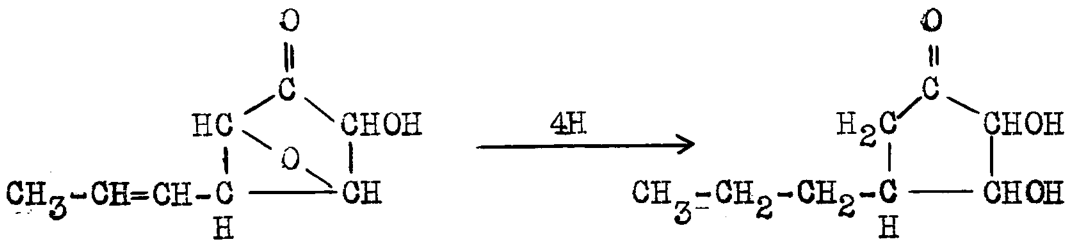
Oxford and Raistrick point out that the structure of the side chain of (LXIV) is uncertain and that it may be either one or other of the following: $-CO-CHOH-CH_3$, $-C(OH)=C(OH)-CH_3$, $\underbrace{-CH-C}_{O}(OH)-CH_3$ or $-\underbrace{C(OH)-CH}_{O}-CH_3$ or an equilibrium mixture of several of the 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.,



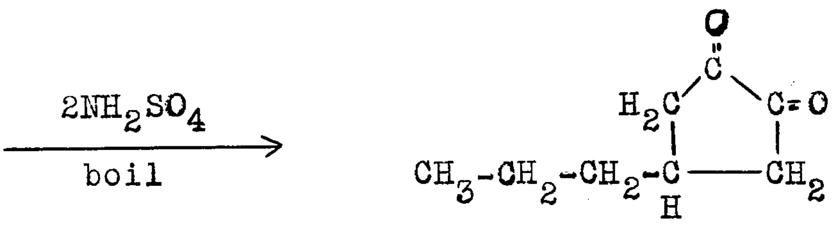
In most cases the principal constituent of the metabolic mixture was the diketone (LXIII). The yield of (LXV) was in general quite small - about 3% of the mixture. In the case of a few species, the benzoin (LXIV) was apparently the chief constituent.

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 Aspergillus terreus Thom 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.



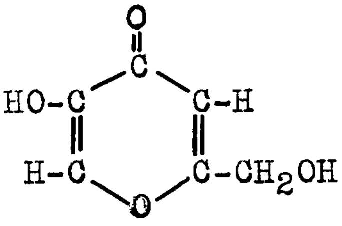
(LXVIa) (LXVIb)



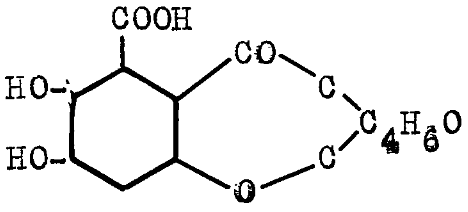
(LXVIc)

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 *A. flavus-oryzoe* 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.



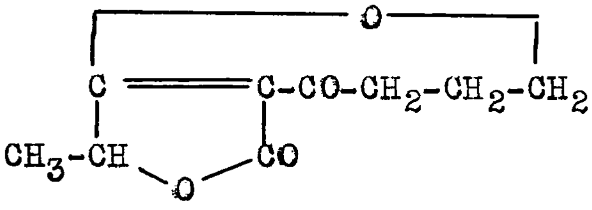
(LXVII)



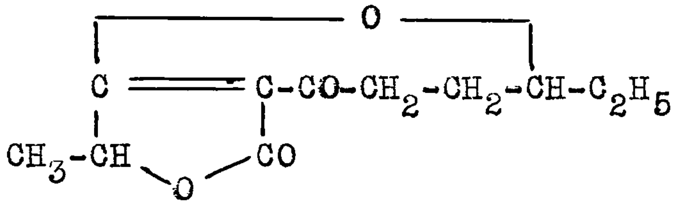
(LXVIII)

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) ($C_{17}H_{17}O_6Cl$); 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.

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 soft-woods (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).

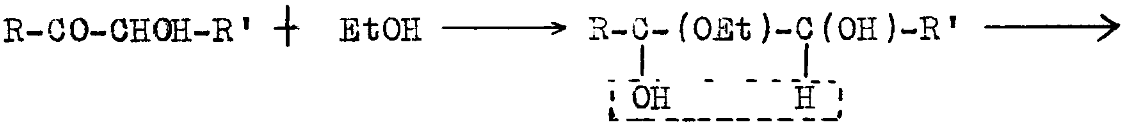
A more complete understanding of the relationship of α-hydroxy-ketones, 1,2-diketones, enediols and monoketones to the mechanism of plant synthesis and their probable role as building units from which lignin is derived, can be obtained by a consideration of the general properties of these compounds.

(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 tomethylate 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.

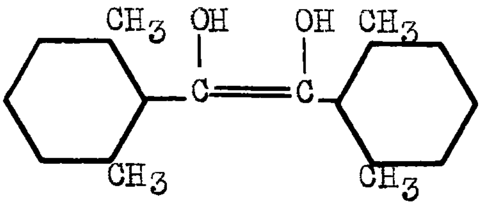


$$R\text{-}CO\text{-}CHOH\text{-}R' + EtOH \longrightarrow R\text{-}\underset{OH}{C}\text{-}(OEt)\text{-}\underset{H}{C}(OH)\text{-}R' \longrightarrow$$

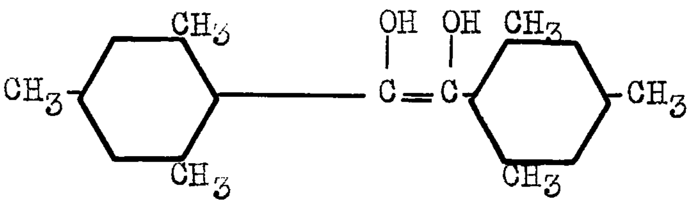
$$R\text{-}C(OEt) = C(OH)\text{-}R' \longrightarrow R\text{-}C(HOEt)\text{-}CO\text{-}R'$$

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.

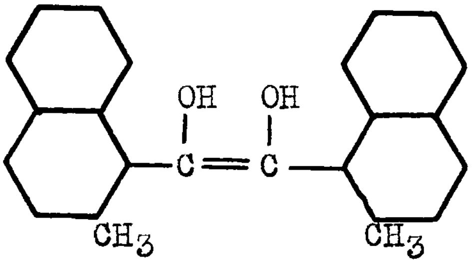


(LXXI)

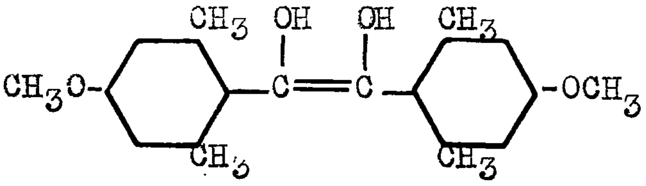


(LXXII)

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



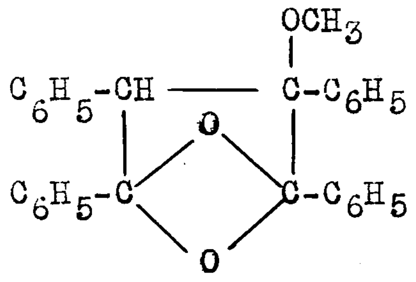
(LXXIII)



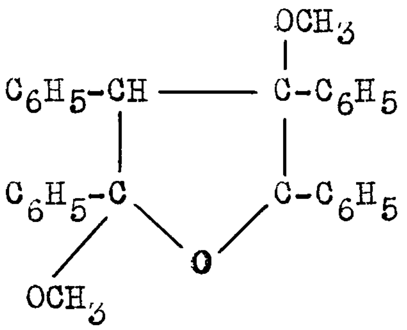
(LXXIV)

The stability of enediols is also determined by their stereo-configuration. Thus the <u>trans</u> modification is much more stable towards oxidation and isomerization than the <u>cis</u> 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)



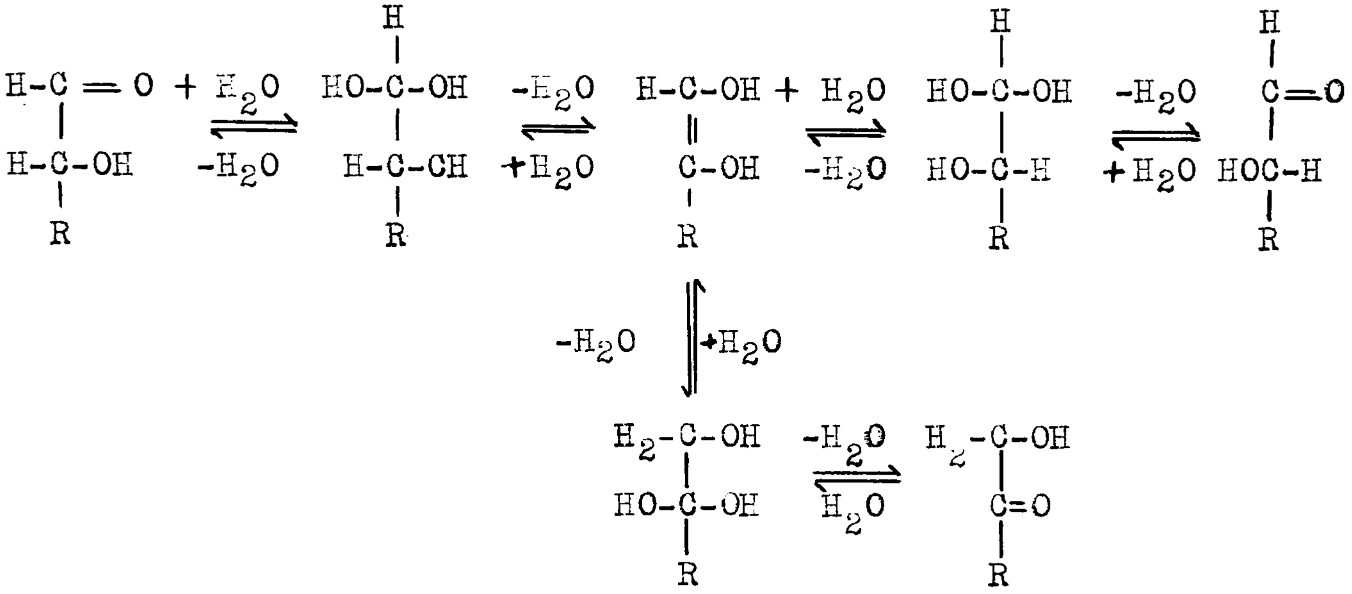
(LXXVI)

(2) Dismutation Reactions of Hydroxy-ketones

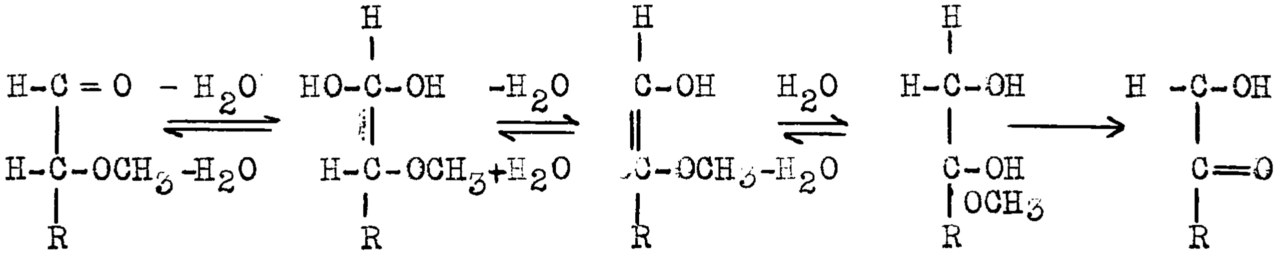
One of the most interesting properties of hydroxy-ketones is their marked tendency to undergo intramolecular dismutation transformations, such as typified below:

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

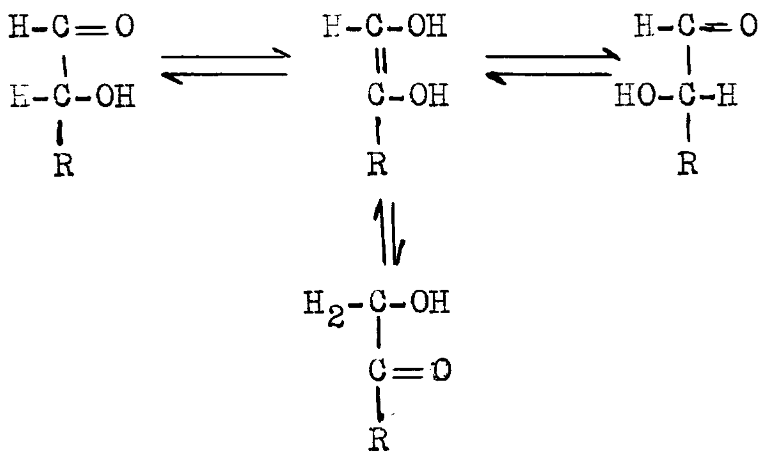
The best known examples of such intramolecular dismutation rearrangements are those which occur in the field of carbohydrate chemistry. The well known Lobry de Bruyn transformations, embracing the equilibrium system, glucose, mannose, fructose (122) and that of glyceric aldehyde and dihydroxy acetone (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:



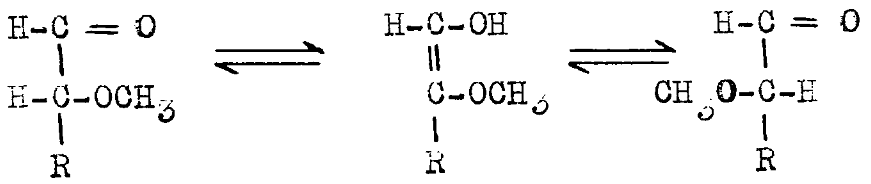
Lewis and co-workers (127), however showed that such a mechanism was not tenable - at least in the case of methylated sugars. Thus, for example, on the basis of Nef's hypothesis, tetramethyl glucose should be converted by alkali into methylated fructose by the loss of methanol from the intermediate hemiacetal:



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:



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:



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.

$$C_6H_5CO\text{-}CHOH\text{-}CH_2\text{-}CO\text{-}CH_3 \longrightarrow C_6H_5\text{-}CHOH\text{-}CO\text{-}CH_2\text{-}CO\text{-}CH_3$$

(LXXVII) (LXXVIII)

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

$C_6H_5\text{-}CHOH\text{-}CO\text{-}CH_3$ $C_6H_5\text{-}CO\text{-}CHOH\text{-}CH_3$

(LXXIX) (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 semicarbazide, 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.

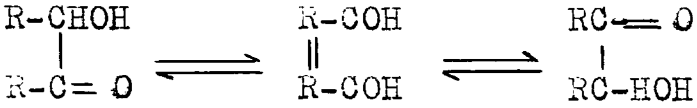
Recently she (135, 136) has extended this work to a study of the two isomeric ketols, ethyl benzoyl carbinol (LXXXI) and phenyl propionyl carbinol (LXXXII).

$C_6H_5CO\text{-}CHOH\text{-}CH_2\text{-}CH_3$ (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 (157) assumed that benzoin transformations represent equilibrium reactions proceeding through the enediol stage:



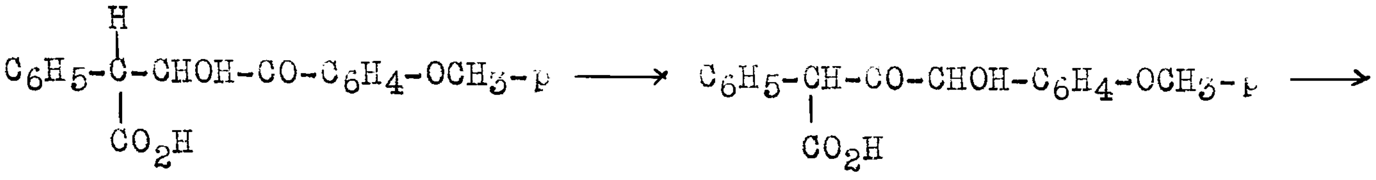
$$\begin{array}{c} R\text{-}CHOH \\ | \\ R\text{-}C=O \end{array} \rightleftharpoons \begin{array}{c} R\text{-}COH \\ \| \\ R\text{-}COH \end{array} \rightleftharpoons \begin{array}{c} RC=O \\ | \\ RC\text{-}HOH \end{array}$$

Kohler and Kimball (158) 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).

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

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

Kohler and Leers (159) 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).



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

(LXXXV)

$$C_6H_5-CH_2-CO-CHOH-C_6H_4-OCH_3-p \longrightarrow C_6H_5-CH_2-CHOH-CO-C_6H_4-OCH_3-p$$

(LXXXVI) (LXXXVII)

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

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

$$C_6H_5-CH_2-CHOH-CO-C_6H_4-Cl-p \rightleftharpoons C_6H_5-CH_2-CO-CHOH-C_6H_4-Cl-p$$

(LXXXVIII) (LXXXIX)

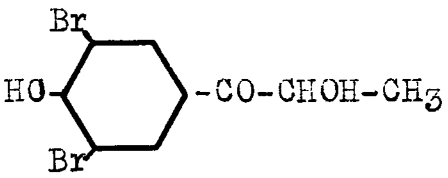
Starting with either isomer he was able to show that under the influence of aqueous alcoholic sodium carbonate both isomers were present in the reaction mixture. He concluded that probably all such 1,2-ketols represent, in solution, equilibrium mixtures of dismutation isomers, and that the failure to 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

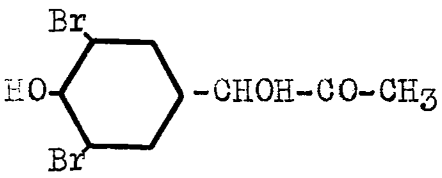
$C_6H_5\text{-CHOH-CO-CH}_3$ (LXXIX)

$C_6H_5\text{-CO-CHOH-CH}_3$ (LXXX)

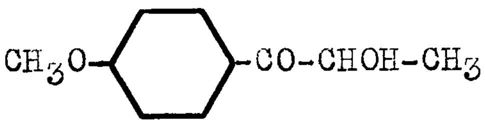
form, this stability is reversed by introducing substituents in the benzene ring. Thus (XC) and (XCII) are more stable



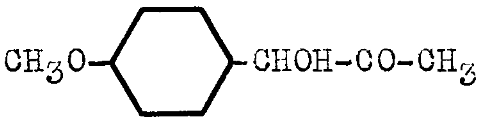
(XC)



(XCI)



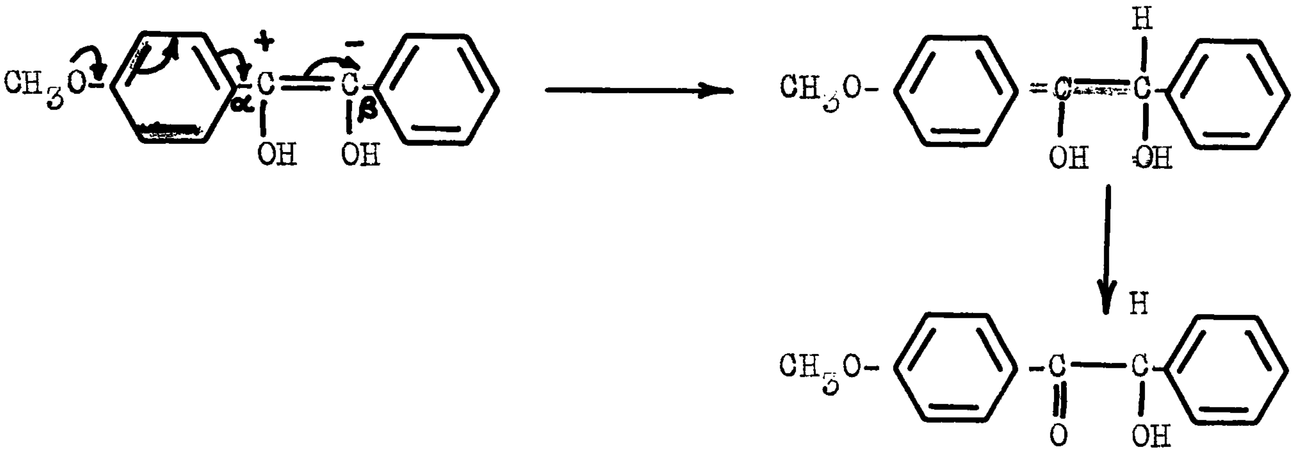
(XCII)



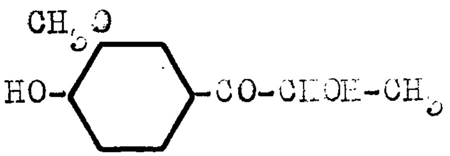
(XCIII)

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

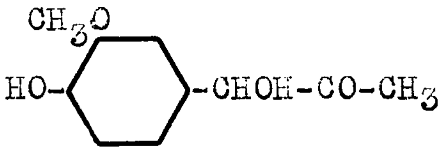
This inversion of stability due to the presence of a para-methoxyl group is also evident in the work of Kohler and co-workers (138, 139). Thus while $C_6H_5\text{-CHOH-CO-CH}_2\text{-}C_6H_5$ appears to be the stable form of the unsubstituted benzoin, carbon followed by ejection of a proton from the alpha-hydroxyl, the stable benzanisoin resulting:



The ethanolysis of spruce and maple woods yields α-ethoxypropiovanillone which is presumably derived from α-hydroxypropiovanillone (XXVI), or more likely from condensation products formed by linkage through its aliphatic hydroxyl group. α-Hydroxypropiovanillone contains the ketol grouping as do the compounds discussed above, and, therefore, the presence of the ethoxy derivative or its dismutation isomer (XCVII).



(XXVI)

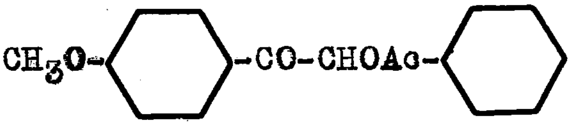


(XCVII)

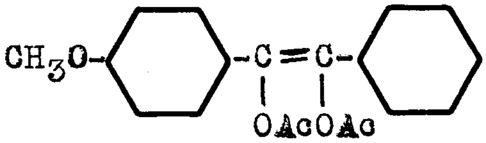
in the ethanolysis reactive mixture is to be expected. The author has shown this to be the case.

$C_6H_5-CO-CHOH-CH_2-C_6H_5$, 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

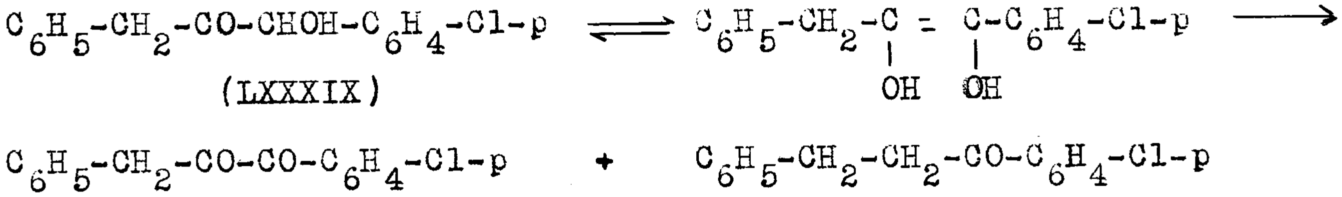
(3) Oxidation and Reduction Reactions of Keto-alcohols

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 consist in 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.



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

$$C_6H_5\text{-}CH_2\text{-}CO\text{-}CO\text{-}C_6H_4\text{-}Cl\text{-}p \quad + \quad C_6H_5\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}C_6H_4\text{-}Cl\text{-}p$$

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.

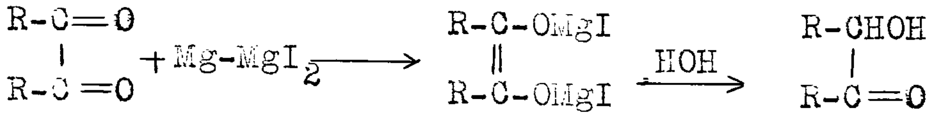
(4) Properties of 1,2-Diketones

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.

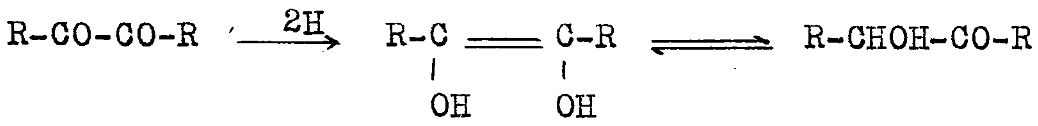
The mechanism of the reaction has been proven beyond doubt to consist of the formation of the magnesium glycolate (XCVIII) followed by hydrolysis and rearrangement.



(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 desoxybenxoins. 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 (158) (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.



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

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).

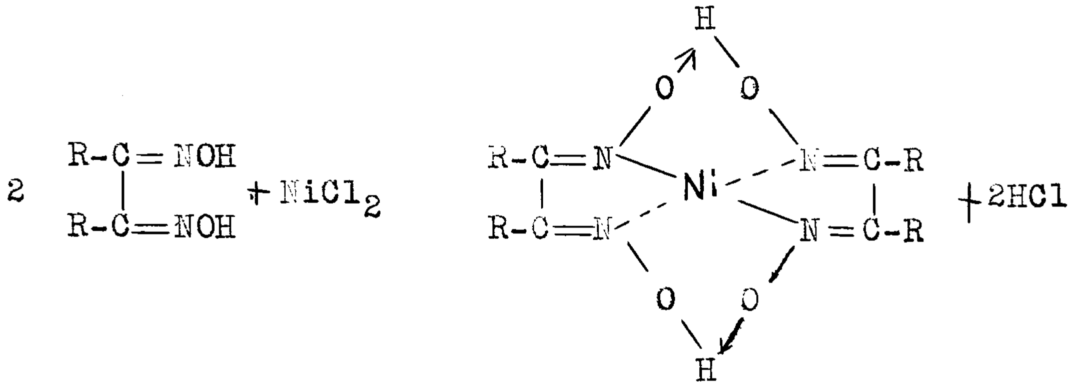
The ease of reduction of 1,2-diketones is of great significance for it indicates a close relationship among the ethanolysis products of wood, e.g. between vanilloyl methyl ketone, α-hydroxypropiovanillone, and (4-hydroxy-3-methoxyphenyl)-

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

A characteristic test for 1,2-diketones is cleavage by alkaline hydrogen peroxide which results in the formation of two moles of monobasic acid per mole of diketone (160) (161). Cyclic diketones yield, when cleaved by alkaline hydrogen peroxide, 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.

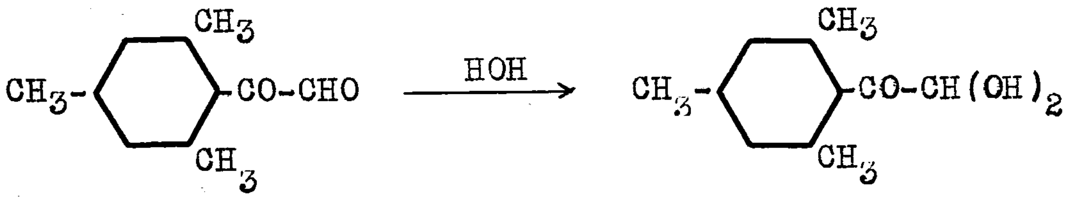
A very important property of 1,2-diketones is their ability to form dioximes in acid or alkali media, which in the presence of nickel, cobalt or iron salts produce insoluble glyoximates (XCIX). This property has been made use of in the separation of 1,2-diketones from monocarbonyl and other compounds. 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-momoximes. The apparent rearrangement is probably a matter of relative stability.

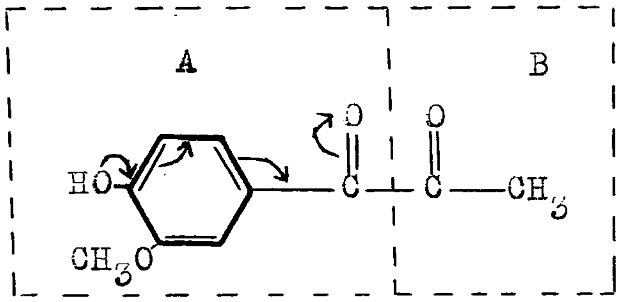
The two carbonyl groups of symmetrical 1,2-diketones are of equal reactivity, the symmetrical aliphatic diketones being more reactive than the aromatic. Unsymmetrical diketones such as the aryl-alkyl compounds, contain one highly reactive carbonyl, and one comparatively unreactive. It is an accepted fact that substituted aryl radicals 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 treatment. 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:

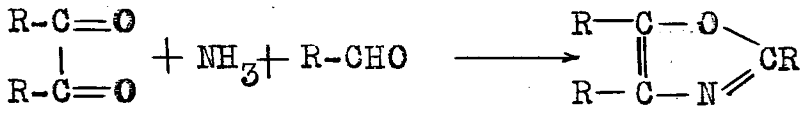


The hydroxyl group has a strong tendency towards electron release, giving rise to an electronic strain in the aromatic nucleus. The para-carbonyl together with the phenolic-hydroxyl form a neutralized system (fragment A) similar to that in a carboxyl group. The reactivity of the para-carbonyl is thus 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).

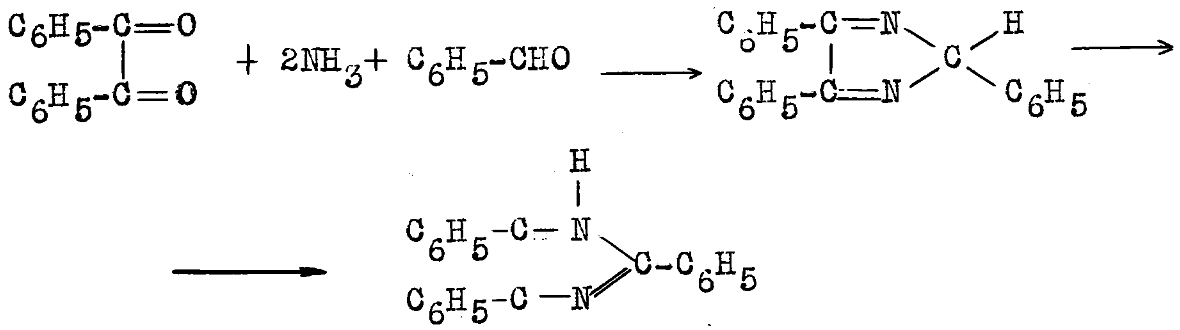
The course of the reaction is indicated by the following equations:

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



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.

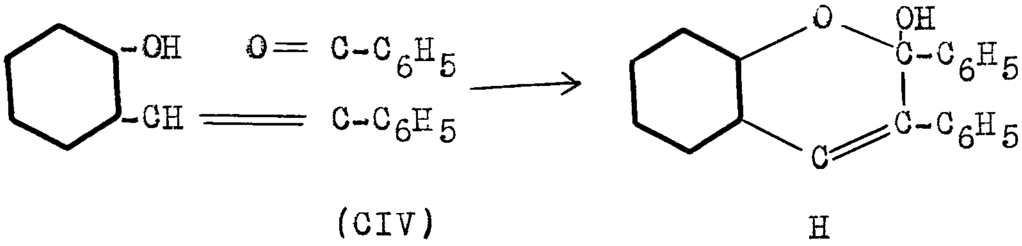
(5) Properties of Desoxybenzoins

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.

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

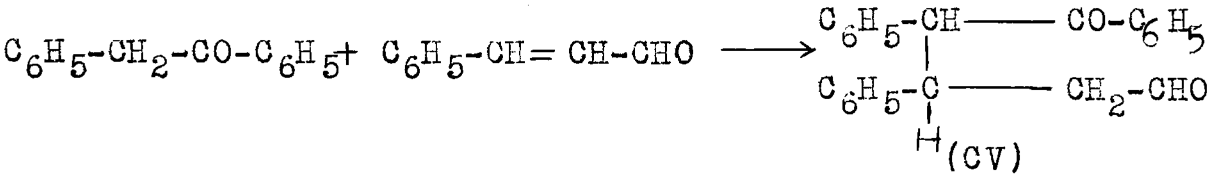
The methylenic group is an active functional group and undergoes the characteristic oxidation and condensation reactions. Specific oxidizing agents such as selenium dioxide (173, 174), nitrous acid (175) and nitrogen tetroxide (176) convert desoxybenzoins to 1,2-diketones. With nitrous acid and nitrogen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



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

(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.

II. Review of Experimental Results

A. Nature of the Problem

The problem assigned to the writer was that of a re-investigation of the ethanolysis of spruce wood with a view to (a) increasing the yield of ethanolysis products; and (b) isolating and identifying hitherto unknown constituents present in the ethanolysis mixture. A parallel project using an angiosperm, namely maple wood, instead of a gymnosperm (spruce), was commenced simultaneously in these laboratories by Mr. M. Kulka. The numerous investigations of Hibbert and co-workers over the last six years have clarified the question of lignin structure to a remarkable degree, inasmuch as prior to that period there was relatively little evidence in support of the essentially aromatic nature of lignin, and the assumption of the presence of a three carbon side chain attached to a guaiacyl nucleus had no actual experimental basis.

The isolation in these laboratories of aromatic products of the types C_6-C (vanillin, syringaldehyde); C_6-C_2 (acetovanillone, acetosyringone); and C_6-C-C-C (α-ethoxypropiovanillone and syringone; vanilloyl- and syringoyl methyl ketone) from the ethanolysis products of wood is discussed in considerable detail in the historical introduction (pp. 8 - 11) and represents, according to von Erdtman (3), "one of the most important results of lignin research in recent years".

Prior to the commencement of the writer's project, only two C_6-C-C-C units (α-ethoxypropiovanillone and vanilloyl methyl ketone) had been isolated from spruce ethanolysis products (41a, 43a) to the extent of ca. 2% (combined yields) whereas the yield from maple was much higher (ca. 4%) (41b, 42).

On the other hand, the actual amounts of the C_6-C units (vanillin, syringaldehyde) isolated by alkaline oxidation of woods (27) are considerably higher as shown in Table I.

Table I

Alkaline Nitrobenzene Oxidation of Plant Materials

Plant Material	Aldehyde as % of initial Klason lignin
Angiosperms	
Maple	42
Aspen	44
Ash	49
Elm	41
Gymnosperms	
Spruce	24
White Pine	20

Included in same are a few recent values for various woods obtained recently by Mr. R. Creighton in these laboratories. This work (concerned with some 40 species of wood) has established beyond doubt the presence of syringaldehyde in all angiosperms and its absence in all gymnosperms. Although this latter evidence is very important in establishing the aromatic nature of lignin, it provides no data on the structure of the side chains, the only known source for same at the present time being the ethanolysis products under investigation in these laboratories.

Recent developments on the hydrogenation of wood at high pressures (30, 31) have shown that both in the case of spruce and maple, identified cyclohexane derivatives, having three-carbon side chains, are present to the extent of at least thirty-six to forty per cent (based on the original Klason lignin) thus providing definite proof of the presence of at least that amount of lignin aromatic components of the type R-C-C-C (where R is guaiacyl or syringyl).

The Relationship of Ethanolysis Products to Native Lignin

In order to place the question of the structure of "native lignin" (that is, lignin as present in an unchanged condition in the plant prior to extraction) on a sounder basis, it is important that (i) its degradation or transformation products((a) ethanolysis, (b) alkaline oxidation, and (c) hydrogenation) should be isolable in higher yields and (ii) their structures established either as fragments of original building units of the native unchanged lignin or as stabilized end products, the latter originating (as shown by well-established experimental proof) from other more labile and reactive products forming and representing the true lignin progenitors (70). The importance of this latter feature of lignin research has been commented upon recently by von Erdtman (5), who points out that the wood ethanolysis products isolated by Hibbert and co-workers possibly represent not the true lignin progenitors, but stabilized end products of the same — a point of view now supported by Hibbert.

Based on purely theoretical views, Hibbert has suggested (Historical Introduction, pp. 22-25) the existence in higher plants of a respiratory system of plant hydrogen transportation catalysts, analogous to that of Szent-Györgyi for the animal cell.

	Animal System (Szent-Gyorgyi)		Plant System (Hibbert)
A	$HOOC-CH_2-CO-COOH$ (Oxal acetic acid)	A'	$R-CH_2-CO-CH_2OH$
	$+2H \; \downarrow\uparrow \; -2H$		$+2H \; \downarrow\uparrow \; -2H$
B	$HOOC-CH_2-CH(OH)-COOH$ (Malic acid)	B'	$R-CH_2-CH(OH)-CH_2OH$
	$\downarrow \; -H_2O$		$\downarrow \; -H_2O$
C	$HOOC-CH=CH-COOH$ (Fumaric acid)	C'	$R-CH=CH-CH_2OH$ (Coniferyl alcohol)
	$+2H \; \downarrow\uparrow \; -2H$		$+2H \; \downarrow\uparrow \; -2H$
D	$HOOC-CH_2-CH_2-COOH$ (Succinic acid)	D'	$R-CH_2-CH_2-CH_2OH$

Only one of the suggested members of the Hibbert system, namely coniferyl alcohol (7) is known to exist in plants and is present only in the early stages of plant growth. It apparently is present in all plants and disappears in the later stages of cell wall development. Recent work by Mr. J. Bower in these laboratories on the hydrogenation of spruce tips (buds) in various stages of development has indicated that C_6-C-C-C units do not appear in appreciable quantities until after the fourth month of growth (181).

In spite of careful work by numerous investigators, it has not been possible, as yet, to isolate any of the three remaining aromatic components A', B' or D', either as such or in the form of simple polymers isolable by ethanolysis or other reactions. There are presumably two main reasons for this lack of success: (i) the subjection of these assumed lignin respiratory catalysts to an oxidizing environment in the later stages of cell activity, especially during the post mortal period, and (ii) the transformation, due to their outstanding reactivity under the environmental conditions existing during the post mortal stage of plant activity or especially in the process of lignin isolation, into more stabilized end products either of a monomeric or, more frequently, polymeric (possibly essentially dimeric) type.

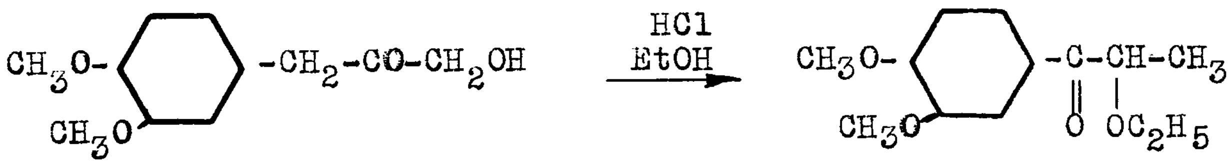
These assumptions were made by Hibbert in an earlier review (4) on lignin structure in which it was postulated that in all probability substances of the general type embodied in the first member of the series, viz, $R\text{-}CH_2\text{-}COCH_2OH$ (where R is guaiacyl or syringyl), undergo, under the influence of mild chemical reagents, an allyl shift as shown below:

$$(1)\ R\text{-}CH_2\text{-}CO\text{-}CH_2OH \longrightarrow (2)\ R\text{-}CH{=}C(OH)\text{-}CH_2OH \longrightarrow$$

$$(3)\ R\text{-}CH(OH)\text{-}C(OH){=}CH_2 \longrightarrow (4)\ R\text{-}CH(OH)\text{-}CO\text{-}CH_3 \rightleftharpoons$$

$$(5)\ R\text{-}C(OH){=}C(OH)\text{-}CH_3 \rightleftharpoons (6)\ R\text{-}CO\text{-}CH(OH)\text{-}CH_3$$

Recently, in these laboratories, this has been shown by Mr. A. Eastham (70) actually to be the case. He finds that when the veratryl analogue of (1) is warmed with ethanolic hydrogen chloride it is converted in high yield into α-ethoxy propioveratrone (B), which is the analogue of the ethyl ether of (6).



(A) (B)

Veratryl hydroxymethyl ketone (A) apparently possesses a strongly enolic character; the enol form, it is important to observe, being no other than methylated α-oxyconiferyl alcohol. The unmethylated keto form represents the first member of the Hibbert system of aromatic-type plant respiratory catalysts and it appears highly probable that their non-isolation, hitherto, is due to their remarkable reactivity and ease of transformation into stabilized end products such as α-hydroxypropiovanillone (isolated as the ethyl ether). This is a necessary corollary of their postulation as hydrogen transportation catalysts since there cannot exist any large free energy increments between the four different members.

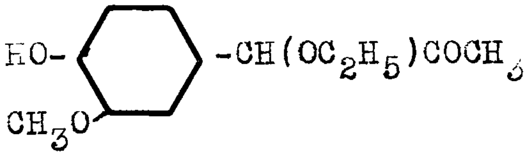
In view of the above, it seemed reasonable to expect that in the event that the dismutation changes postulated between (4), (5) and (6) (above) should occur during the ethanolysis procedure at a rate slow enough to permit etherification of the presumably primarily formed dismutation isomer of α-ethoxypropiovanillone.

$$R\text{-}CH(OH)\text{-}CO\text{-}CH_3 \longrightarrow R\text{-}CH(OC_2H_5)\text{-}CO\text{-}CH_3$$

(C)

the corresponding ether (C) should be present in the ethanolysis products, and the author has succeeded in proving this to be the case. Its presence, or that of a related isomer had been indicated in previous work in this field carried out by A.S. MacInnes (182). He showed that (i) α-ethoxypropiovanillone was present in all of the ethanolysis products obtained from a series of angiosperms and gymnosperms (184), (ii) that the alkali-soluble portion of the water-soluble lignin fraction isolated from the spruce wood ethanolysis mixture contained material other than α-ethoxypropiovanillone and (iii) that the latter could be separated from the balance of the phenol fraction as its ether-insoluble ammonium salt by treating an anhydrous ether solution of said fraction with dry gaseous ammonia.

The author has succeeded in showing that a portion (39%) of the ether-ammonia-soluble part of the phenol fraction consists of a compound, viz. 1-(4-hydroxy-5-methoxyphenol)-1-ethoxypropanone-2,



not isolated heretofore from the ethanolysis products of spruce wood.

B. Improvements in the Ethanolysis Procedure

(1) Status of the procedure prior to the author's investigation

The method used for the extraction of lignin from spruce and other woods has undergone numerous modifications by various workers in these laboratories (41a, 41b, 43a, 110) and at the time the author commenced the re-investigation of spruce ethanolysis products the procedure consisted of the following steps (Fig. 1).

(a) Preliminary extraction of the wood meal with organic solvents and then with water to remove fats, waxes, tannins, resins and soluble carbohydrates, and drying in a vacuum oven at 50°.

(b) Refluxing the wood meal with anhydrous (or approximately so) ethanolic hydrogen chloride (2g./100 cc.) in an inert atmosphere for 48 hours and filtering off the residual wood meal.

(c) Concentration of the reaction liquor followed by precipitation into a large volume of water and filtration of the ethanol lignin.

(d) Concentration of the filtrate under reduced pressure followed by extraction of the concentrate with benzene to obtain the crude water-soluble ethanolysis oils.

(e) Separation of the crude water-soluble ethanolysis oil into bisulfite-, bicarbonate-, alkali-soluble and "neutral" fractions.

(2) Separation of adsorbed oil

Previously, the removal of oil adsorbed on the precipitated ethanol lignin had been effected by shaking it several times with benzene (110). This procedure had the inherent disadvantage that, in addition to the adsorbed water-soluble oil, some benzene-soluble, water-insoluble lignin also went into solution and later, because of its water-insolubility, this separated out during the subsequent fractionation into solubility groups. Elimination of this difficulty has been effected by dissolving the ethanol lignin in acetone and reprecipitating into a large volume of water (183). Two such reprecipitations were found to be sufficient.

A more complete removal of amorphous ethanol lignin and low molecular weight lignin ethanolysis oils from the ethanolysis wood residue was obtained by extracting the residue with ethanol in a Soxhlet extractor after the usual washing carried out in the Büchner funnel.

(3) Separation of lignin ethanolysis oils from lignin tars

The concentration of the aqueous filtrate (Fig, 2, page-108) was generally accompanied by the separation of a considerable amount of tar. After unsuccessful attempts to reduce or eliminate this tar formation by keeping the concentration temperature below 45°, it appeared that it either was formed as a result of the prolonged heating at 45°, or was already present in the ethanolysis products and appeared in the concentration step only because of its low water-solubility. For these reasons, and the fact that the aqueous distillate was found to contain an alkoxyl- and methoxyl-bearing oil (0.6% of the initial Klason lignin), direct extraction of the precipitation liquors without preliminary concentration was adopted as a new standard procedure and applied successfully to the ethanolysis products of maple wood (105).

In spite of the above precautions, some tarry material still continued to appear with the crude ethanolysis oils and caused considerable difficulty and loss in the fractionation scheme (Figure 1).

A modification of the petroleum ether precipitation technique (183) was adopted in order that purer ethanolysis oils could be obtained without resorting to distillation since this generally resulted in partial charring of the tar and the oil. This procedure consisted in pouring an acetone solution of the crude ethanolysis oils into a large volume of petroleum ether, resulting in precipitation of the tarry material the water soluble lignin oils remaining in solution. Several such consecutive precipitations were needed to separate the tar completely from the non-tarry oil which dissolved in the supernatant liquids. These latter were combined, the solvent removed, leaving the clear, light-yellow colored oil. The fractionation of this into solubility groups was entirely unaccompanied by the usual formation of amorphous and tarry material.

(4) Fractionation into solubility groups

In the old fractionation procedure no cognizance had been taken of the fact that the water-solubility of the ethanolysis oil might cause an overlapping of the various solubility groups, so that, the bisulfite-soluble fraction might well contain portions of the true bicarbonate-soluble, alkali-soluble and "neutral" fractions. Back extraction with benzene of the combined extracts of each group eliminated this difficulty. However, in the case of the alkali-soluble fraction, it was found that the formation of petroleum ether-insoluble tar (presumably an alkaline polymerization product) could be kept at a minimum by acidifying each alkaline extract immediately after it was separated. At the end of the alkaline extraction, the acidified extracts were combined, made alkaline and back extracted with benzene.

In spite of all the above precautions, some polymerization was found to take place during the course of the fractionation into solubility groups, and in order to have purer material for the subsequent investigations, the tar was again separated from each fraction of the oils by petroleum ether precipitations.

The modified ethanolysis procedure, incorporating the author's improvements, is summarized in Fig. 2.

As a consequence of the above alterations in the ethanolysis procedure a considerably improved overall yield was obtained, as well as more sharply defined end-products. The yields (expressed as per cent of the initial Klason lignin without corrections for the ethoxyl content) obtained by the writer from the ethanolysis of old spruce wood and fresh spruce sapwood are given in Table II together with the yields obtained by earlier workers (186).

Table II

Yields[*] from the Ethanolysis of Wood

Ethanolysis products	Earlier results on old spruce wood	Author's results	
		Old spruce wood	New spruce sapwood
Residual wood meal	64.0	58.7	57.5
Ethanol lignin	16.3[x]	31.9	29.2
Tar separating during concentration	-	3.5	7.8
Oil separated from aqueous distillate (Fig. 2)	-	-	0.6
Crude water-soluble oil	11.5	12.1	14.0
Total recovery	91.8	106.2	109.1

[x] This value represents the sum of the ethanol lignin and "benzene shakings".

[*] Expressed as per cent of the initial Klason lignin without corrections for the ethoxyl content.

Because of the differences in the experimental procedures, it is difficult to make an exact comparison, but in general the author's procedure results in a greater overall yield. The new procedure, the details of which are given in the experimental section, embodies the following main changes and advantages:

(i) A higher yield of ethanol lignin is obtained, due mainly to the Soxhlet extraction of the wood residue.

(ii) Precipitation of the crude water soluble oil into petroleum ether affords a means of separating the tar from the oil and hence facilitates the fractionation procedure.

(iii) The modified fractionation procedure results in a sharper separation into solubility groups with a minimum of polymerization.

C. Re-investigation of the Alkali-Soluble Fraction of the Water-Soluble Spruce Lignin Fraction from Ethanolysis of Spruce Wood

(1) Isolation of vanillic acid

Prior to the development of the author's new fractionation procedure involving acidification of the alkaline extract of the water-soluble spruce ethanolysis oils after separation, it had been found that a crystalline material separated from both the benzene back-extraction solutions ((M) Fig.3, p.114) and also from the petroleum ether precipitation liquor on concentration ((C) Fig.4, p.118). On recrystallization from water to constant melting point this product proved to be vanillic acid (M.P. and mixed M.P.) and amounted in one instance to as much as 4.3% of the total alkali-soluble oils.

Inasmuch as the benzene extract of water-soluble lignin ethanolysis products had been extracted with sodium bicarbonate solution, (in which vanillic acid is soluble) prior to treatment with alkali, it appeared probable that vanillic acid was being formed as a degradation product of one of the constituents of the alkali-soluble fraction. It was found by A. MacInnes (182) that a phenol differing in structure from α-ethoxypropiovanillone was present and this unknown product has been shown by the present author to yield appreciable quantities of vanillic acid when shaken with cold dilute alkali. To prevent this cleavage, and also to reduce any tendency towards intermolecular condensation of the low molecular weight fraction of the oils, the practice of acidifying the alkaline extract immediately after separation was adopted. As a consequence, the yield of vanillic acid decreased to about 0.1% of the alkali-soluble oils in contrast to over 4% obtained previously by the old procedure. A corresponding increase in the yield or the phenol precursor was obtained and this is discussed in some detail in a later section.

(2) Improved method for isolating α-ethoxypropiovanillone

As mentioned above, the occurrence along with α-ethoxypropiovanillone of another phenolic product had already been established but no completely satisfactory method has been found for its separation and identification. The author, after considerable experimentation with the preliminary process developed by MacInnes (182), was able to devise a much more satisfactory procedure (Fig. 3, page 114).

This separation is based on the fact that α-ethoxypropiovanillone can be removed from the accompanying phenolic constituents by conversion into its ammonium salt by the action of anhydrous gaseous ammonia on an anhydrous ethyl ether solution of the purified alkali soluble fraction. On acidification this ammonium salt yielded an oil which was shown to be largely α-ethoxypropiovanillone by methylation to α-ethoxypropioveratrone using either diazomethane or dimethyl-sulfate. The large losses occurring on methylation of the phenol and in the purification of its methyl ether by recrystallization led to a search for a more suitable means of establishing the complete identity of the ammonium salt. Decomposition of an aqueous solution of the latter with dilute acid and back-extraction with benzene (which also is attended by appreciable losses) gave an oil which, upon fractional distillation, yielded a constant boiling fraction (n^{25} 1.5510) in an amount corresponding to seventy-five percent of the ammonium salt and equivalent to fifty percent of the total petroleum ether soluble phenol fraction. The ammonium salt thus consists essentially of α-ethoxypropiovanillone although it is probably accompanied by small amounts of other phenolic constituents.

(c) Isolation of a new phenolic constituent in the form of its semicarbazone

The yellowish colored viscous oil (I, Fig.4, p.118) left after evaporation of the supernatant liquors from the ammonium salt precipitation yielded a pale yellow limpid oil (n^{25}_{D} 1.5093) on distillation which could not be induced to crystallize. The semicarbazone, however, was readily obtainable in good yield and after suitable purification, the pure product melted at 171-172°.

Its analyses corresponded to the theoretical values for a semicarbazone of α-ethoxypropiovanillone. Inasmuch as the melting point of the semicarbazone of α-ethoxypropiovanillone was found to be somewhat indefinite, it became necessary to reconvert the unknown product into the pure original phenolic material for purposes of comparison with pure synthetic α-ethoxypropiovanillone.

(4) Purification of the newly isolated phenolic constituent.

This was accomplished by hydrolysis of the semicarbazone with dilute sulfuric acid and fractionation of the recovered phenol. A high yield of an almost colorless oil, giving a semicarbazone of identical M.P., in over 90% yield, indicated no degradation had taken place during the reconversion stage. Its refractive index (n_D^{25} 1.5241) was quite different from that of a purified sample of α-ethoxypropiovanillone (n_D^{25} 1.5511).

The purity of the new phenol was further shown by careful fractionation, in the highly efficient Cooke-Bower column, of the original phenol product left after removal of α-ethoxypropiovanillone. About a third of the distillable oils, comprising the first fraction, had almost identical refractive indices, namely 1.5240 at 25°.

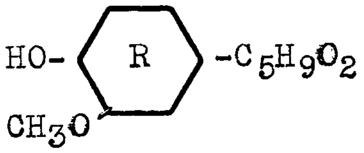
(5) Analysis and molecular weight of the new phenolic constituent and of its semicarbazone

Results of the analyses of the free phenol and of its semicarbazone (Experimental Part, page 128) indicated their respective empirical formulae as $C_{12}H_{16}O_4$ and $C_{13}H_{19}O_4N_3$ which, as pointed out above, are also those of the isomeric form of α-ethoxypropiovanillone and its semicarbazone.

In view of this a thorough chemical investigation was necessary to establish the structure of the unknown phenol constituent.

(6) Degradation of the new phenolic constituent to vanillic acid as proof of the presence of the vanill nucleus

The presence of the 3-methoxy-4-hydroxyphenol (vanill) nucleus was confirmed by conversion of the unknown semicarbazone into vanillic acid by treatment with dilute aqueous sodium hydroxide. In spite of the low yield (ca. 6%) the isolation of the pure acid definitely established the presence of the vanill nucleus in the original phenolic product. Its empirical formula $C_{12}H_{16}O_4$ can therefore be expanded to



leaving only the structure of the side chain for elucidation.

(7) Structure of the side chain

Consideration of the experimental data shows the presence of an ethoxyl and a carbonyl group in the side chain indicating the structure as R-$C_2H_4(OC_2H_5)(CO)$. (R-vanill).

Its insolubility in aqueous bisulfite solution indicates that the compound is ketonic, rather than aldehydic, and further proof of this is given in the section on "chromic acid oxidation".

The presence of a carbonyl group precludes the presence of an isopropyl side chain, thus leaving for consideration only four possible structures:

I. $R\text{-}CO\text{-}CH(OC_2H_5)\text{-}CH_3$
α-ethoxypropiovanillone

II. $R\text{-}CO\text{-}CH_2\text{-}CH_2OC_2H_5$
β-ethoxypropiovanillone

III. $R\text{-}CH_2\text{-}CO\text{-}CH_2OC_2H_5$
1-vanill-3-ethoxypropanone-2

IV. $R\text{-}CH(OC_2H_5)\text{-}CO\text{-}CH_3$
1-vanill-1-ethoxypropanone-2

A comparison of their refractive indices measured at 50°, and shown below, eliminates the known structures I and II whose properties have been established previously (41a, 192).

	n_D^{50}
(I) α-ethoxypropiovanillone	1.5390
(II) β-ethoxypropiovanillone	1.5420
New Phenolic Constituent	1.5160

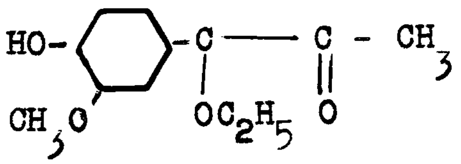
A further proof of the non-identity of the new phenol with I (α-ethoxypropiovanillone) and II (β-ethoxypropiovanillone) is to be found in the fact that the ultraviolet absorbtion spectra of the two latter show a maximum at ca. 980f. (unpublished work of Mr. R. F. Patterson of these laboratories) characteristic of a carbonyl group in conjugation with the guaiacyl nucleus while this band is completely absent in the spectrum of the new phenol.

Elimination of structures I and II leaves only III and IV calling for consideration.

(8) <u>Oxidation with chromic acid</u>

Treatment with chromic acid of organic products of the type in question (i.e., containing end methyl groups) gives an equivalent of acetic acid for each methyl group present. Thus α-ethoxypropiovanillone gives two equivalents of acetic acid and β-ethoxypropiovanillone only one (190). A similar oxidation of the semicarbazone of the new phenolic constituent yielded two equivalents of acetic acid, indicating the structure of the original phenol as IV (acetic acid = 2 equivalents) and not III (acetic acid = 1 equivalent). This evidence also serves to eliminate any straight chain aldehyde structure as this could yield only <u>one</u> equivalent of acetic acid. The fact that the semicarbazone grouping does not interfere with the chromic acid oxidation technique was shown by the absence of acetic acid formation in the oxidation of vanillin semicarbazone by chromic acid under the same conditions.

The structure of the new phenolic constituent is thus 1-vanill-1-ethoxypropanone-2.



The absence of a positive iodoform test, considered characteristic of most methyl ketones (191) is possibly to be attributed to the rapid degradation caused by warm alkali. Vanilloyl methyl ketone also fails to give a positive test (43a).

(9) Proof of structure by direct synthesis

Final proof of the structure of the unknown phenol constituent as 1-vanill-1-ethoxypropanone-2 was furnished by direct comparison (mixed melting point) of the semicarbazones of the natural product and of the compound itself synthesized in these laboratories (see Experimental Part, page 131).

(10) Improvement in yields of identifiable products from the alkali soluble fraction of water-soluble spruce ethanol lignin.

Initial work was carried out on the products obtained from two duplicate ethanolyses of long-stored spruce wood, while in the third experiment freshly-cut material was used.

New method of isolation with improved yield

The yield of 1-vanill-1-ethoxypropanone-2 isolated by (a) fractional distillation of the oil left after removal of α-ethoxypropioveratrone amounted to 25.8%; whereas (b) a primary conversion into semicarbazone followed by purification, regeneration of the free ketone, and distillation of same increased the yield to 27.2%. The latter amount is a minimum value since no attempt was made to recover additional quantities from either the reaction- or mother liquors.

As a result of the improved ethanolysis procedure and recovery technique an accurate estimation of the yields of pure ethanolysis products present in the alkali soluble fraction is now possible and these values are given in Table IV. The yield of 1-vanill-1-ethoxypropanone-2 was obtained by calculating the amount of phenol equivalent to its pure semicarbazone.

Table III

Yields of Products Isolated from the Alkali-Soluble Fraction of the Water-Soluble Spruce Ethanolysis Oils

	Percent of the Alkali Soluble Fraction	Percent of the Initial Klason Lignin
α-Ethoxypropiovanillone	45.9	2.0
1-Vanill-1-ethoxypropanone-2	11.7	0.6
Vanillic acid	0.1	0.005

(11) Significance of the presence of 1-vanill-1-ethoxypropanone-2 in the spruce ethanolysis mixture

Isolation of 1-vanill-1-ethoxypropanone-2, the new isomer of α-ethoxypropiovanillone, would seem to provide additional support for Hibbert's theory of lignin formation as arising from members of a group of plant hydrogen-transporting respiratory catalysts, the first member of which, $R-CH_2-CO-CH_2OH$ (R - guaiacyl or syringyl), corresponds to oxalacetic acid in the Szent-Györgyi system of animal cell hydrogen transportation catalysts. As pointed out by Hibbert, such a product as 1-vanill-3-hydroxypropanone-2 (in the form of its phenol methyl ether) is known to undergo ready transformation in the presence of ethanol-HCl into α-ethoxypropiovanillone (70). This reaction apparently involves a series of changes consisting of a primary allyl shift to a 1:2 ketol and dismutation of the latter to α-hydroxypropiovanillone:

(1) $R-CH_2-CO-CH_2OH \longrightarrow$ (2) $R-CH=C(OH)-CH_2OH \longrightarrow$

(3) $R-CHOH-C(OH)=CH_2 \longrightarrow$ (4) $R-CHOH-CO-CH_3 \rightleftarrows$

(5) $R-C(OH)=C(OH)-CH_3 \rightleftarrows$ (6) $R-CO-CHOH-CH_3$

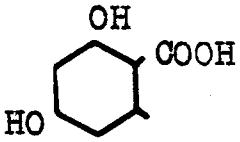
The presence of the ethyl ethers of the intermediary dismutation forms (4) and (6) in the spruce ethanolysis products thus provides valuable support for the theory in question since, in all probability, they represent stabilized end products of 1-vanill-3-hydroxypropanone-2, the latter being the first member of the new Hibbert series of plant respiratory catalysts.

D. Reinvestigation of the Bisulfite-Soluble Fraction of the Water-Soluble Ethanolysis Products

(1) Introduction

In the historical introduction reference was made to the close similarity of the side chains of the C_6-C-C-C- lignin building units isolated from the ethanolysis products of spruce and maple woods to those isolated by Raistrick and co-workers (see page 41) from aqueous solutions containing the metabolic products formed by the action of molds of the Penicillium family on aqueous glucose solutions (glucose representing the sole organic substrate).

Raistrick and co-workers

R = 

(A) $R-CO-CO-CH_3$

(B) $R-CH(OH)-CO-CH_3$

(C) $R-CH_2-CO-CH_3$

Hibbert and co-workers (78)

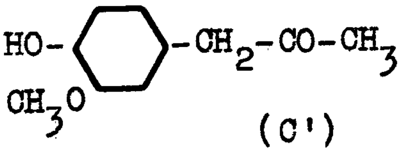
R' = vanill or syringyl

(A') $R'-CO-CO-CH_3$

(B') $R'-CH(OH)-CO-CH_3$

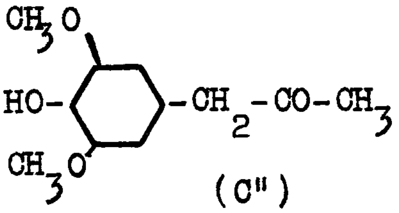
(C') $R'-CH_2-CO-CH_3$

In view of the striking parallelism between the first two members of each series (A,A' and B,B') it seemed not unlikely that lignin derivatives (C') having side chains identical with those of (C) might also be present in the still unidentified portion of the bisulfite-soluble fraction of the water-soluble spruce and maple ethanolysis products. An extended re-investigation of the maple bisulfite fraction just concluded by Kulka in these laboratories has, in fact, shown the presence therein of two such new products, namely vanillpropanone-2 (C') and syringpropanone-2 (C")



(C')

and



(C")

This led the writer to carry out a similar investigation on the corresponding fraction of spruce water-soluble ethanolysis oils, with the resulting isolation and identification of vanillpropanone-2.

It was also possible to establish the presence of vanillin as a definite component of the bisulfite-soluble fraction, a point of interest in view of the conflicting results previously obtained by various workers in these laboratories (42). Discrepancies in these earlier results apparently were due to the losses occurring during the lengthy operations involved in concentration of the aqueous precipitation liquors as well as also the less efficient methods used for the final isolation of the vanillin.

(2) Isolation and identification of vanilloyl methyl ketone, vanillin and vanillpropanone-2 from the bisulfite fraction of the spruce water-soluble ethanolysis reaction mixture

(a) Separation of vanilloyl methyl ketone

The procedure for the separation of the diketone (vanilloyl methyl ketone) was that developed previously by Kulka for maple wood (43b), and consisted (Fig.5, p.133) in treating an aqueous solution of the petroleum-ether soluble, bisulfite-soluble spruce ethanolysis oils with hydroxylamine sulfate and nickel chloride to form the insoluble nickel salt of the dioxime. Hydrolysis of the latter with 7N sulfuric acid, and extraction with benzene, yielded the free diketone, which on vacuum distillation gave a large fraction of a low-boiling yellowish-colored oil

(B.P. 160°/0.4 mm.) and a small amount (5%) of a high-boiling, high-melting material which was not identified. The low-boiling distillate solidified on standing overnight and was purified by crystallization from water. It was shown to be vanilloyl methyl ketone by melting point and mixed melting point (M.P. 68-69°) determinations. The aqueous mother liquors from the recrystallizations of the diketone were reworked and yielded additional quantities of this substance as well as a small amount of product which melted over a wide range (65 to 122°) and which has not as yet been purified and identified. Since the yield of pure diketone from the crude oil obtained from hydrolysis of the nickel salt was only 45%, and an appreciable amount of higher melting products was obtained, it would seem that that portion of the bisulfite fraction separated as the nickel salt contains a constituent, or constituents, other than vanilloyl methyl ketone.

(b) Separation of vanillin and vanillpropanone-2

The oil remaining after separation of the nickel salt should, by analogy with Kulka's products, contain both vanillin and the oxime of vanillpropanone-2, and use of his method (193) has permitted of their isolation, separation and identification. A benzene solution of the residual oil left after removal of the nickel salts was extracted with sodium bisulfite to remove the free vanillin and leave the oximes in solution. Evaporation of the benzene and hydrolysis of the residual oil with 7N sulfuric acid yielded the free ketone which was extracted from the reaction mixture with benzene. Evaporation of the benzene left an oil which, upon treatment with thiosemicarbazide, gave crystalline vanillpropanone-2-thiosemicarbazone, identified by analysis and melting-point and mixed melting point determinations.

The isolated vanillin proved to be an impure product requiring a number of recrystallizations so that only a low yield of pure product was obtained.

The overall combined yield of purified vanillin and vanillpropanone-2 amounted to only 7.5% of the total oil remaining after separation of the nickel salts of vanilloyl methyl ketone.

(c) Separation of vanillin and vanillpropanone-2 by direct hydrolysis followed by fractional distillation

In view of the fact that the above separation, based on the sodium bisulfite solubility of vanillin, resulted in a low recovery of both vanillin and vanillpropanone-2, the procedure was modified as follows:

Following the removal of the nickel salt of vanilloyl methyl ketone the residual oil containing oximes and free vanillin, was hydrolyzed with 7N sulfuric acid. The total carbonyl derivatives were then recovered by extraction with benzene, the solvent removed and the residual oil submitted to fractional distillation, whereby a series of low-boiling crystalline fractions and higher-boiling oils were obtained. Recrystallization of the former fractions from water yielded pure vanillin, while the latter gave crystalline vanillpropanone-2-thiosemicarbazone on treatment with thiosemicarbazide. The yields obtained by the two methods of recovery, calculated on the weight of carbonyl-containing crude oil (C) left after removal of the diketone are shown below:

	Percentage Yields Obtained	
	(a) By extraction	(b) By fractional distillation
Vanillin	6.15	9.95
Vanillpropanone-2	1.4	4.97

The second method thus gave much better yields and was adopted for the subsequent separations.

(d) Determination of the maximum yields of vanillin and vanillpropanone-2 from spruce sapwood ethanolysis products

The details of the separation finally used are given in the Experimental Part pages 136-40, (Fig.6, p.134) and the yields of pure crystalline products are summarized below:

Table IV

Yields of Pure Compounds from the Bisulfite-Soluble Fraction of Spruce Sapwood Ethanolysis Oils

Yields	Calculated on percentage of		
	Original Klason lignin	Total petroleum ether-soluble bisulfite fraction	Total residual oil left after separation of the diketone and prior to hydrolysis
Vanilloyl methyl ketone	0.20	13.5	
Vanillin	0.11	7.43	16.6
Vanillpropanone-2	0.017	1.18	2.65
Total	0.33	22.1	19.3

The petroleum-ether soluble portion of the bisulfite fraction has thus been identified to the extent of 22.1%. The very large working losses (78%) are possibly due to those occasioned by recrystallization, non-controllable polymerization and to the existence of other, as yet unknown constituents the presence of which was indicated by a small amount of high-boiling material found in the fractionation of the crude products.

E. Summary of Results

In addition to the products isolated by previous workers from the water-soluble fraction of the ethanolysis products from spruce, namely, vanillin (42), α-ethoxypropiovanillone (41a) and vanilloyl methyl ketone (43a); the author has now isolated, separated and identified vanillic acid, vanillpropanone-2 and 1-vanill-1-ethoxypropanone-2. The results provide additional support for Hibbert's conception of lignin as a mixture of stabilized products arising from a series of very reactive plant respiratory hydrogen-transportation catalysts as outlined in the previous discussion dealing with the isolation and identification of vanillpropanone-2.

A summary of yields obtained in the ethanolysis of spruce sapwood is given in Table V.

Table V

Summary of Yields from the Ethanolysis of Spruce Sapwood

Product	Weight g.	Klason Lignin %	Klason Lignin Weight g.	Initial Klason Lignin %
Starting material, sapwood meal	828	29.2	242	100
1) Wood residue	510	27.3	139	57.5
2) Ethanol lignin	70.8			29.2
3) Tars from water concentration	18.9			7.83
4) Oil from aqueous distillate	1.45			0.60
5) Crude water-soluble oil	33.8			14.0
(Total recovery of initial Klason lignin)*				109.1
6) Petroleum ether, water-soluble oil	24.6			10.2
7) Vanillin				0.108
8) Vanilloyl methyl ketone				0.200
9) Vanillpropanone-2				0.017
10) Vanillic acid				0.006
11) α-Ethoxypropiovanillone				2.05
12) 1-Vanill-1-ethoxypropanone-2				0.603
(Klason lignin recovered as pure compounds)				2.98

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* No correction is made for added ethoxyl groupings

III. EXPERIMENTAL

The ethanolysis oils used in this investigation were obtained from ethanolyses carried out on several one kilogram quantities of extracted spruce wood meal. Both seasoned wood and freshly cut sapwood were employed as starting materials, the experimental procedure being identical in both cases. A complete description of the author's improved method of lignin extraction and separation into solvent groups is given below for a spruce sapwood run.

A. Preparation of Wood Meal

A four-foot section of a freshly-felled, twenty-six year old spruce tree was sawn into transverse sections and these divided into sapwood and heartwood by band-sawing parallel to the annular rings. The sapwood blocks were chipped and Wiley-milled while still wet and the wood meal leached with ethanol for six hours, then air-dried overnight and finally re-milled to pass the medium screen of the Wiley-mill (1/32 inch diameter holes).

Part of the wood meal so obtained (1041 g.) was immediately extracted with a mixture of 1500 cc. of ethanol and 1500 cc. of benzene in a modified Soxhlet apparatus for 48 hours and then for 24 hours with three liters of ethanol. The extracted wood meal was washed with hot water for about

12 hours; leached with ethanol; air-dried for 6 hours and finally dried in a vacuum oven at 65°C and 25 mm. for 48 hours.

B. Ethanolysis

The ethanolysis procedure and the subsequent separation of the extracted lignin into solvent groups are outlined in the flow-sheets of Figures 1 and 2 and the letters in parentheses in the following pages are those given in these Figures.

The dry wood meal (856 g., 3.28% moisture) was transferred to a 12 liter flask and 9 liters of absolute ethanol added. The flask was swept free of air with carbon dioxide and then 1000 cc. of 20% ethanolic hydrogen chloride were added. The flask was fitted with a mechanically driven, glass hook stirrer operating through a short straight condenser and also with a long, bulb-type condenser through the upper end of which a constant stream of carbon dioxide was passed. The mixture was heated to reflux temperature on a water bath for 48 hours, allowed to cool and filtered through a cloth filter on a Büchner funnel, the residual wood meal being washed with a little pure ethanol, and then further extracted with ethanol for 24 hours in a Soxhlet extractor.

C. Separation of Extracted Lignin into Solvent Groups

The combined filtrate and washings (A) were neutralized by adding an excess of solid sodium bicarbonate and stirring

FIGURE 1

Ethanolysis of Spruce Wood Meal

Separation of the Ethanol Lignin into Solvent-extracted Groups

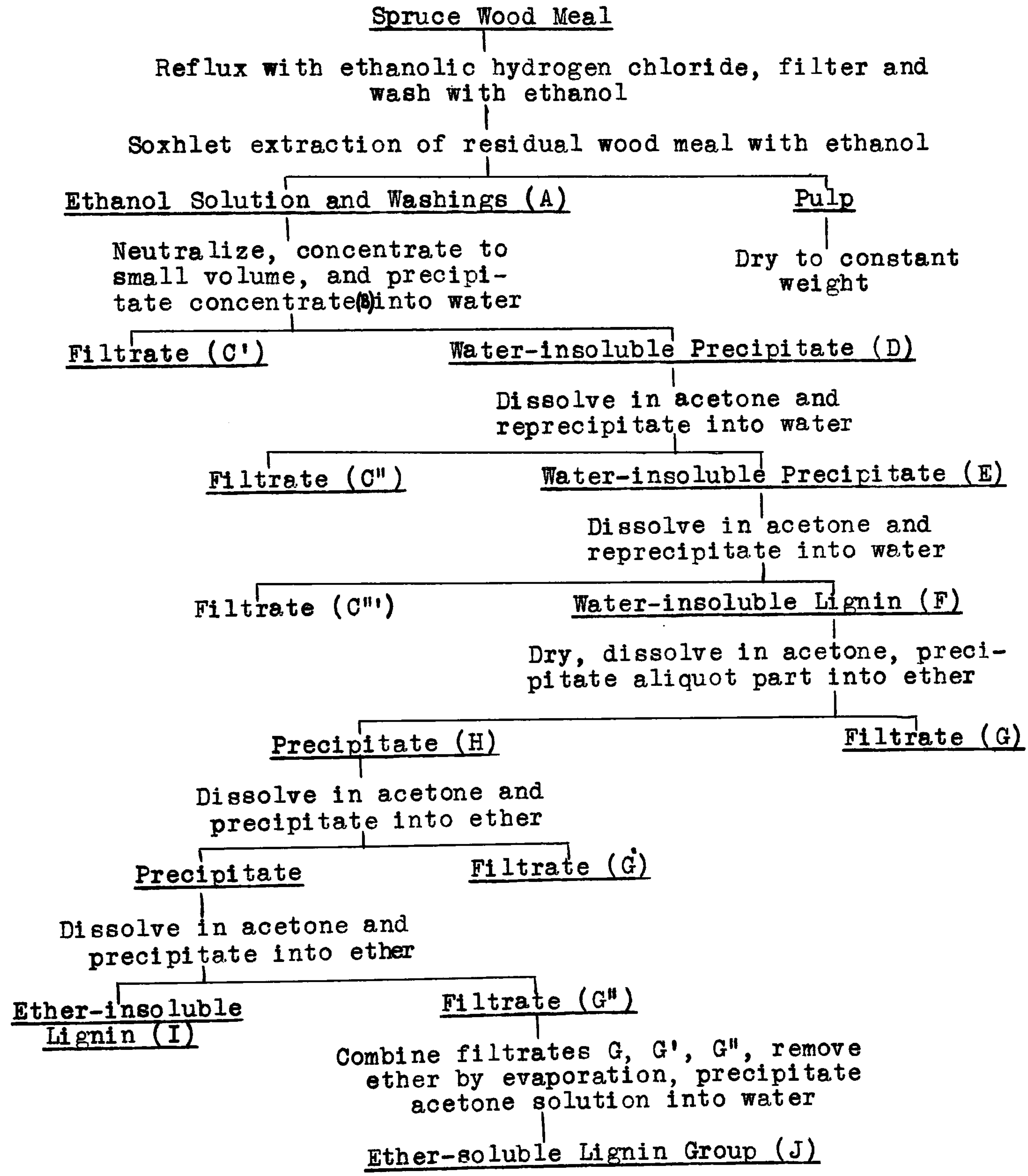
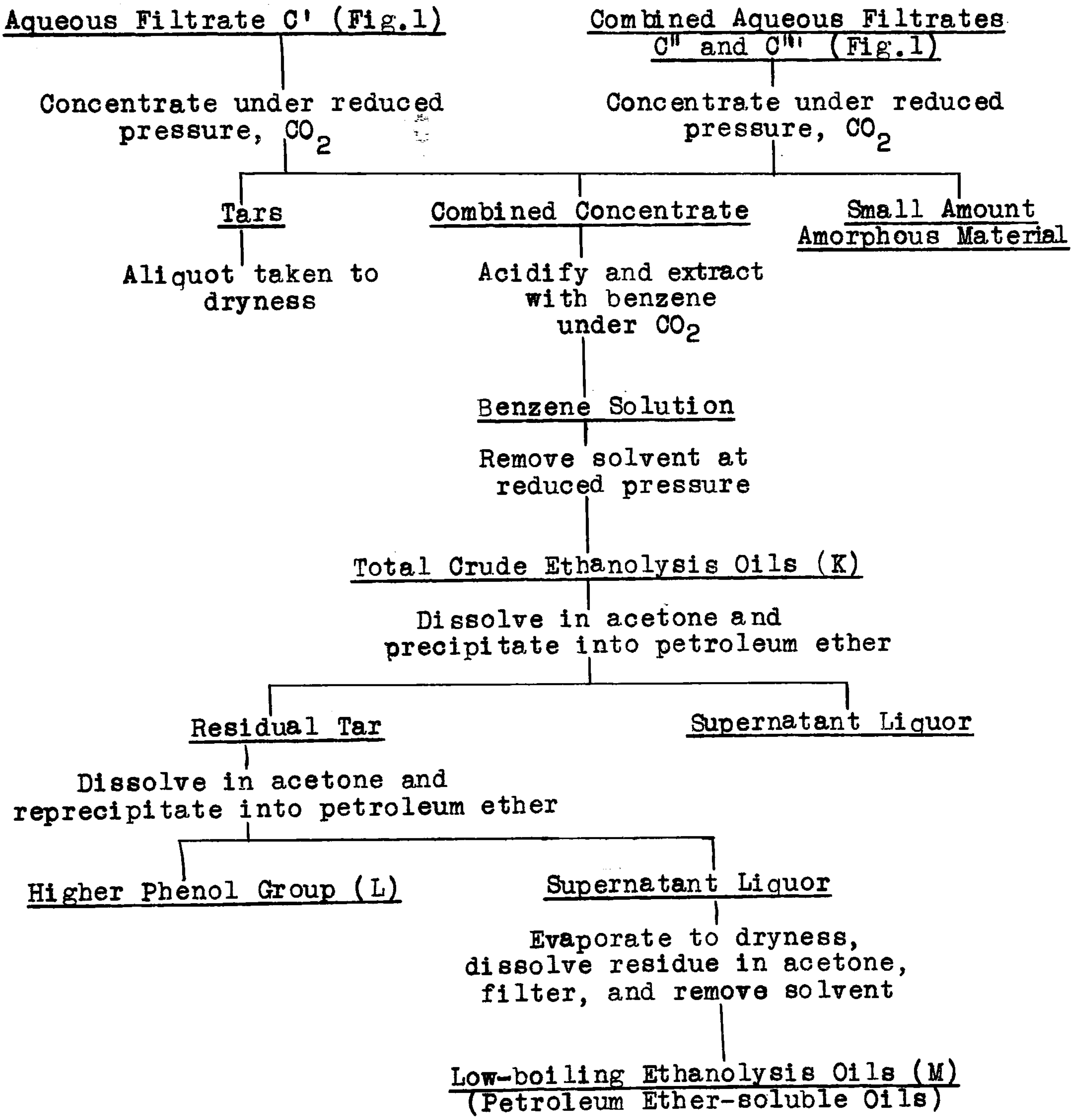


FIGURE 2

Separation of Water-soluble Ethanolysis Oils Into Solubility Groups



the mixture for about 8 hours. (170 g. of $NaHCO_3$ were used). The sodium chloride so formed was filtered off and the alcoholic extract concentrated from approximately 10 liters to 1700 cc. at 45°-50°C. and 50 mm. and this latter solution (B) run through a fine nozzle into 15 liters of distilled water with vigorous mechanical stirring and in the presence of a carbon dioxide atmosphere. The precipitate was allowed to agglomerate and settle overnight and then separated by siphoning and suction filtration into the aqueous solution (C') and the amorphous, water-insoluble residue (D). This latter was dissolved in 800 cc. of acetone and the solution precipitated into 8 liters of vigorously-stirred distilled water as before. The mixture was allowed to settle and again separated by siphoning and filtering, giving filtrate (C") and the precipitate (E). The latter was precipitated a third time from acetone solution into water (800 cc. of acetone and 8 liters of water) and a third filtrate (C''') and the final amorphous, water-insoluble lignin (F) obtained.

This latter (F) was thoroughly dried under reduced pressure, dissolved in 733 cc. of pure dry acetone and a 200 cc. aliquot run through a fine nozzle into ca. 3500 cc. of pure, sodium-dried diethyl ether with mechanical stirring and in the presence of an atmosphere of carbon dioxide. The mixture was allowed to settle and the clear liquor (G) decanted.

The precipitate (H) was reprecipitated twice from acetone solution (100 cc.) into diethyl ether (1800 cc.) and the final dark-brown, amorphous, ether-insoluble lignin (I) (6.26 g.) thoroughly dried at reduced pressure (20°C./0.06 mm.).

The ether filtrates (G, G', G") were combined and the ether removed by evaporation until a small volume (ca. 200 cc.) of acetone solution remained. This was precipitated into 2 liters of distilled water and the ether-soluble lignin group (J), which was a golden-brown amorphous powder, then filtered off and dried at reduced pressure. (Wt. of J = 7.50 g.). The remainder of (F) was reserved for similar treatment.

The entire aqueous filtrate (C') from the first precipitation (approximately 16 liters at pH 5.6) was concentrated to one liter at 45°C/22 mm. in an atmosphere of carbon dioxide (final pH 5.3). In the last stages of the concentration a considerable amount of tar formed which was separated by decantation of the mother liquor. The tar was dissolved in 200 cc. of ethanol, and a 10 cc. aliquot of the solution evaporated to dryness. Weight of residue was 0.927 g., or a total weight of tar equal to 18.94 g. The aqueous filtrates (C'') and (C''') were combined (total volume approximately 20 liters) and concentrated to one liter as before. In this case, while no tar precipitated out on concentration, a very small amount of amorphous lignin-like material separated out. The two aqueous concentrates were combined (total volume 2 liters), made acid to congo red with 15% sulfuric acid, and extracted continuously with benzene in a carbon dioxide atmosphere. Time of extraction and yields of extracted material are given in Table VI.

Table VI

Benzene Extraction

Time of extraction		Yield of extracted oil		
Period	Cumulative	Weight, g.	Percent of total	
			Per extraction	Cumulative
8 hrs.	-	22.03	65.2	65.2
3 days	3 days	6.73	19.9	85.1
4 "	7 "	2.65	7.9	93.0
10 "	17 "	2.05	6.1	99.1
8 "	25 "	0.31	0.9	100.0
	Total	33.77		

A part (32.8 g. or 97.2%) of the total crude oil (K) obtained from the above benzene extractions and subsequent removal of the solvent under reduced pressure was dissolved in 328 cc. of acetone and the solution run through a fine orifice into 6560 cc. of 30-50° petroleum ether with vigorous mechanical stirring and in an inert atmosphere. The mixture was allowed to stand for several hours and then the supernatant liquor was decanted and filtered. The residual tar was dissolved in 50 cc. of acetone and reprecipitated into 2 liters of 30-50° petroleum ether, yielding an almost colourless supernatant liquor and a viscous orange-brown oil (L), the "higher phenol" or "water-soluble lignin" group (7.98 g.). The combined petroleum ether solutions from the above two precipitations were evaporated to dryness, leaving a clear, light-yellow oil which was dissolved in acetone, filtered and again taken to dryness (low-boiling oil group (M), 23.82 g.). Weights and yields for this petroleum ether precipitation are given in Table VII.

Table VII

Petroleum Ether Precipitation

Lignin Group	Weight,*g.	% of original oil	% of Klason lignin
Crude water-soluble oil	32.80	100.0	13.9
Petroleum ether-insoluble (higher phenol group)	7.98	24.4	3.4
Petroleum ether-soluble (low-boiling oils)	23.82	72.6	10.1
Lost in manipulation	1.00	3.0	0.4

* Actual weight obtained. In calculating the percentage of the Klason lignin correction has been made for the fact that only 97.2% of the original crude oils were used in this separation.

D. Fractionation of the Petroleum Ether-soluble Group (M)**

(1) Extraction with sodium bisulfite

The petroleum ether-soluble oil ((M), Figure 2) (23.82 g.) was dissolved in benzene (300 cc.) and the solution extracted with eight 25 cc. portions of sodium bisulfite solution (20 g. $NaHSO_3$/100 cc. of solution). The combined bisulfite extracts (B) (refer to Figure 3) were back-extracted with benzene and the extract added to the original benzene solution (A). The bisulfite solution (B) was then acidified, freed of sulfur dioxide under reduced pressure and extracted exhaustively with benzene (shaken with four 25 cc. portions of benzene and then extracted continuously for four days in an atmosphere of carbon dioxide). Removal of the benzene under reduced pressure yielded 3.88 g. of crude "bisulfite-soluble" oil (D).

(2) Extraction with sodium bicarbonate

The benzene solution (A) was next extracted with five 25 cc. portions of sodium bicarbonate (8 g. $NaHCO_3$/100 cc. of solution) and the combined extracts (E) were twice back-extracted with 25 cc. portions of benzene, then made acid to congo red with 15% sulfuric acid and extracted continuously with benzene for four days. The benzene solution (F) was dried over anhydrous sodium sulfate and then evaporated to dryness under

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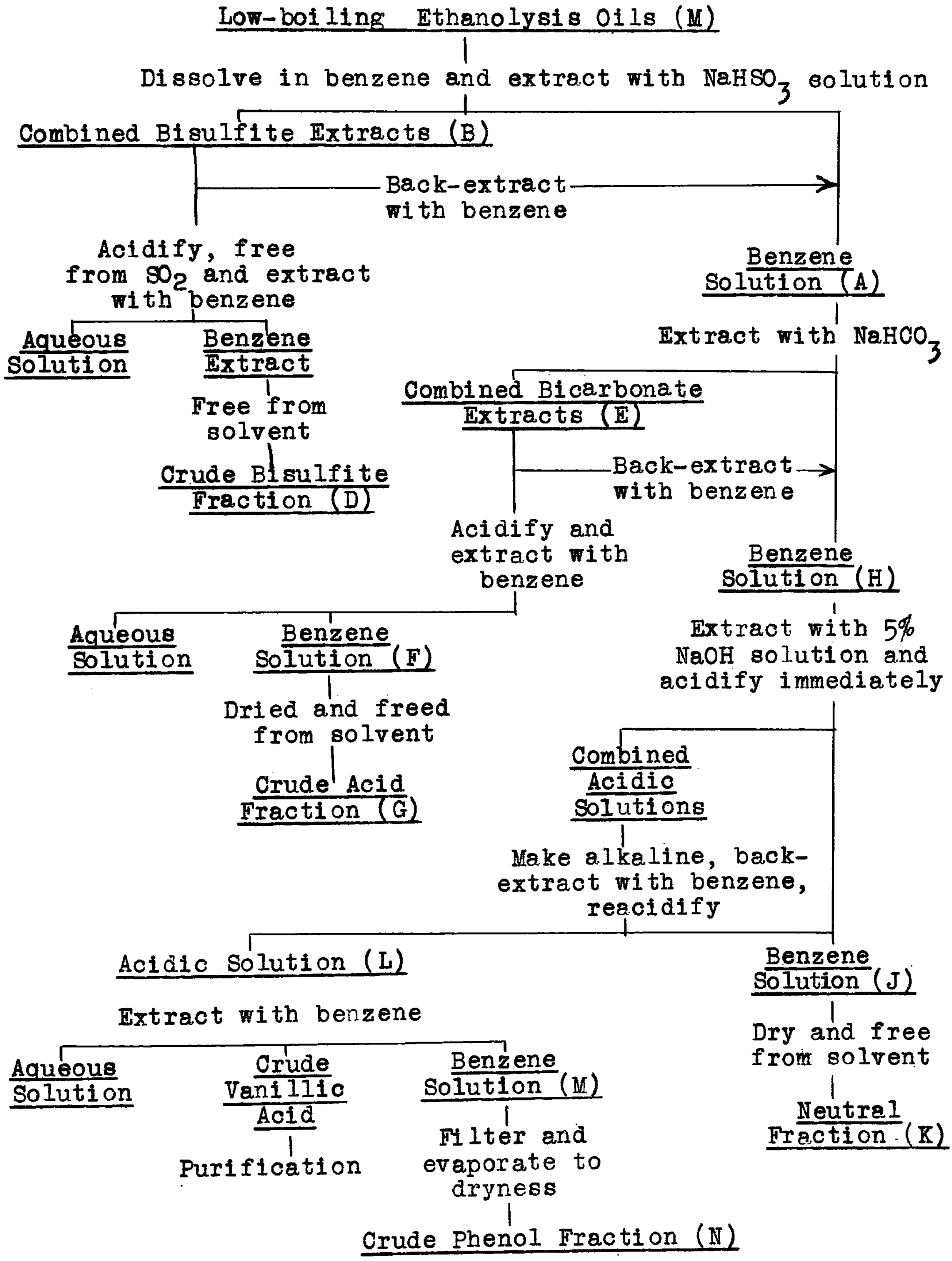
** See Figure 3, page 114.

FIGURE 3

Fractionation of Spruce Low-boiling Ethanolysis Oils



reduced pressure, leaving a dark-coloured oil (G), the "acid fraction" (0.348 g.).

(3) Extraction with alkali and isolation of vanillic acid

The benzene solution (H) was extracted with ten successive 25 cc. portions of 5% sodium hydroxide solution in the following manner: The alkali (25 cc.) was added to the benzene solution in a separatory funnel, the mixture stirred vigorously with a motor driven stirrer for 4 minutes, allowed to separate for 6 minutes and then separated by drawing off the alkaline layer directly into an excess of cold 15% sulfuric acid. The acidic solutions were later combined, neutralized and brought to 5% alkalinity with 30% sodium hydroxide solution, back-extracted with three 50 cc. portions of benzene and then immediately re-acidified (L). The combined benzene solution and back extractions (J) were dried with anhydrous sodium sulfate, filtered and evaporated under reduced pressure (CO_2 bubbler), leaving a clear light-yellow oil containing a considerable proportion of crystalline material. This mixture is termed the "neutral" fraction (K) (1.797 g.).

The acidified alkaline extract (L) was extracted first with three 100 cc. portions of benzene and then in a continuous liquod-extractor with benzene for three successive periods of six hours, two days and one day. A small amount of brown, crystalline material separated from the second benzene extract and yielded a mat of white needle-like crystals (0,015 g.) (m.p. 207-207.5, uncorr.) upon recrystallization from water. A mixed melting point with vanillic acid showed no depression. % OCH_3; calcd. - 18.45%; obsvd.- 18.17, 18.20.

The combined benzene extracts (M) were dried with anhydrous sodium sulfate, filtered and freed from solvent at reduced pressure to obtain the "phenol" fraction (N) (12.925 g.), a clear brown oil.

A complete summary of the yields of the various fractions obtained by the above procedures from the low-boiling ethanolysis oils is given in Table VIII.

Table VIII

Fractionation of Spruce Petroleum Ether-Soluble Ethanolysis Oils (M)

Fraction	Weight, g.	% of starting material	% of initial Klason lignin
Starting material	23.82	100.0	10.1
Bisulfite	3.88	16.3	1.65
Bicarbonate	0.35	1.5	0.15
Alkali *	12.92	54.2	5.48
Neutral	1.80	7.5	0.76
Recovery	-	79.5	8.04

* This does not include the 0.015 g. of vanillic acid.

E. Investigation of the Phenol Fraction from Spruce Sapwood

(1) Petroleum ether precipitation

The crude phenol fraction (A) (see Figure 4, page 118) (12.92 g.) was dissolved in 65 cc. of acetone and the solution poured in a fine stream into 2600 cc. of 30-50° petroleum ether. The tarry residue was separated from the supernatant liquor by decantation and dissolved in acetone (20 cc.) and reprecipitated into 400 cc. of petroleum ether. The residue was reprecipitated a third time from acetone into petroleum ether and the final tarry precipitate (B) dissolved in acetone, filtered and the solvent removed under reduced pressure at 61°C. The filtered supernatant liquors were combined (C) and freed from solvent under a reduced pressure of carbon dioxide (giving fraction D). The yields obtained in this petroleum ether separation are given in Table IX.

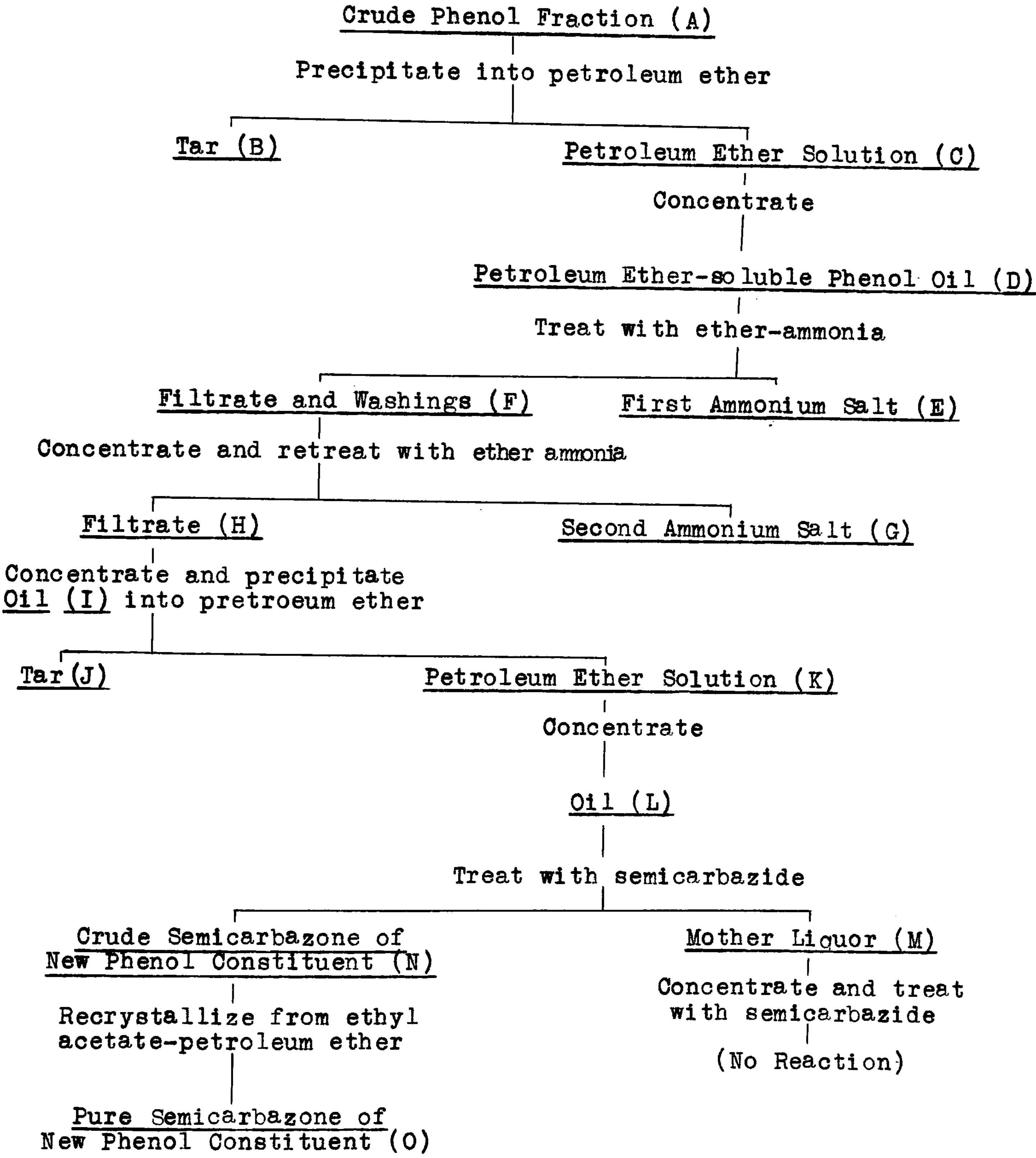
Table IX

Petroleum Ether Precipitation of the Crude Phenol Fraction

	Weight, g.	% of crude phenol fraction
Crude phenol fraction (A)	12.92	100.0
Petroleum ether-soluble oil (D)	12.02	93.0
Petroleum ether-insoluble tar (B)	0.89	6.9

FIGURE 4

Investigation of the Phenol Fraction from Spruce Sapwood



(2) Isolation and purification of α-ethoxypropiovanillone

(a) Separation as the ammonium salt

The petroleum ether-soluble portion (D) (Figure 4) (p.118) of the crude phenol fraction (A) was dissolved in 300 cc. of anhydrous diethyl ether and the solution cooled in an ice bath and treated with gaseous ammonia for 30 minutes. A bright-yellow crystalline precipitate (E) formed and was filtered off through a sintered glass funnel, washed with three 100 cc. portions of ether and dried in a vacuum desiccator (5.07 g.) (all operations were at 0-2°C.). The ether filtrate and washings were combined (F) and the solvent removed under reduced pressure and in a nitrogen atmosphere, leaving a residual oil which was again dissolved in ether and subjected to a second treatment with gaseous ammonia. The precipitate was filtered off, washed with ether saturated with ammonia and then dried in a vacuum desiccator (G) (3.31 g). The combined filtrate and washings from this second treatment (H) were freed from solvent under reduced pressure (nitrogen atmosphere) leaving 4.00 g. of a brown oil (I) which were dissolved in acetone (20 cc.) and precipitated into 800 cc. of 30-50°C. petroleum ether. The residue was dissolved in acetone and reprecipitated in the same way. The final yield of petroleum ether-soluble, ether-ammonia-soluble oil (L) was 3.59 g.

(b) Methylation of the ammonium salt

The first ammonium salt precipitate (E) (5.07 g.) was transferred to a 100 cc. three-necked flask fitted with a stirrer, a nitrogen inlet tube and two burettes. On the addition of sodium hydroxide (30cc. of a 5% solution) the salt dissolved to give a reddish-brown solution, to which dimethyl sulphate (15 cc.) and sodium hydroxide (15 cc. of a 30% solution) were added simultaneously in 1 cc. portions at two minute intervals. The colour of the solution became lighter during this addition and at the same time a flocculent precipitate separated and the temperature rose slightly (25°C. to 30°C.).

The reaction mixture was allowed to stand at room temperature for three hours, the precipitate removed by filtration, washed with water, air dried and recrystallized from ethanol-water (1:1). Yield 3.155 g. of impure α-ethoxypropiovanillone, M.P.. 71-80°C. A portion (0.188 g.) of this impure α-ethoxypropioveratrone was recrystallized from water-ethanol (1:1) and gave 0.12 g. of pure material; M.P. 81-81.5°C.; mixed M.P. with authentic α-ethoxypropioveratrone 81-81.5°C.; M.P. of authentic material 81-81.5°C. Additional dimethyl sulphate (4 cc.) and sodium hydroxide (4 cc. of 30% solution) were added to the first filtrate and the mixture warmed at 90°C. for three hours but no further precipitation occurred. Extraction of this liquor with three 50 cc. portions of ether yielded 0.526 g. of an oil which solidified partially upon standing but was not further investigated.

(c) Hydrolysis of the ammonium salt and fractional distillation of α-ethoxypropiovanillone

The ammonium salt used in this determination was obtained from the phenol fraction of the water-soluble oils from the ethanolysis of seasoned spruce wood (same method as in Fig.4) The salt (7.72 g.) was dissolved in 200 cc. of water, transferred to a separatory funnel, made acid to congo red with 15% sulfuric acid and extracted with six successive 50 cc. portions of benzene. The extraction solutions were combined, dried over anhydrous sodium sulfate, filtered, concentrated and freed of solvent under feduced pressure (CO_2 bubbler). (Yield 7.29 g. of oil). Further extraction of the remaining aqueous solution with ether yielded 0.061 g. of oil and indicated that the extraction was essentially complete.

A preliminary distillation of the oil (7.81 g.) at 130-140°C./0.020 mm. yielded 7.00 g. of distillate. A second sample of this oil (3.227 g.) was fractionally distilled in a 12" fractionating column equipped with a vacuum jacket and spiral gauze packing. The results are summarized in the following Table (Table X, page 122).

Table X

Fractional Distillation of α-ethoxypropiovanillone

Fract.	Wt., g.	% of starting	Temp., °C.		Press., mm.	n_D^{25}	Remarks
			Bath	Column			
Starting Material	3.227	100				1.5511	Yellow color
1	0.157	4.9	173	98	20	1.5518	Yellow color
2	0.357	10.9	188	105.5	20	1.5513	Yellow (lighter)
3	0.134	4.1	188	105.5	20	1.5512	Very light yellow
4	0.344	10.7	184	106	20	1.5511	"
5	0.254	7.9	178	104.5	20	1.5511	
6	0.290	9.0	177	103.5	15	1.5510	
7	0.556	17.2	184	104	15	1.5511	n_D^{46}=1.5409; n_D^{50}=1.5390
8	0.431	13.3	184	102.5	15	1.5511	
9	0.093	2.9	190	100	15	1.5511	
	2.616	80.9					
Residue in column	0.183	5.7					
Rec.*	0.198	6.1					
		92.7					
Residue in pot.	0.011	0.3					
		93.0					

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* The distillate condensing in the receiver was recovered and weighed. The weight given is a minimum value.

(3) Isolation and purification of the unknown phenol constituent

(a) Separation and purification as the semicarbazone

The crude oil remaining after the separation of the α-ethoxypropiovanillone (L) (Figure 4, page 118) (0.105 g.) was dissolved in 0.5 cc. of ethanol, the solution diluted with 3.5 cc. of water and 0.06 g. each of semicarbazide hydrochloride and potassium acetate were added. Crystallization started after about 24 hours and after two days a copious precipitate (N) (Figure 4) had formed, which, after filtering, washing and drying melted at 169-170°C. (Yield = 0.078 g.). A mixed melting point with semicarbazide hydrochloride (M.P. 172°C.) melted at 142-145°C. indicating that the material was not semicarbazide hydrochloride. A series of successive recrystallizations from four different solvents was carried out, yielding materials of the following melting points:

From:		
	(1) Ethyl acetate.	170-171°C.
	(2) Ethanol - petroleum ether (30-50°C.) (1:1)	170.5-171.5°C.
	(3) Water	171-172°C.
	(4) Water	171-172°C.

(b) Reconversion of the semicarbazone to the free phenolic ketone

The semicarbazone of the new phenol constituent (0.163 g.) was placed in a centrifuge tube with 10 cc. of benzene and 3.1 cc.

of 2% sulphuric acid (10% in excess of the theoretical requirements for the formation of semicarbazide acid sulphate). The tube was stoppered, shaken vigorously at room temperature for 20 minutes and the contents centrifuged. The supernatant benzene was removed with a pipette and replaced with fresh benzene and the above procedure repeated twice. After these three treatments the semicarbazone had dissolved, but three additional extractions were carried out to insure complete removal of the oil. The combined benzene solutions were dried over anhydrous sodium sulphate, filtered, concentrated, and freed from solvent under reduced pressure. The product was a light yellow oil (Yield 0.128 g.; 95% of the theoretical). It was dissolved in 1.2 cc. of acetone and directed in a fine stream into 25 cc. of petroleum ether 30-50°C. No tar was formed. Filtration and evaporation of the petroleum ether solution again yielded 0.128 g. of a light yellow oil. Distillation of 0.113 g. of this material at 120-128°C./0.05 mm. gave 0.108 g. of an almost colourless oil (n_D^{25}= 1.5241), representing 83.1% of the theoretical yield from the semicarbazone. The semicarbazone was regenerated by dissolving 0.053 of the oily distillate in 0.5 cc. of ethanol, diluting to 10 cc. with water, adding 0.03 g. each of semicarbazide hydrochloride and potassium acetate and allowing the mixture to stand overnight. The while precipitate, after filtration, washing and drying (0.060 g.), had a melting point, and mixed melting point with a sample of the original semicarbazone, of 171-172°C.

(c) Comparison of the efficiency of separation of the new phenol constituent by semicarbazone formation and by fractional distillation

The petroleum ether-soluble oil (L) (Figure 4, page 118), obtained by the ethanolysis of seasoned spruce wood, was used in this comparative study.

(i) Separation as the semicarbazone - Using the procedure given above, 0.096 g. of the oil (L) was converted to the crude semicarbazone (0.036 g.), which, upon recrystallization from water gave 0.025 g. of material having a melting point of 171-172°C. If, as in section (b) above, an 83.1% yield of the new phenol constituent were obtained from the semicarbazone, then this latter would represent 27.2% of the petroleum ether-soluble oil (L).

(ii) Separation by fractional distillation - A portion of the petroleum ether-soluble oil (L) (Figure 4) (2.43 g.) was subjected to a preliminary distillation (120-170°C./0.025 mm.) and a light-yellow distillate (n_D^{25} = 1.5195) obtained; the yields being as follows:

	Starting Material	Distillate	Residue in Flask
Weight (g)	2.43	2.01	0.36
% of the starting material	100	82.7	14.8

Part of the distillate (2.013 g.) was redistilled under reduced pressure in a 12", 8-theoretical-plate fractionating column (195). A complete account of the conditions of distillation and yields is given in Table XI.

Table XI

Fractional Distillation of the Oil Remaining After the Separation of α-Ethoxypropiovanillone

Fraction	Wt., g.	% of starting material	Temps. °C. Bath	Column	Press. mm.	n_D^{25}	Remarks
Starting material	2.013	100				1.5093	
1	0.102	5.06	186	96	50	1.5185	Partially crystalline
2	0.050	2.48	184	94	250	1.5206	" "
3	0.105	5.21	192	91	750	1.5223	" "
4	0.144	7.15	189	94	750	1.5232	
5	0.109	5.41	193	93	750	1.5240	
6	0.129	6.41	194	91	750	1.5241	
7	0.100	4.96	194	96	750	1.5240	n_D^{50}, 1.5160
8	0.080	3.97	187	95	750	1.5240	
9	0.118	5.86	192	94	750	1.5240	
10	0.047	2.34	207	94	750	1.5243	
11	0.060	2.98	210	95	750	1.5244	n_D slightly fuzzy
12	0.141	7.00	213	97	200	1.5275	
13	0.172	8.55	237	1065	80	1.5470	Partially crystalline n_D indefinite.
	1.357	67.38					
Residues Col.	0.131	6.51					
Pot	0.386	19.19					Completely sol in acetone. Brown color.
	1.874	93.08					
Loss		6.92					Cold trap not worked up.

Fractions 5 to 11, inclusive, weighed 0.643 g. and account for 25.8% of the petroleum ether-soluble portion of the oil left after separation of the α-ethoxypropiovanillone (L).

(d) Final modified procedure for the isolation of the new phenol constituent

The crude oil remaining after the separation of the α-ethoxypropiovanillone (I)(Figure 4,page 118) was twice precipitated into petroleum ether (30-50°C.) using ratios of oil:solvent:precipitate of 1:5:200. The results are summarized below:

	Weight g.	% of crude oil
Crude ether-ammonia-soluble oil	4.00	100.0
Petroleum ether-soluble oil	3.59	89.7
Petroleum ether-insoluble oil	0.37	9.2

Semicarbazide hydrochloride (1.7 g.) and potassium acetate (1.7 g.) were added to a solution of 3.389 g. of the petroleum ether-soluble oil (L) in 34 c.c. of ethanol and 100 c.c. of water After standing overnight, the copious precipitate was filtered off, washed and dried (N) (Yield 1.823g.,M.P 169-170°C.). A portion of this crude material (1.066 g.) was recrystallized from a mixture of ethyl acetate-petroleum ether (1:3) and yielded 0.977g. of pure semicarbazone, M.P.171-172°C. Retreatment of the reaction liquors left after removal of the semicarbazone with additional potassium acetate (1.0 g.) and semicarbazide hydrochloride (1.0 g.) failed to give more semicarbazone The yields obtained by this final separation procedure were as follows:

Total weight of pure semicarbazone	1.760 g.
Total weight of free ketone equivalent to the semicarbazone	1.403 g.
Percent of the petroleum ether-soluble oil remaining after separation of α-ethoxypropiovanillone (L)	35.1%
Percent of petroleum ether-soluble, alkali-soluble oil (i.e. crude phenol fraction (A))	11.7%
Percent of initial Klason lignin	0.603%

(4) Characterization of the new phenol constituent

(a) Analytical data

Table XII

	Semicarbazone	Pure Ketone
Carbon		
Observed values	55.8, 55.9	64.0, 64.3
Average observed value	55.9	64.2
Theoretical value*	55.5	64.3
Hydrogen		
Observed values	7.00, 7.44	7.74, 7.61
Average observed value	7.3	7.7
Theoretical value	6.77	7.14
Nitrogen		
Observed values	14.74, 14.94	
Average observed value	14.8	
Theoretical value	14.94	
Total Alkoxyl as Methyl		
Observed values	21.88, 21.83	27.2
Average observed value	21.9	27.2
Theoretical value	22.05	27.66
Methoxyl		
Observed value	10.4	
Theoretical value	11.03	

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* Calculated for 1-vanill-1-ethoxypropanone-2 and its semicarbazone.

(b) Rast molecular weight determination.

The procedure used was that given by Shriner and Fuson (188).

Weight of camphor................ 0.10342 g.

Weight of semicarbazone........... 0.01090 g.

Melting point of camphor......... 177°C.

Melting point of mixture.......... $\left\{\begin{matrix}162\\162.5\end{matrix}\right\}$ 162.3°C.

$$\text{Molecular weight} = \frac{(39.7)(1000)(0.01090)}{(14.7)(0.1034)} = 285(\pm\ 14)$$

(c) Ultraviolet absorption data*.

A sample of the purified new phenol fraction was weighed out (0.0039 g.) and dissolved in 10 cc. of absolute ethanol. A portion of this solution (1.3 cc.) was diluted with 8.7 cc. of absolute ethanol, giving a 0.0051% solution with which the absorption spectral determinations were made. The absorption curve, obtained with a Hilger Spekker photometer and Hilger E2 quartz spectrograph, showed a single well defined maximum at 1060 fresnel units ($E_{max.}$=1.66) and a minimum at 1145 fresnel units ($E_{min.}$=1.06), after which the curve rose sharply to the limit of the photometric range (1224 f at E=2.0).

(d) Proof of aromatic nuclear structure by degradation to vanillic acid.

The semicarbazone of the new phenol constituent (0.053 g.) was dissolved in 10% sodium hydroxide solution (10 cc.) and heated at 50°C. overnight. Subsequent acidification of the solution

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* determined by R.F. Patterson.

with 15% sulfuric aicd gave a small amount of brown flocculent precipitate that could not be recrystallized from water. Four extractions of the aqueous solution with 5 cc. portions of ether yielded a brown oil containing a small amount of crystalline material. Additional overnight treatment of this oil with sodium hydroxide (10 cc. of 10%) at 50 °C. followed by acidification, yielded a small amount of brown flocculent material which, as in the first case, could not be recrystallized from water. The acidified mother liquor was extracted with ether, the ether solution dried over sodium sulphate, filtered, concentrated and freed from solvent under reduced pressure (61°C./50 mm.). The residue, a semicrystalline mass, was dissolved in water, shaken with charcoal, filtered and recrystallized (m.p. 203-204°C.). A final recrystallization of this material from water, yielded a crystalline product (0.002 g.) having a melting point of 207-207.5°C. and a mixed melting point with pure vanillic acid of 207-207.5°C.

(e) Oxidation with chromic acid.*

Chromic acid oxidation of the semicarbazone of the new phenol constituent was carried out using a modification (190) of the Kuhn and L'Orsa (189) method. (In the modified procedure an acetyl determination on the distillate serves as a check on the first titration with acetic acid.)

Titration of the acetic acid produced by oxidation of the semicarbazone of the new phenol constituent with chromic acid required 10.80 cc of 0.103 N alkali (corrected for blank), equivalent to 39.6% acetic acid or 92.3% of the theoretical amount.

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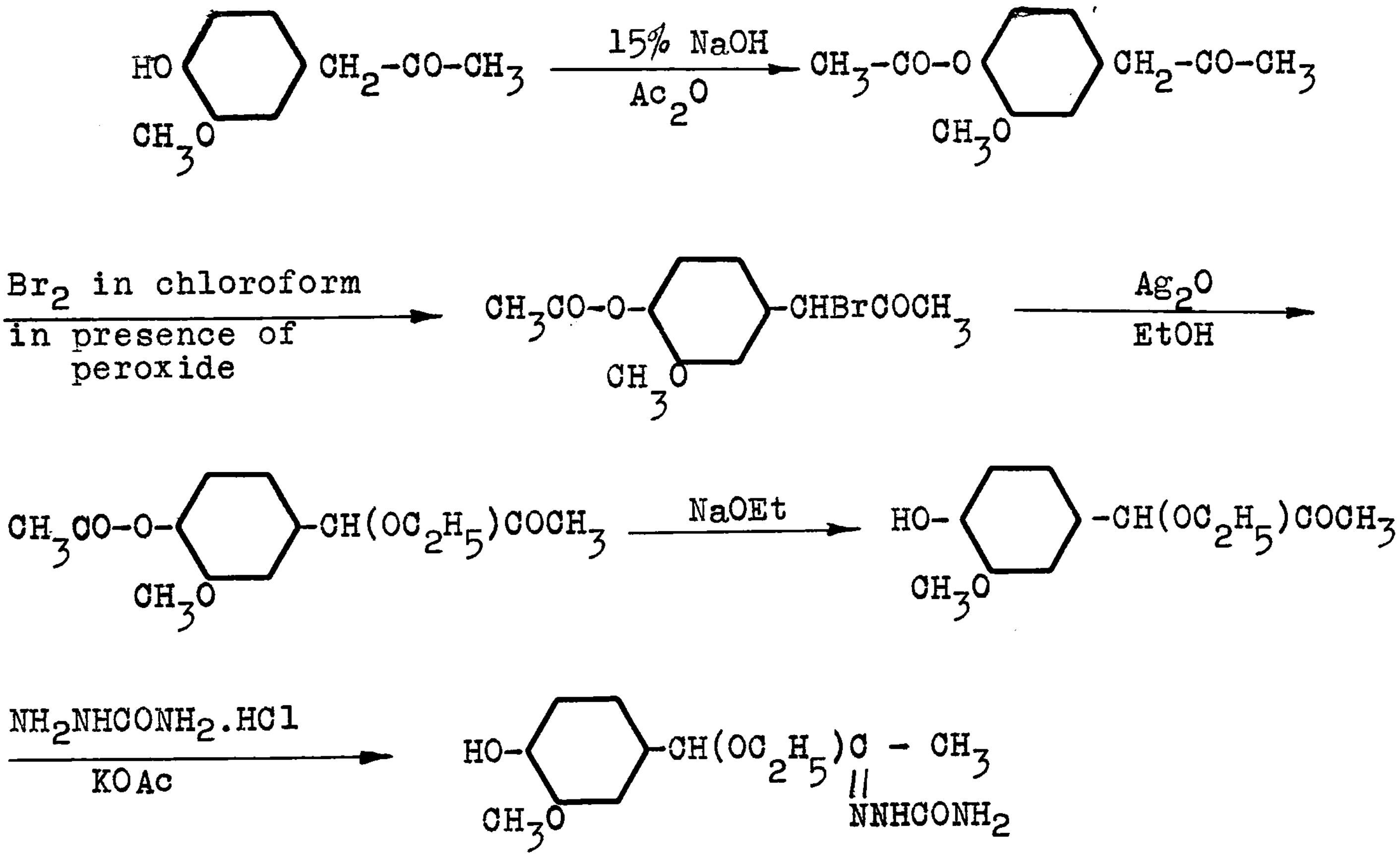
* The author's thanks are due to Mr. W.S. MacGregor for these values.

Oxidation of 0.177 g. of vanillin semicarbazone gave acetic acid equivalent to 0.33 cc. of 0.103 N alkali. This corresponds to a yield of 0.52% of acetic acid and shows that the semicarbazone group does not interfere with the determination.

(5) Synthesis of 1-vanill-1-ethoxypropanone-2

Direct synthesis of the new phenol constituent, 1-vanill-1-ethoxypropanone-2 has been successfully accomplished in these laboratories (194) according to the following steps:



Melting point of synthetic semicarbazone	173-174°C.
Melting point of natural semicarbazone	171-172°C.
Mixed melting point	172-173°C.

F. Investigation of the Bisulfite-soluble Fraction

The procedure described below is the improved technique used in the present investigation of the petroleum ether-soluble,sodium bisulfite-soluble spruce sapwood ethanolysis oil (D-Figure 3, page 114). Flow-sheets outlining the work are given in Figures5 and 6 pages 133 and 134.

(1) Petroleum ether precipitation

The crude bisulphite soluble oil from the spruce sapwood ethanolysis was dissolved in 39 c.c. of acetone and poured in a fine stream into 780 c.c. of petroleum ether (30-50°). The supernatant liquors were combined and freed from solvent under reduced pressure leaving a light yellow oil. The yields obtained in these precipitations are given below:

	Weight g.	% of crude bisulfite soluble oil
Crude bisulfite-soluble oil	3.88	100.0
Petroleum ether-soluble oil	3.38	87.1
Petroleum ether-insoluble tar	0.43	11.2

(2) Separation of vanilloyl methyl ketone

(a) Nickel dioxime formation

Petroleum ether-soluble, bisulfite-soluble spruce ethanolysis oil (3.38 g.)((A), Figure 5, page 133) was dissolved in 1500 c.c. of water in a three litre flask fitted with a reflux

FIGURE 5

Fractionation of Bisulfite-soluble Ethanolysis Oils

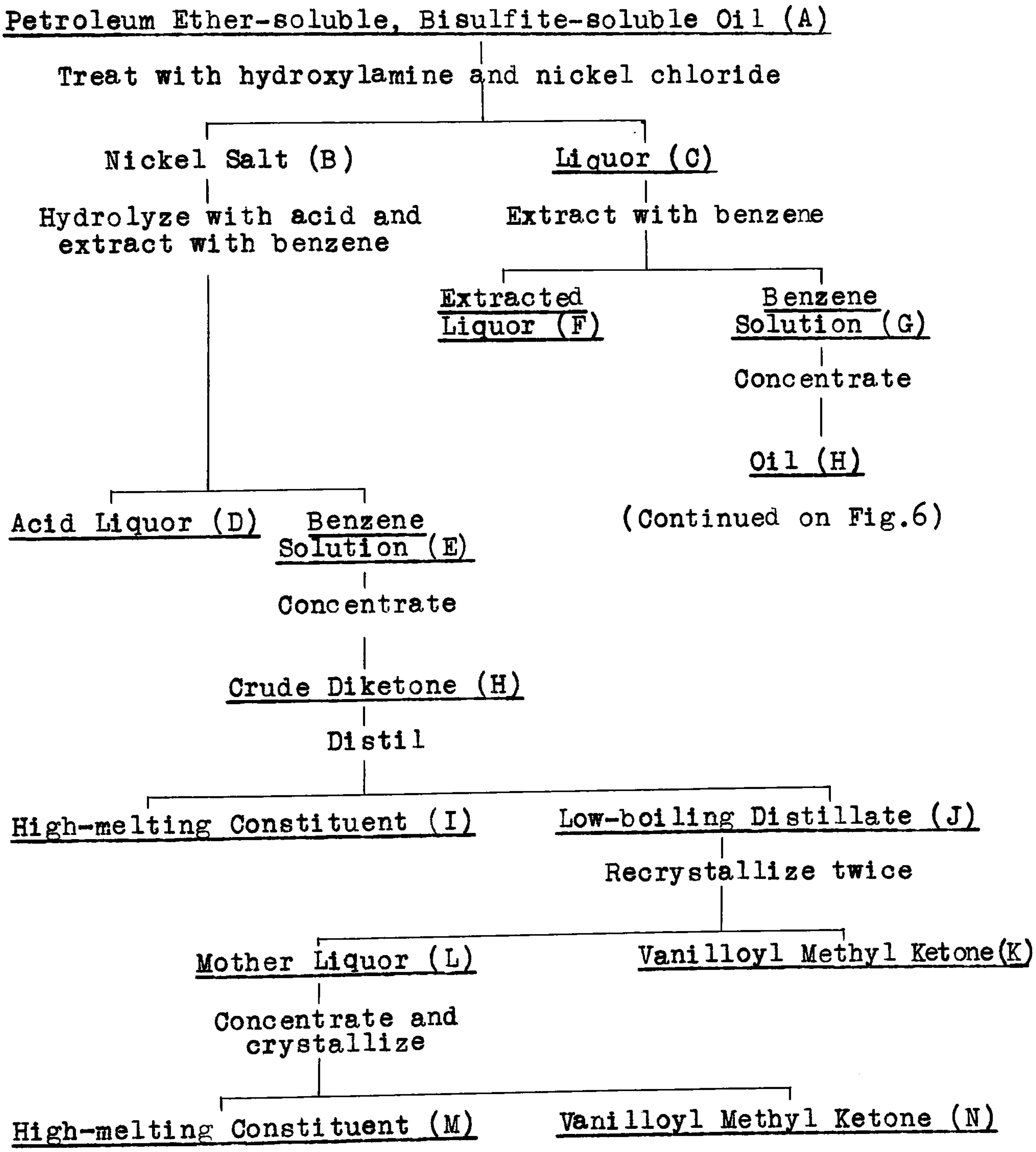
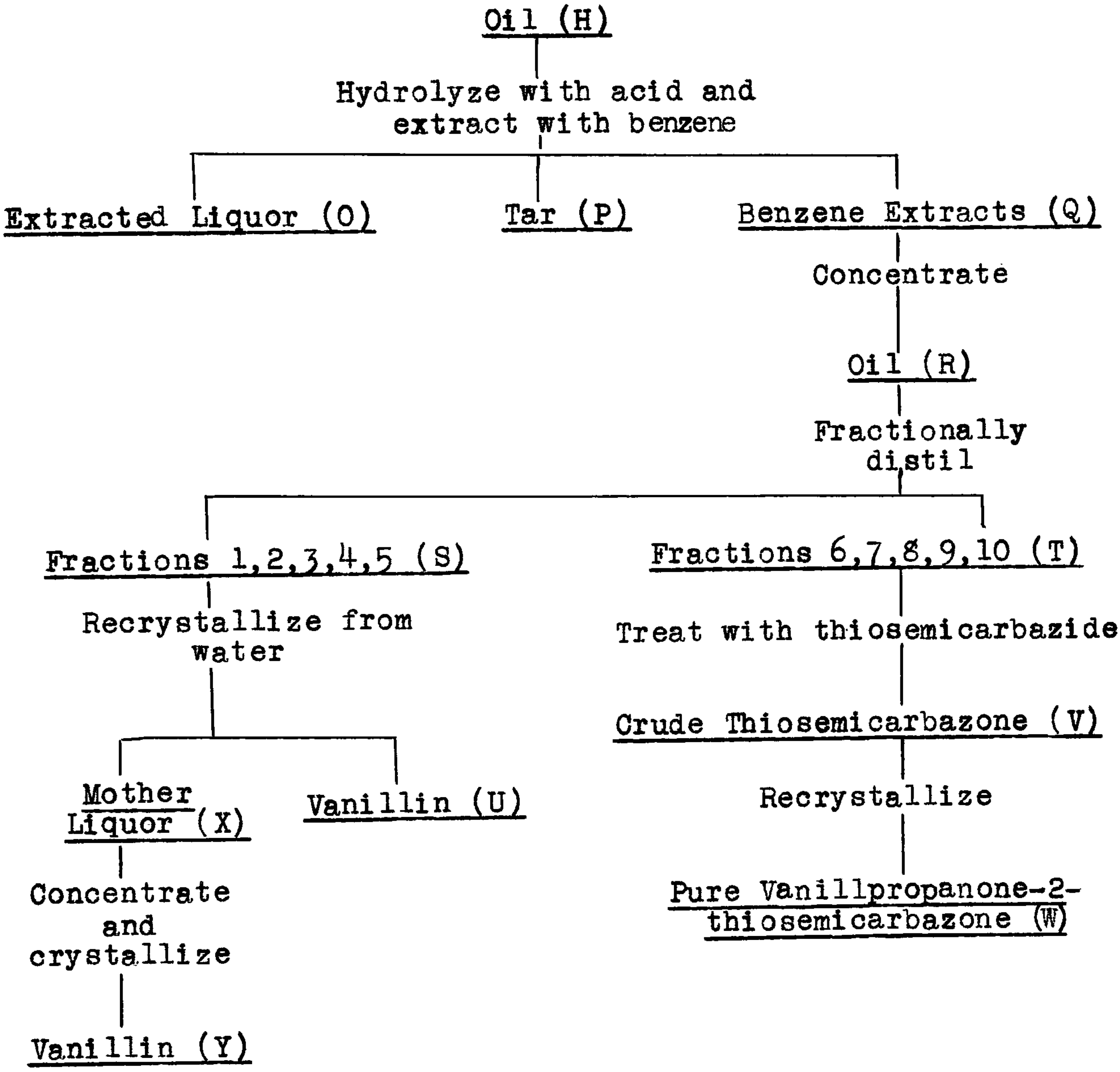


FIGURE 6

Fractionation of Bisulfite-soluble Ethanolysis Oils

(Continued from Fig.5)



condenser and a carbon dioxide inlet. To this solution was added sodium acetate (33.8 g.), nickel chloride solution (44.3 cc. of 5% solution) and hydroxylamine sulfate (4.3 g.). The mixture was heated for two days on a steam bath, then cooled to 2°C. and the red nickel salt filtered off on a tared, sintered glass funnel, washed and dried at 105°C.

The filtrate was returned to the flask, a further quantity of nickel chloride solution (3 cc.) and hydroxylamine sulfate (0.30 g.) added and the mixture heated at reflux for two days. The solution was again cooled, the salt filtered off and the same procedure repeated three times, after which no more nickel salt was formed. The total weight of nickel salt (B) was 1.732 g.

The final filtrate (C) was made acid to congo red by adding 15% sulfuric acid, and the solution exhaustively extracted in a continuous liquid extractor. Removal of the benzene from the combined extracts at reduced pressure (40°C./25 mm.) yielded 1.510 g. of oil (H).

(b) <u>Hydrolysis of the nickel salt and isolation of vanilloyl methyl ketone.</u>

The combined nickel salts (B) (1.732 g.) were macerated with 12 N sulfuric acid (400 cc.) at 0°C., the mixture stirred and then filtered. The filtrate was extracted with ten successive 75 cc. portions of benzene, the extracts combined, shaken with solid sodium bicarbonate, dried over anhydrous sodium sulfate, filtered, concentrated and freed from solvent under reduced pressure. The product, a viscous yellow oil (H) (1.020 g.), was transferred to a Späth bulb and distilled at 140-160°C./0.4 mm. Near the end of the distillation, a material, which was not vanilloyl methyl ketone, sublimed into the neck of the Späth bulb. This material was removed separately (I) (0.048 g.) but not further investigated.

The main distillate (J) (0.820 g.), which crystallized on standing overnight in the cold room (2°C.), was recrystallized from water and yielded 0.514 g. of crystalline material melting at 67.5-68.5°C. A final recrystallization from water gave 0.390 g. of pure vanilloyl methyl ketone (K) having a melting point and a mixed melting point with an authentic sample of 68-69°C.

The mother liquor (L) was reworked and yielded 0.066 g. of vanilloyl methyl ketone (N) melting at 68-69°C. and 0.015 g. of a crystalline material (M) which melted over a wide range (65-122°C.) and was not investigated further. The yield of pure vanilloyl methyl ketone is summarized as follows:

The total weight of pure vanilloyl methyl ketone was 0.456 g., and represented 56.8% of the distillate (J); 44.7% of the oil obtained by hydrolysis of the nickel salt (B); 13.5% of the petroleum ether (30-50°C.) soluble, bisulphite-soluble oil; and 0.20% of the initial Klason lignin.

(3) Separation of vanillin and vanillpropanone-2 by fractional distillation

(a) Hydrolysis of the oil remaining after the separation of the nickel salt of vanilloyl methyl ketone dioxime

The oil (H) remaining after the separation of the nickel salt (1.510 g.) was treated with 7 N sulfuric acid at 0°C. for one hour, then at room temperature for 24 hours and finally at 40°C. for four hours. A small amount of tar (P) separated from the reaction liquor and was removed (0.234 g.) by siphoning off the mother liquor.

The acid hydrolysis liquor was extracted with ten 20 cc. portions of benzene, the combined extracts (Q), shaken with solid sodium bicarbonate, dried with anhydrous sodium sulfate, filtered, concentrated and freed from solvent under reduced pressure (40°C./25 mm.). The oil (R) (0.880 g.) was distilled under reduced pressure (120-150°C./0.06 mm.) and yielded 0.790 g. of distillate which was redistilled in a 6", 8 plate, Cooke-Bower column (195). A complete account of yields and conditions is given in Table XIII (page 138).

(b) Purification of the vanillin fractions

Fractions 1 to 5 inclusive (S) (0.361 g.) were combined and recrystallized. Yield of vanillin (U) (0.171 g.), identified by its melting point and mixed melting point with an authentic sample. The mother liquor (X) was recovered and yielded two additional crops of pure vanillin (Y) (0.074 g.). The total yield of vanillin was 0.245 g., and the percentages of the bisulfite-soluble oil and of the initial Klason lignin, etc, are given in Table IV (page 102).

Table XIII

Summary of Fractional Distillation of the Aldehyde-monoketone Mixture (R)(Fig.3) from the Bisulfite Fraction

Fractions No.	 Wt.g.	 % of 0	Temp., °C. Bath	 Column	Press. Microns	n^{25}_{D}	Remarks
0*	0.790	100					
1	0.043	5.4	135	80	30	-	Crystalline
2	0.63	8.0	134	79	15	-	"
3	0.050	6.3	139	80	50	-	"
4	0.134	17.0	140	79	40	-	Crystalline, MP. 78-79°C.
5	0.071	9.0	153	76	25	-	Semicrystalline
6	0.057	7.2	148	81	20	1.5525**	Oil
7	0.088	11.1	158	83	25	1.5440	"
8	0.060	7.6	150	87	25	1.5434	"
9	0.046	5.8	148	87	25	1.5443	"
10	0.073	9.2	180	110	25	1.5490	"
11	0.034	4.3	180	110	25		Semicrystalline high melting sublimate
	0.719	90.9					
Res.	0.060	7.6					
	0.779	98.5					
Loss	0.011	1.5					

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* Starting material.

** ± 0.0003; 1.5444 for synthetic R-propanone-2.

(c) Preparation of vanillpropanone-2-thiosemicarbazone from the higher boiling fractions

Fraction 6 from the above fractionation (less a small refractive index sample) (wt. 0.052 g.) was dissolved in 0.4 cc. of ethanol and the solution diluted with 4 cc. of water. Thiosemicarbazide (0.021 g.) was added and the solution warmed to 70°C. for a two hour period and then placed in the cold room (2°C.) for three hours. A yellowish-brown precipitate formed and was filtered off, washed, dried, weighed (0.046 g.), dissolved in ethanol, treated with charcoal and recrystallized from ethanol solution. The white recrystallized product (0.020 g.) melted at 183-184°C.

Fraction 10 and the combined fractions 7, 8 and 9 were treated in the same manner. The yields are given in Table XIV.

Table XIV

Thiosemicarbazone Formation

Product and Operations	Fractions 6	 7,8,9 (combined)	 10
Weight of oil used (g.)	0.052	0.184	0.067
Weight of crude thiosemicarbazone (g.)	0.046	0.174	0.027
Weight of product recrystallized from ethanol (g.)	0.020	0.119	0.010
Simultaneously determined melting points. (M.P.)	183-184	183-184	179-181
Mixed M.P. (with authentic sample)	186-187	185-186	181-182
M.P. (authentic sample)	186.5-187	186.5-187	186.5-187

Since the thiosemicarbazones obtained from fractions 6, 7, 8 and 9 appeared to be equally pure they were combined, recrystallized once from ethanol and twice from ethyl acetate and yielded 0.053 g. of thiosemicarbazone having melting point and mixed melting point identical with that of synthetic vanillpropanone-2 thiosemicarbazone (187-188°C.).

Analysis -

Carbon: found 52.1, 52.0; calcd., 52.2.
Hydrogen: found 6.5, 6.3; calcd., 6.0.

Applying corrections for the material lost in the refractive index determinations, the calculated yield (from the thiosemicarbazone) of pure vanillpropanone-2 is 0.040 g. The calculated yields of vanillpropanone-2 and of vanillin are given in Table IV, page 102.

SUMMARY

I. The ethanolysis procedure for spruce wood has been re-investigated and certain improvements introduced:

- A. A more complete separation and a larger overall yield of ethanolysis products has resulted from (1) extraction of the residual wood meal with ethanol and (2) the separation of tars ("higher-boiling phenols") from the ethanolysis oils by a new petroleum ether-precipitation technique.
- B. It has been shown that a decrease in the time of exposure of the water-soluble oils to alkali in the fractionation procedure brings about a reduction in (1) the degradation of one of the constituents to vanillic acid and (2) the amount of tar formed by the polymerization of the simple units.

II. A re-investigation of the bisulfite-soluble, water-soluble ethanolysis oil has established the presence of vanillin as a definite constituent of that fraction. Vanillpropanone-2, which previously had been found in the ethanolysis products of maple, now has been isolated from spruce ethanolysis products for the first time by a modification of the procedure used for maple.

III. A new substance, 1-vanill-1-ethoxypropanone-2, an isomer of α-ethoxypropiovanillone, has been isolated from the alkali-soluble, water-soluble spruce ethanolysis oils. The isolation of this compound provides additional support for Hibbert's theory of lignin as a mixture of simple and more complex stabilized products related to oxyconiferyl alcohol and derived from lignin progenitors constituting a group of hydrogen-transportation respiratory catalysts.

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