# THE STRUCTURE OF LIGNIN

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**A Thesis**

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for the Degree of Doctor of Philosophy

by

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# INTRODUCTION

Up to the present time numerous investigators have prepared lignin by a variety of methods. In their preparation drastic reagents have, in general, been employed and only a small percentage of the total lignin present in the wood has been isolated. This has long been recognized by Hibbert and co-workers but, in spite of the successes previously achieved, no satisfactory solution has yet been found. This is due to the remarkable sensitivity of native lignin (as present in wood) to the action of chemical reagents. Hibbert and co-workers recently attempted to isolate an unchanged lignin from wood meal (previously extracted with solvents to remove gums and resins) by first methylating with dimethyl sulphate and alkali at room temperature and then removing the methylated cellulose by hydrolysis with methyl alcohol at around 100°C. using hydrochloric acid as a catalyst. A methylated lignin was obtained in good yield (more than 50 per cent) with a methoxyl content of about 28 per cent,

but unfortunately it was not homogeneous and, after complete methylation with the same reagents, could be separated into fractions having methoxyl values ranging from 32 to 36 per cent. Some chemical change, apparently, had taken place in the lignin molecule during isolation. In view of this, the author was encouraged to undertake a new line of attack, in the hope of obtaining an unchanged homogeneous lignin in quantitative yield, by the use of mild reagents at room temperature.

## HISTORICAL REVIEW

It is unnecessary to discuss the previous work on lignin in great detail since this has been reviewed at length by Fuchs (1) for the period prior to 1926 and also, including the later work, by Phillips (2) in a recent publication. Freudenberg (3) has published a recent article on his own researches in the field of lignin chemistry and related compounds.

Along with these, several theses have been submitted from the Division of Industrial and Cellulose Chemistry of this University containing historical reviews of this subject, that of Dr. Buckland being, perhaps, the most complete.

Because of these excellent general reviews only the more important facts bearing directly on this subject need be mentioned.

### Present Conception Regarding the Structure of Lignin

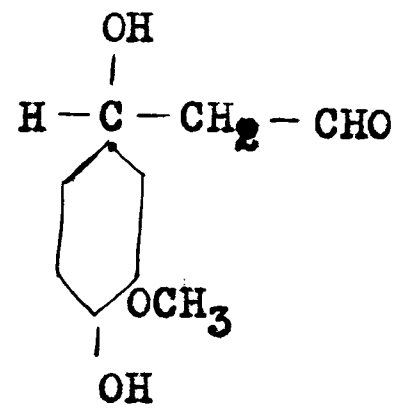
The bulk of the evidence as to whether lignin belongs to the aliphatic, aromatic, hydroaromatic or heterocyclic series points to the presence of aromatic nuclei or at

least cyclic rings readily convertible into aromatic substances (viz. hydroaromatic).

Willstätter and Kalb (5) support the theory of an aliphatic structure, while Jonas (6) favors the heterocyclic form and suggests that lignin is made up of furan nuclei. Both views are supported by only very weak experimental evidence. Freudenberg (7) is probably the most prominent supporter of the aromatic structure for the lignin molecule.

Klason (8) was the first to propose an aromatic structure for lignin. In his original theory, he suggested, in order to explain the $\alpha$ and $\beta$ forms of lignin sulphonic acid, that lignin is a condensation product of coniferyl aldehyde. He has continued to modify his theory concerning the mode of linkage and type of condensation product from time to time in order to keep pace with new data acquired by him or other workers. In 1932 he advanced the theory that lignin contains the building unit

(A)

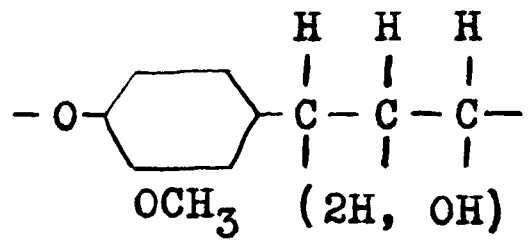


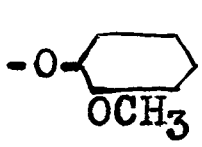
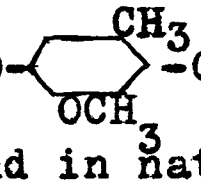
since Freudenberg's work indicated that there is no open chain ethylenic linkages in the aliphatic part of the molecule. The conversion of (A) into an acrolein compound is necessary to account for sulphonic acid formation.

Klason also believes in the principle of a chain mechanism in which an attempt is made to correlate the unions of the para-phenol groups by regarding them as entering into reaction with the aldehyde group in the following building unit with formation of a half acetal union. Freudenberg (9), in a recent communication, disproves this formula of Klason's. Such addition products of aldehydes and alcohols (half acetals) on account of their instability cannot be present in the lignin molecule. The half acetal formed from acetaldehyde and ethyl alcohol which is assumed to be present in a mixture of these two components (Meerwein) is decomposed into its components by simple distillation. Half acetals from phenols and aldehydes have never been isolated, a fact which undoubtedly is related to their ease of decomposition, if they are actually capable of existence, which seems doubtful. If lignin were formed from a series of molecules

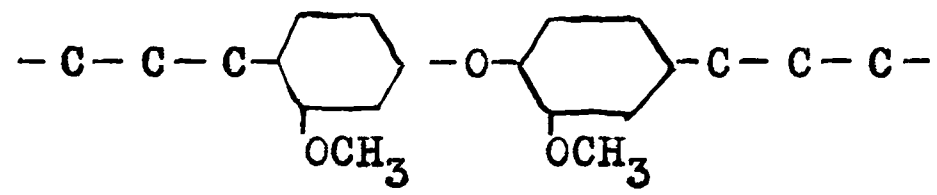
of coniferyl aldehyde joined together by free half-acetal linkages, it would be expected that the union between such units would be very unstable. It is much more probable that we are dealing with a condensation product in which one unit is irreversibly united to the next by a carbon to carbon linkage. Freudenberg proves this by the isolation of iso-hemipinic acid from the methylation products of partly degraded lignin.

For the building unit of lignin Freudenberg (10) assigns the expanded empirical formula $C_9H_8(OCH_3)(OH)$-(-O-) and for the structural arrangement



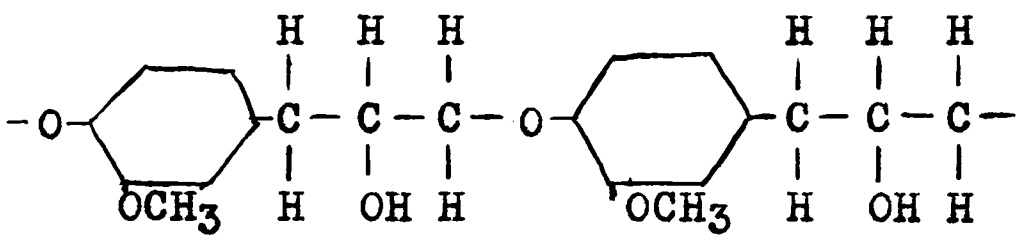
The three carbon atoms are in open chain formation since this arrangement denotes the simplest formula for lignin, and also because other types, for example  and  according to Freudenberg are seldom found in nature. The presence of the latter radical would be recognized in the alkaline

fusion product as methyl protocatechuic acid. The ether oxygen linkage cannot be present in the form of a diphenyl oxide linkage, for example

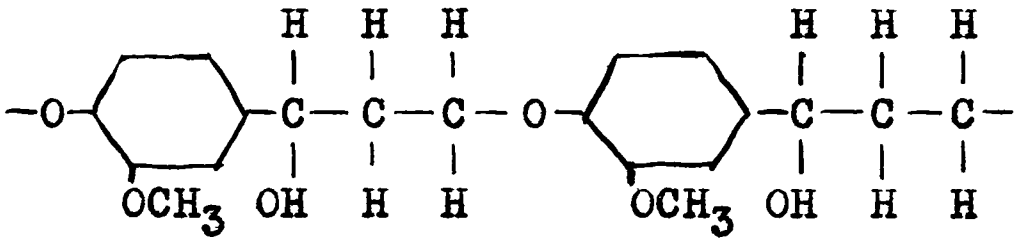


because in lignin there is, statistically, one ether oxygen for every benzene nucleus. In this building unit the aromatic nucleus must be connected with one of the aliphatic carbon atoms of the next building unit. On the other hand, if ether linkage occurs through the para position without nuclear condensation and methylation in meta (vanillin type) the following probabilities occur

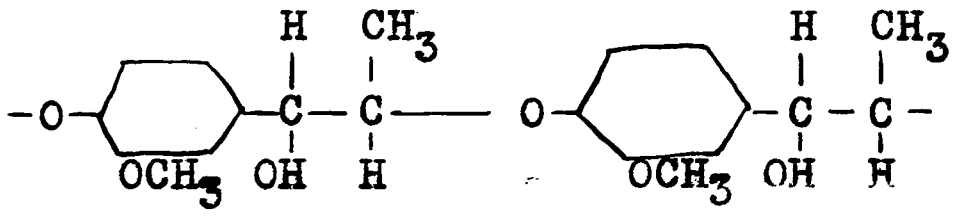
(a)



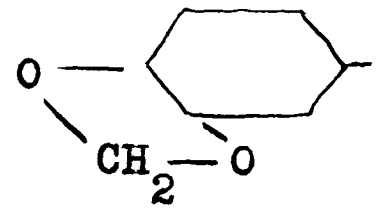
(b)



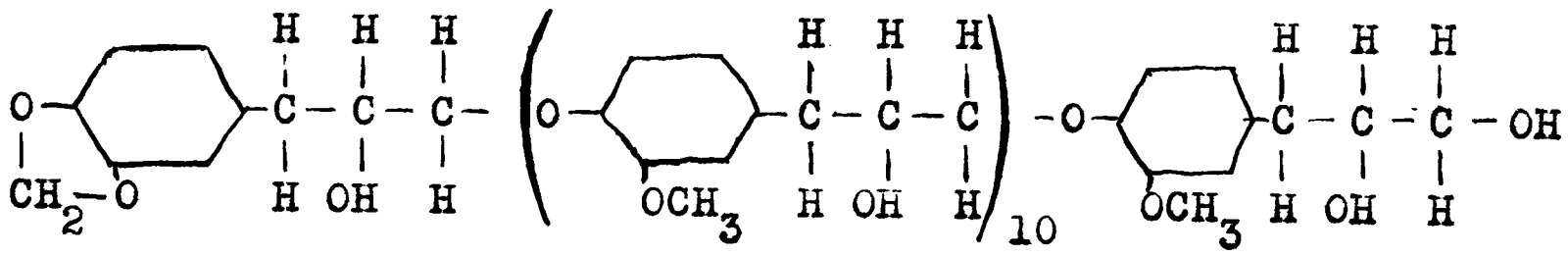
(c)



Further possibilities are excluded since no primary hydroxyls are present and the hydroxyl cannot be attached to the same carbon as the ether oxygen. The isovanillin type is possible instead of vanillin. Finally the terminal unit can be present as a methylene dioxide group



From the amount of formaldehyde split off Freudenberg concludes there are twelve building units in one chain. Such a chain corresponds to primary lignin (using (a) as a basis).



He also suggests that this primary lignin may condense in more than one plane to produce indefinite numbers of these building units, forming a secondary lignin of enormous molecular weight.

In a recent address (62) on the chemical constitution of spruce wood lignin, he summarizes his conclusions regarding the structure of spruce lignin as

follows: It may be regarded as proven that for every ten carbon atoms present in lignin six of them belong to a benzene nucleus; one of these carbon atoms is present as a methoxyl or a methylene dioxide group and the remaining three are present as a side chain.

From a study of other highly polymerized substances the conclusion may be drawn that lignin is formed by a step-wise polymerization of building units which may vary within certain limits and are capable of undergoing condensation in various ways. The building unit is probably dihydroeugenol (propyl guiacol) in the side chain of which three hydrogen atoms are replaced either by the hydroxyl group or an oxygen atom. For the combination of these units it is indispensable that the spruce wood lignin should possess no free phenol groups and for each ten carbon atoms there must be present approximately one bridged oxygen atom. There are numerous experimental results which support the point of view that this oxygen atom is present as part of a benzofuran or a benzopyran system formed from the oxygen and the benzene nucleus of the one building unit and two or three carbon atoms from a side chain of the following building unit.

The explanation of the changes underlying the condensation-oxidation of isoeugenol investigated by H. Erdtman provide confirmation for such systems.

According to Freudenberg this structural formula for lignin fulfils all the requirements of the known established facts : (a) absence of phenolic hydroxyl groups; (b) presence of aromatic methoxyl; (c) dioxymethylene and secondary hydroxyl groups in proportions shown in the formula; (d) completely amorphous nature; and (e) results of mercuration and bromination.

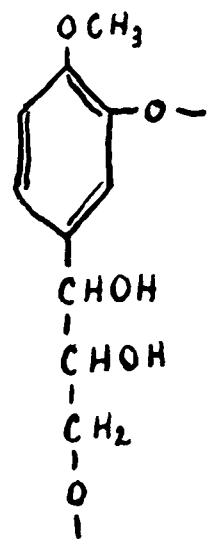
Although this scheme classifies many observed facts, it is entirely unsatisfactory in many points. One would expect the ether linkage to be severed by the action of hydriodic acid at 150°C. but this is not the case, the reduction products having high molecular weights. Further, it gives no explanation for the mechanism of the condensation of primary lignin. The conclusion that the formaldehyde obtained from lignin originates from a dioxymethylene group is questioned by many workers. Further criticism of Freudenberg's formula will be given near the end of the discussion of experimental results of this thesis.

In a recent article, Fuchs states that according

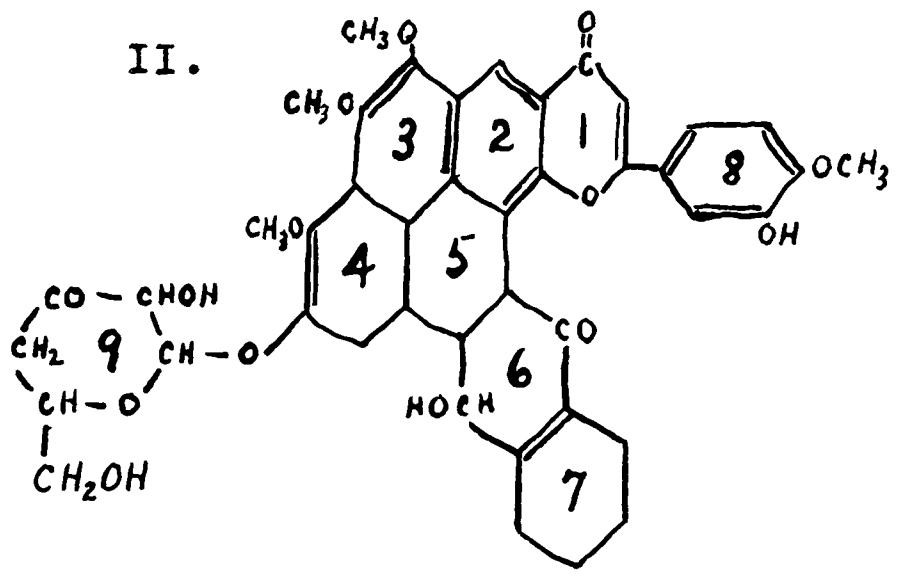
to Freudenberg's (50) conception, lignin is a giant molecule entirely built up of units shown in Formula I. Originally only linkages as indicated in this figure were assumed while later also other linkages comprising carbon linkages were taken into consideration. It may be mentioned that Klason (51) and others usually assumed the same building unit (I).

Since lignin is not a uniform product, according to Fuchs, a lignin formula cannot have the same meaning as in the case of uniform crystalline substances. Nevertheless, he has recently proposed a structure representing a hypothetical substance capable of satisfying all the known facts brought to light in the investigation of spruce lignin. This is represented in Formula II.

I.




II.



Such a compound would have the following properties:

1. The formula is $C_{43}H_{42}O_{14}$, the molecular weight 782, the ultimate composition 66.0 per cent C, 5.3 per cent H, 28.7, O and 15.9 per cent $OCH_3$. The oxygen functions comprise 4 methoxyl groups, 4 hydroxyl groups (one of them being phenolic in nature), 2 oxygen bridged rings and 3 cyclic keto groups. The average composition of spruce lignin complies with these properties and it has been found, for instance, that lignin in wood, as well as isolated lignin, on treatment with diazomethane, reacts like a phenol with one OH group in a molecule of 800 (52).

2. The Formula (II) contains in rings 1, 2 and 8, the flavon grouping, ring 8 being a guaiacol radical. Cleavage of ring 8 should give about 20 per cent protocatechuic acid (53) and a variety of guaiacol derivatives, for instance, vanillic acid, eugenol, propylcatechol (54). Fission of ring 1 would be expected to give mono oxybenzoic acids such as o- or p-oxybenzoic acid, and mono-oxy polybenzoic acids. Such results actually have been obtained with lignin.

3. The rings 5, 2, 3, 4, and 6 represent a condensed system which, on oxidation, should give benzene

carboxyllic acids. Such results have been obtained with spruce lignin (55) and also with humic acids which are closely related to spruce lignin (56).

4. Ring 7 can be expected to give on oxidation adipic acid and to undergo exhaustive bromination. Evidence for both is to be found in lignin research (57) (58).

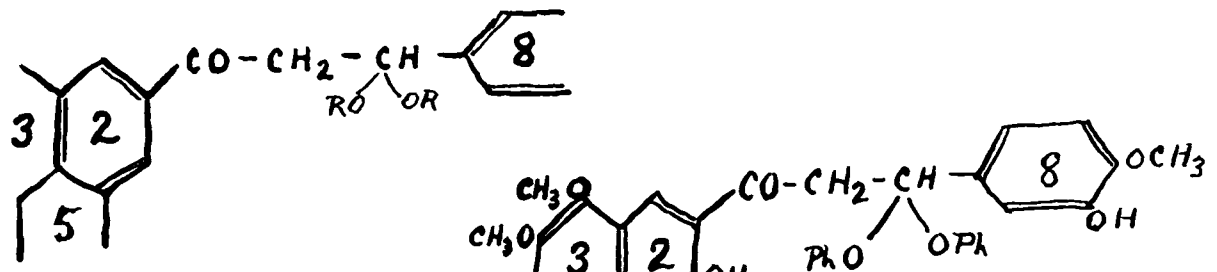
5. Ring 9 imparts to the combination the nature of a glucoside in which the sugar is unsaturated. Therefore, the combination can be expected to be very sensitive toward both alkalies and strong acids. This also applies to lignin and results obtained on treating wood with benzoyl peroxide point to the presence of an unsaturated sugar radical in lignin (59). This may possibly explain the formation of oxymethyl furfurol(60) from lignin as well as of formaldehyde.

6. The formula offers a possible explanation for the formation of certain lignin derivatives such as phenol lignins and alcohol or glycol lignins.

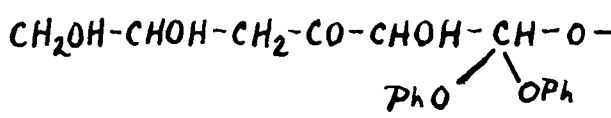
On treatment of wood with organic hydroxylic compounds, such as alcohols, glycols or phenols, in the presence of small amounts of catalysts, such as hydrochloric acid, lignin derivatives are formed which are

easily soluble in various organic solvents and in alkali. It was shown in 1929 (61) that the reaction most probably consists in opening an oxygen bridge similar to ring 1, Formula II, and therefore the products may be represented by Formula III and IV.

III.




IV.




## Views of Hilpert and Co-workers

Within the last year or two Hilpert (11) and co-workers have published a number of papers in which somewhat radical theories are advanced relative to the structure of lignin and its mode of formation from non-lignin plant constituents under the influence of strong chemical reagents.

Hilpert and Littmann (12) point out that lignin is the term used to indicate the product formed during the lignification of plant tissue, and that its presence serves to differentiate between lignified and non-

lignified woody tissues. Chemically speaking, lignin is characterized in that it cannot be converted by treatment with acids into soluble products as is the case with polysaccharides. It is, in fact, left as an insoluble residue when plant tissue or fibres are treated with strong mineral acids. For this hydrolysis König and Rump (13) use 72 per cent sulphuric acid while Willstätter and Zechmeister (14) use 42 per cent hydrochloric. The procedure varies but the principle is the same. The assumption has been made in all cases that the sugars present do not react with the acid to form insoluble material.

Hilpert and co-workers (12) have shown that this assumption is incorrect. They point out that carbohydrates under the influence of mineral acids readily undergo conversion into dark-colored,insoluble products, referred to generally as humic acids. It is therefore to be expected that water-insoluble polysaccharides present in wood would give rise to such derivatives if treated under similar conditions. This separation of dark material is not an uncontrollable decomposition but a constantly recurring phenomenon under controlled conditions. The sugars differ in a characteristic way,

in that some yield a very small amount (less than 1%) of dark residue with acids, while others yield as high as 51 per cent. In the series of hexose sugars galactose is the least susceptible to the action of acids, and similar to glucose and mannose in that it yields only a very slight insoluble precipitate when treated with sulphuric acid or hydrochloric acid, while the much more reactive fructose is very susceptible to change and yields more than 25 per cent insoluble matter.

From these facts it follows that any lignin determination which depends on the estimation of insoluble matter formed in total hydrolysis by use of acids leads to false results, particularly when the hydrolyzed carbohydrates contain fructose or xylose. For the same reason it is difficult, or impossible, to separate and determine fructose in resistant polyoses by hydrolysis with acids.

From its elementary analysis "fructose lignin" is seen to contain less hydrogen than the lignin obtained from plants. In spite of this, its composition falls within the experimental limits of the analytical values for lignin. An essential difference is the methoxyl

content which, in lignin isolated from wood, amounts to about 15 per cent, increasing on complete methylation to 28 per cent. "Fructose lignin" on complete methylation with dimethyl sulphate contains 12 per cent $OCH_3$ and the methylated product has a brown color similar to that of lignin prepared from wood by the use of 43 per cent hydrochloric acid. The former, however, is so similar to the lignin isolated from plants that its presence, based on such characteristics, in mixtures with true lignin cannot be determined.

The pentoses also are quite sensitive to the action of acids. Xylose gave a 36 per cent yield of water-insoluble product after treatment at room temperature for 48 hours with 72 per cent sulphuric acid followed by dilution, while over a period of about one week this was increased to nearly 52 per cent. The "pentose lignin" in contrast with "fructose lignin" is completely insoluble in alkali, is methylated with great difficulty, and in composition approximates that of lignin. The same is true of "arabinose lignin" containing C, 61.0 per cent and H, 4.5 per cent compared with Fisher and Schrader's spruce wood lignin C, 60.6 per cent and H, 4.5 per cent.

The action of highly concentrated hydrochloric acid on hexoses and pentoses is slight and less water-insoluble product is formed than with sulphuric acid, so that the product from hydrochloric acid should be more like that of true lignin. It is quite possible that some of the sugars, very sensitive to acids, are united with the polysaccharides as an insoluble complex (for example, the pentoses in union with pentosans) and these carbohydrates along with other unidentified complex sugars may be converted into water-insoluble "sugar lignins" during hydrolysis, thus contaminating the lignin that originally existed in wood. Thus many of the recorded observations in the literature are clarified, especially those relating to the dark color of sulphuric acid lignin. Willstätter and Kalb attempted to reduce spruce lignin by treating with concentrated hydriodic acid at 250°C. in order to obtain the basic lignin skeleton hydrocarbon. This unidentified dark colored material was similar to that obtained by Hilpert from carbohydrates under the same conditions. From which the latter concludes that a close relationship exists between both carbohydrates and lignin.

In a more recent publication Hilpert and Hellwage (16) claim that beech wood lignin is a decomposition product of carbohydrates present in the wood and that so-called "lignin" has no actual pre-existence in the wood but is formed in every case from the carbohydrates and polysaccharides present.

Beech wood differs from spruce in having a higher methoxyl value (21% as compared with 15%), a result, in Hilpert's opinion, not in harmony with the idea of a uniform lignin of definite building unit.

The "lignification" of certain sugars by acids takes place rapidly, according to Hilpert, at temperatures as low as -10°C. With fuming hydrochloric acid the reaction proceeds so rapidly that the yield of water-insoluble product after two hours was 12 per cent, the product having a composition midway between wood and true lignin

| | %C | %H | %$OCH_3$ |
|---|---|---|---|
| Wood | 49.3 | 6.0 | 6.2 |
| Residue I | 56.15 | 5.6 | 12.8 |
| Lignin I | 60.0 | 6.2 | 16.7 |

After 24 hours the reaction-product approaches lignin

so closely in character that the assumption of the liberation of the latter from other constituents in the wood is rendered doubtful. With more dilute acids (HCl, d-1.19) products were obtained intermediate between wood and lignin. Further treatment of Residue I at 15-20°C. gave Lignin I, similar to Hess' lignin (17).

Treatment of beech wood with strong acids brings about solution in large measure and, on dilution, a flocculent precipitate having a composition corresponding very closely to that of methylated cellulose anhydride $2C_6H_{10}O_5H_2O$ is obtained, having one methoxyl group per two cellulose anhydride groups. Treatment of this with hydrochloric acid at 15-20°C. gave a new lignin (II) in 8 per cent yield with a methoxyl value of 21 per cent. This is a maximum methoxyl value and corresponds to that reported in the literature as the value for beech wood lignin. From the properties of the solution it is evident that it can contain in every case only carbohydrates and water-soluble compounds, but no lignin in the usual sense. Thus, according to Hilpert, "beech lignin" is not an actual constituent of wood, but a reaction product. Its variation in composition with temperature indicates that the entire

lignin is formed by the action of acids on carbohydrates. That methoxyl groups were attached to carbohydrates and passed over partially into the insoluble reaction product was shown in one specific instance.

In a somewhat similar way Hilpert and Wagner (18) claim to prove that lignin does not exist in the leaves of plants, but is a product formed by the reagents used for its extraction.

The views of Hilpert and co-workers are at variance with the findings of all previous workers in lignin chemistry and will, therefore, be discussed critically. They are based on very incomplete experimental evidence and the conclusions drawn are quite unwarranted.

Many cases are known where furan derivatives are prepared by the action of acids on sugars. It is quite possible that Hilpert's 25 per cent "fructose lignin" is a condensation or polymerization product of some furan derivative such as hydroxymethylfurfural which has been prepared from fructose by the use of acids, and that its percentage composition resembles that of lignin.

Plunguian (25) has shown that humic acid is

prepared by the action of 72 per cent sulphuric acid on various carbohydrates, the procedure being identical with that of Hilpert. The elementary analysis was almost identical with that of the black "lignin" obtained by Hilpert and co-workers. The humic acids obtained from various mono- and di-saccharides by Plunguian could be methylated to a value of 21.9 per cent methoxyl, about the same as the "fructose lignin" obtained by Hilpert, and on which he bases his theory that "fructose lignin" is the same as beech lignin ($OCH_3$, 21%). Evidently Hilpert was working, not with lignin, but with humic acid, or a compound very closely related to it and this seems rather incredible since many other investigators have obtained humic acid from carbohydrates by the same chemical treatment.

Furthermore, it has been shown by Hibbert and Tomlinson (19) that vanillin forms a definite part of the lignin molecule, there being at least one vanillin group per building unit, a result entirely incompatible with Hilpert's view.

Hibbert and Brauns (20) have prepared a methanol spruce lignin by extracting spruce wood meal with

absolute methyl alcohol, using 2 per cent hydrochloric acid as a catalyst. The lignin obtained had a methoxyl value of 21.6 per cent. It was partially methylated with diazomethane ($OCH_3$, 24.8%), acetylated, and fully methylated with dimethylsulphate.($OCH_3$, 32.3%). From the elementary analysis and the ratio of methoxyl in the original methanol lignin to that of the diazomethane methylated and fully methylated compounds, a formula for the smallest building unit of the methanol lignin and for native lignin can be derived. The latter is represented by the empirical formula $C_{47}H_{52}O_{16}$ or, expanded, $C_{42}H_{32}O_6(OCH_3)_5(OH)_5$. The methanol lignin ($OCH_3$, 21.6%) when methylated completely with dimethyl sulphate and 30 per cent sodium hydroxide at 50-60°C. yielded a lignin containing 32.2 per cent $OCH_3$ and corresponded to the formula $C_{42}H_{32}O_6(OCH_3)_{10}$. Recently Harris, Sherrard and Mitchell (21) in an investigation on the progressive methylation of Klason lignin (72% sulphuric acid) with dimethylsulphate and sodium hydroxide obtained a completely methylated lignin with methoxyl content of 32.2 per cent, in agreement with the value obtained for fully methylated methanol lignin. Later, it was shown by Hibbert and co-workers that this

expanded empirical formula not only applied to methanol lignin but also to glycol lignin (22), Willstätter and Freudenberg lignins (24).

## Future Work on Structure of Lignin

What are the primary problems for solution in respect to the structure of lignin?

(1) Lignin must be isolated from wood in higher yields, and in an unchanged condition by using lower temperatures and milder reagents.

(2) The nature of the free OH groups and the OH groups corresponding to $OCH_3$ groups in native lignin must be determined.

(3) The relation between lignin present in soft and hard woods.

(4) Evidence for the different types of ring structure in lignin,(synthetic).

(5) Means must be sought for the breaking down of the lignin complex into aromatic and heterocyclic ring compounds.

This thesis is concerned chiefly with the first four problems and is the answer to problem three.

## DISCUSSION OF EXPERIMENTAL RESULTS

### Extraction of Oak Lignin from Oak Wood

Previous investigators have not been able to isolate lignin from wood by mild extraction methods in yields higher than 25 per cent except at elevated temperatures (above 100°C.) and then only in yields of around 30-40 per cent. Moreover, there seems to be a marked tendency for the formation of secondary products under the latter conditions (47)(27)(31)(46).

In view of this it was decided to investigate the extraction of acetylated lignin by the procedure involving acetylation of the wood, as worked out by Suida and Titsch (26). It was found that in the case of oak wood a crude fully acetylated lignin could be isolated in yields as high as 75 per cent of the total lignin present, and the pure deacetylated native lignin in yields higher than 50 per cent. Their original procedure was modified considerably in that all reactions were run at room temperature (20-25°C.). It was decided to investigate the preparation of oak lignin under mild conditions and to ascertain its structural relation to the lignin from soft woods.

Oak wood flour (300-500 mesh) previously extracted with alcohol-benzene (1:1), water, and 5 per cent alkali, was acetylated with a mixture of glacial acetic acid and acetic anhydride, using 0.5 per cent sulphuric acid as a catalyst. The acetylated product was dissolved in chloroform and the insoluble inorganic and other insoluble compounds filtered off. The final acetylated product containing acetylated cellulose, lignin and pentosans was extracted with acetone, in which the acetylated lignin is very soluble. The acetone solution was precipitated into water and finally purified by precipitation from dioxane into ether. This crude acetylated lignin represented about 30 per cent of the total acetylated product and had a methoxyl value of about 10 per cent. Repeated precipitations from dioxane into ether showed that the methoxyl value could be raised to about 11.1 per cent. The acetylated lignin still contained attached pentosans or cellulose, as showed by a pentosan determination (about 10%). An investigation of acetylated xylan showed that its solubilities in various solvents are very similar to that of the acetylated oak lignin. Hence, it is still a question whether or not the acetylated pentosans are

chemically attached to the acetylated lignin extracted by means of acetone. They are removed, however, during the isolation of the native oak lignin by deacetylation of the acetylated product, being left as an alkali insoluble product admixed with some lignin, the mixture having a methoxyl value of about 4 per cent. The alkali soluble portion (75%) was precipitated by addition of acid and when purified was found to have a methoxyl value of 20.6 per cent. On methylation with diazomethane, this value increased to a constant value of 26.6 per cent. Acetylation of these products, followed by a simultaneous deacetylation and methylation with dimethylsulphate and sodium hydroxide, yielded two fully methylated lignins each having a methoxyl value of about 37.6 per cent and being, apparently, identical compounds.

Combustions of the native oak lignin gave the following average values:- carbon, 63.98%; hydrogen, 6.19%; oxygen, by difference, 29.83%. From these values the following empirical formula is obtained: $C_{5.33}H_{6.19}O_{1.86}$ and $(OCH_3)_{0.67}$.

Based on a methoxyl value for native oak lignin of 20.6 per cent, the diazomethane-methylated product of 26.6 per cent and the fully methylated product of 37.6

per cent, the number of methoxyl groups in the smallest building unit can be calculated, if it is assumed that only two methoxyl groups are formed as a result of methylation with diazomethane. It is necessary to assume that two groups are methylated rather than one, as will be shown later, because of the odd number of groups methylated in the preparation of methanol oak lignin and its diazomethane-methylated derivative.

If "x" is equal to the molecular weight of native lignin and "y" equal to the total number of methoxyl groups present, the percentage of methoxyl is given by

$$\frac{31.02y \times 100}{x} = 20.6$$

while for diazomethane-methylated oak lignin the value is represented by

$$\frac{31.02(y+2)100}{x+28} = 26.6$$

from which "y" is found to be 6.04 and, substituting 6 for "y" in the equations, "x" is found to be 903.5 and 904.9 respectively. From these values and from the carbon, hydrogen, and oxygen values an empirical formula can be derived for native lignin, viz.

$$C_{48}H_{56}O_{17}$$

From the methoxyl values of this, of the diazomethane product, and of the fully methylated product, the formula for the isolated oak lignin can be expanded to

$$C_{42}H_{32}O_5(OCH_3)_6(OH)_6 \qquad \text{m.w. } 904.6$$

for diazomethane-methylated oak lignin

$$C_{42}H_{32}O_5(OCH_3)_8(OH)_4$$

and for fully methylated oak lignin

$$C_{42}H_{32}O_5(OCH_3)_{12}$$

## Relation to Spruce Lignin

From the expanded formula for native oak lignin, $C_{42}H_{32}O_5(OCH_3)_6(OH)_6$, it can be seen that it has one less oxygen atom and two more hydroxyl groups than the formula determined by Hibbert and co-workers for methanol spruce wood lignin, $C_{42}H_{32}O_6(OCH_3)_6(OH)_4$. This would suggest the possible loss of one molecule of water from two hydroxyl groups during the synthesis of spruce wood lignin in the plant with formation of an oxygen ring. In native oak lignin one of these hydroxyl groups may exist as a previously methylated radical, thus preventing the removal of a molecule of water during plant synthesis. Since native oak lignin has one more methoxyl group than native spruce lignin (20) this theory would appear feasible.

This oxygen ring theory may also explain why methanol spruce lignin prepared in an autoclave above 100°C. in the presence of hydrochloric acid (2.5%) can be fractionated into lignins with methoxyl values ranging, when fully methylated, from 32 to 38 per cent. It is quite possible that at the higher temperatures the oxygen ring, in the presence of alcoholic hydrochloric acid, undergoes ring fission forming either two new hydroxyl groups

capable of methylation or, in the case of a furan ring, giving rise to a dimethylal derivative. These two types of lignins would have similar solubilities and would, therefore, prove difficult to separate by fractional precipitation from organic solvents. Compton and Hibbert (27) succeeded in separating two different fully methylated compounds ($OCH_3$, 32.9% and 37.8%) along with fractions having intermediate values.

The diazomethane-methylated native oak lignin was acetylated and then subjected to a simultaneous deacetylation and methylation with dimethylsulphate and sodium hydroxide. The resulting product had the same methoxyl value (37.6%) as the compound obtained by direct methylation of the acetone extracted lignin, thus indicating the homogeneity and absence of foreign substances.

Native oak lignin, $C_{42}H_{32}O_5(OCH_3)_6(OH)_6$, contains six methoxyl groups and six hydroxyl groups, and has a molecular weight of 904.5, as compared with native spruce lignin, $C_{42}H_{32}O_6(OCH_3)_5(OH)_5$, molecular weight 872.5 with five methoxyl and five hydroxyl groups, and one more oxygen atom. This difference corresponds to the elements of one molecule of methyl alcohol.

## Spruce Wood Lignin

Spruce wood meal, previously extracted with alcohol-benzene, water, and 5 per cent alkali was methylated several times with dimethylsulphate and alkali until the methoxyl content became constant at 38.8 per cent. The methylated wood meal was subjected to treatment with anhydrous methyl alcohol containing about 4 per cent dry hydrogen chloride in an autoclave at 100°C. for a period of four days. The methylated cellulose was hydrolyzed to methylated methyl glucosides which remained in solution on precipitation of the lignin by water. A yield of approximately 50 per cent of partially methylated lignin ($OCH_3$, 28%) was obtained, which when fully methylated could be fractionated into fractions having methoxyl values ranging from 32 to 35 per cent. Evidently some type of decomposition had taken place and possible ring scission had occurred, forming more hydroxyl groups which could be methylated, or else there was more than one type of lignin present. This was indeed disappointing, as it was hoped that such a method would be excellent for preparing methanol lignin in good yield.

Since the product obtained was not homogeneous,

it was considered advisable to search for a new method of isolating unchanged native lignin. As indicated, a method was found but, unfortunately, it was only applicable to hard wood lignin.

Numerous modifications of the oak-wood acetylation technique were employed with spruce wood meal in which various concentrations of sulphuric acid ranging from 0.1 to 0.5 per cent were used at temperatures varying from 20-30°C. and over periods of two days to six weeks. Crude acetylated lignins were obtained with very low methoxyl contents ranging from 2.9 to 4.5 per cent. The time of reaction seemed to be the most important factor. Thus lignin obtained in an experiment of six weeks' duration using 0.1 per cent sulphuric acid and at 25°C. had a methoxyl content of 4.5 per cent while that of a product obtained by acetylating for a period of three days at 30°C. using 0.5 per cent sulphuric acid was 2.9 per cent. An analysis showed the presence of about 30 per cent pentosans. These pentosans were so firmly attached that they could not be removed by alkaline hydrolysis as in the case of oak lignin, where only a small percentage was present. The deacetylated product was obtained in very small yield and had a methoxyl content of 13 per cent.

This method of extraction by acetylation and solvent recovery which gave such excellent results with oak wood, proved to be worthless with spruce, chiefly because of the very low yield of the latter. The material other than spruce lignin can be removed, however, by a simultaneous deacetylation and methylation of the acetylated product using dimethylsulphate and sodium hydroxide, the methylated pentosans being soluble in water. If any chemical union exists between the lignin and pentosans originally extracted, it is broken during the methylation treatment. The methylated product obtained (about 3 per cent of the original wood) had a methoxyl content of 32.8 per cent, the same as that obtained by the complete methylation of spruce methanol lignin by Hibbert and Brauns (20). This is of particular interest in that it confirms the validity of their work on fully methylated spruce and its homogeneity, and shows the direct difference between spruce and oak lignins prepared by the same procedure.

## Nature of the Hydroxyl Groups

The extent of diazomethane methylation of spruce lignin points to the presence of one phenolic or enolic group, while with native oak lignin, $C_{42}H_{32}O_6(OCH_3)_6(OH)_6$ the presence of two phenolic or enolic groups is indicated. Of the remaining four aliphatic hydroxyl groups, three undergo methylation with methyl alcoholic hydrogen chloride in the preparation of methanol oak lignin and yield a compound having 29.5 per cent methoxyl corresponding to exactly nine methoxyl groups, $C_{42}H_{32}O_5(OCH_3)_9(OH)_3$.

A careful fractionation of this product showed that the dioxane-ether soluble portion (about 25%) was identical with the ether insoluble in so far as the methoxyl content is concerned. Evidently it was a portion of the insoluble which did not precipitate from the 10 per cent dioxane-ether solution, and consequently suggests that the product was homogeneous. This is not the case with spruce wood lignins.

The diazomethane methylation of methanol oak lignin gives a compound with a methoxyl content of 32.4 per cent, indicating that only one hydroxyl group has been methylated, the product corresponding to the formula $C_{42}H_{32}O_5(OCH_3)_{10}(OH)_2$.

It is evident that the two hydroxyl groups in native oak lignin, methylatable with diazomethane, are not alike since only one of them is methylated with alcoholic hydrogen chloride in the preparation of methanol oak lignin. This would suggest the possibility of one phenolic group and one enolic group since the latter can, in some cases, be methylated with methyl alcohol and hydrochloric acid.

Treatment of the diazomethane-methylated native oak lignin, $C_{42}H_{32}O_5(OCH_3)_8(OH)_4$, with triphenylchlormethane gives a compound with a methoxyl content of 17.5 per cent, which corresponds to the replacement of two hydrogen atoms of hydroxyl groups by trityl groups, $C_{42}H_{32}O_5(OCH_3)_8$-$(OC(C_6H_5)_3)_2(OH)_2$, and indicates the presence of two primary alcoholic groups. When this compound is further treated with p-toluene sulphonyl chloride, the remaining two free hydroxyls are tosylated, giving the compound

$$C_{42}H_{32}O_5(OCH_3)_8(OC(C_6H_5)_3)_2(OSO_2C_6H_4CH_3)_2$$

and pointing to the presence of two secondary alcoholic groups.

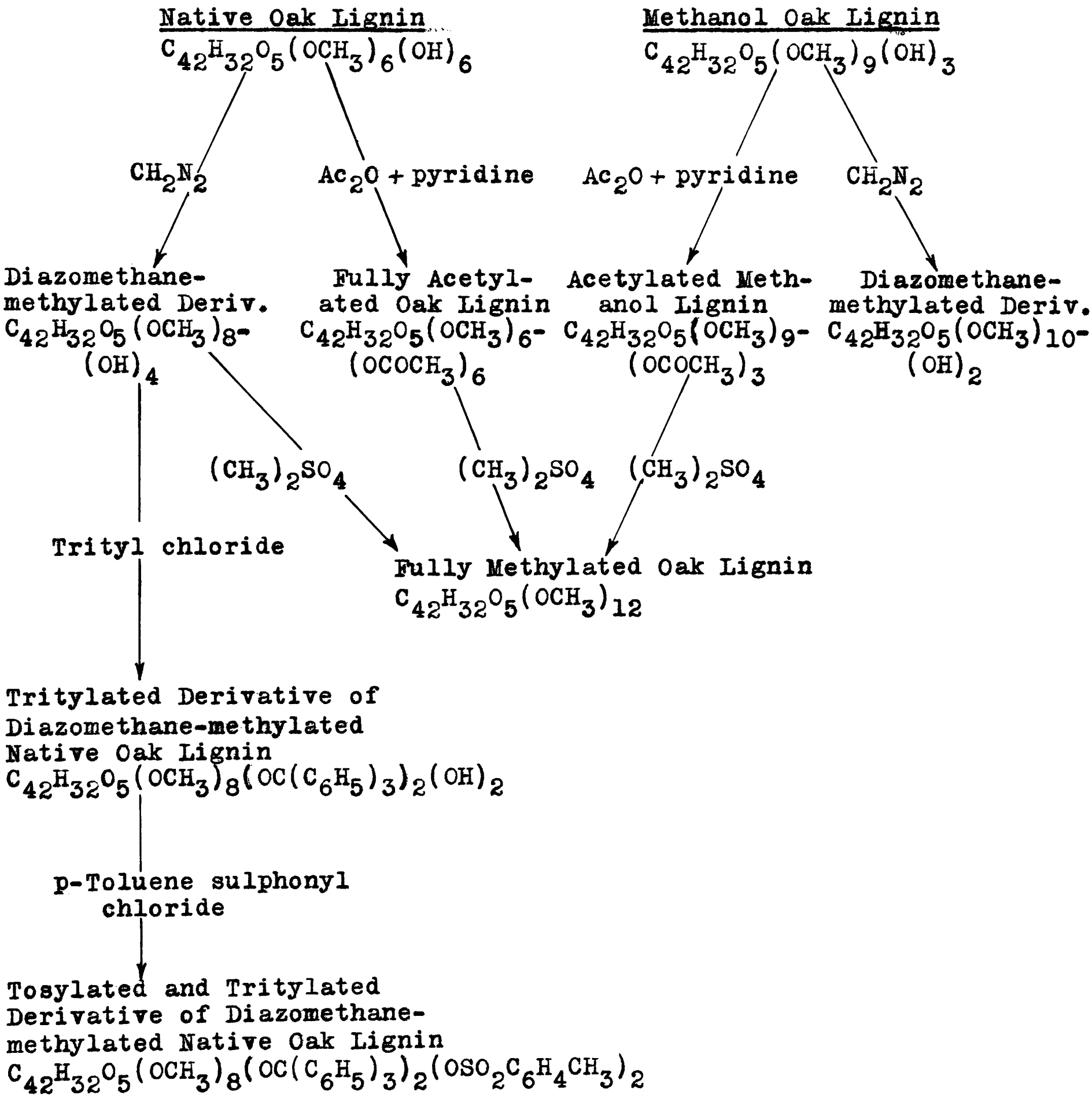
Hence, of the six free hydroxyl groups in native oak lignin one is probably phenolic, another enolic, two are primary, and two secondary. Since aliphatic methoxyl groups

are but rarely found in nature, it seems logical to assume that the six methoxyl groups in native lignin are phenolic in character. Conversely, if we have seven methoxyl groups in lignin, there must be at least two benzene rings, and possibly three or four.

A summary of the methylated products showing the step-wise methylated derivatives of oak lignin follows:

| | |
|---|---|
| Native oak lignin | $C_{42}H_{32}O_5(OCH_3)_6(OH)_6$ |
| Diazomethane methylated native oak lignin | $C_{42}H_{32}O_5(OCH_3)_8(OH)_4$ |
| Methanol oak lignin | $C_{42}H_{32}O_5(OCH_3)_9(OH)_3$ |
| Diazomethane-methylated methanol oak lignin | $C_{42}H_{32}O_5(OCH_3)_{10}(OH)_2$ |
| Fully methylated native or fully methylated methanol oak lignin | $C_{42}H_{32}O_5(OCH_3)_{12}$ |

## Flow Sheet Showing Various Derivatives of Oak Lignin



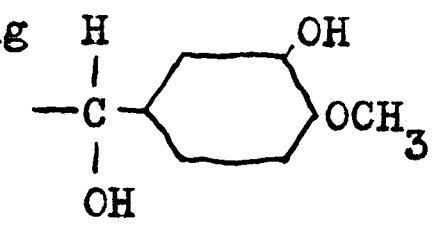
## TABLE I

Comparison of the Analyses of Oak Lignin and some of its Derivatives, with Theoretical Values Calculated on Basis of Proposed Formula for Native Lignin $C_{48}H_{56}O_{17}$

| Substance | Av. Found Values (ash free basis) | | | | Theoretical Values | | | |
|---|---|---|---|---|---|---|---|---|
| | %C | %H | %$OCH_3$ | %$CH_3CO$ | %C | %H | %$OCH_3$ | %$CH_3CO$ |
| Native Oak Lignin | 63.98 | 6.07 | 20.8 | ----- | 63.68 | 6.24 | 20.6 | ----- |
| Diazomethane Methylated | 64.40 | 6.50 | 26.6 | ----- | 64.36 | 6.48 | 26.6 | ----- |
| Fully Methylated | 65.26 | 6.80 | 37.7 | ----- | 65.56 | 6.93 | 37.6 | ----- |
| Fully Acetylated | 62.35 | 5.94 | 16.8 | 22.9 | 62.3 | 5.9 | 16.1 | 22.3 |
| Acetylated Diazomethane Methylated | 62.8 | 6.1 | 21.0 | 16.7 | 63.24 | 6.23 | 22.5 | 15.6 |
| Methanol Oak Lignin | 63.15 | 6.53 | 29.5 | ---- | 63.20 | 6.61 | 29.5 | ----- |
| Diazomethane Methylated Methanol Oak | 64.8 | 6.74 | 32.4 | ---- | 64.97 | 6.72 | 32.3 | ----- |
| Fully Methylated Methanol Oak | 65.53 | 6.96 | 37.8 | ---- | 65.56 | 6.93 | 37.6 | ----- |

Action of Bisulphites on Native Oak Lignin (28)

Hitherto it has not been found possible to convert readily, if at all, an isolated lignin into the corresponding sulphonic acid. Heating with aqueous solutions of bisulphites under pressure either is without effect or else prolonged action is necessary to effect partial solution. On the other hand, it is well known that when untreated wood is subjected to this treatment the lignin is readily separated in the form of a soluble lignin sulphonic acid. The nature of the changes during the isolation of the lignin which gives rise to this behavior is quite unknown. It is certain, however, that the oak lignin used in this investigation possesses quite different properties from other lignins isolated by more drastic methods, in that it forms a lignin sulphonic acid. Owing to lack of time the behavior of the lignin sulphonate from oak wood and oak lignin towards hot alkali could not be investigated, but it is to be expected that either vanillin or a related aldehyde will be isolated in this manner, thus confirming the presence of the grouping




in lignin.

In this event it would be possible to expand the structural formula for native oak lignin as follows:

$$C_{29}H_{21}O_5(OCH_3)_5(>CH.C_6H_3.OCH_3,OH)(-CH=\overset{OH}{C}-)(CHOH)_2(CH_2OH)_2$$

(A) (B)* (C) (D) (E)

Of the five methoxyl groups (A), all are possibly aromatic as aliphatic methoxyl groups rarely occur in nature. The methylation of one hydroxyl by both diazomethane and alcoholic hydrogen chloride suggests the possibility of (C). The presence of two primary hydroxyl groups (E) has been shown by tritylation experiments, while the presence of two secondary hydroxyl groups (D) has been indicated by tosylation experiments of the trityl derivative.

## Heterocyclic Oxygen Rings in Lignin

Since lignin does not contain aldehyde or ketone groups (unless exceedingly inert) it seems logical to assume that the remaining unknown residue $C_{29}H_{21}O_5$ consists of heterocyclic oxygen rings. It has become a matter of increasing importance to determine the exact nature of these oxygen rings, which consist, probably, of a fused system of furan, pyran and benzene rings. It was with this in view that the subject of mercuration was investigated.

* Tomlinson and Hibbert (19)

Use of Mercury Salts for Determination of Ring Structure

According to Dimroth (29) mercuric acetate is one of the best reagents for indicating the presence of an aromatic ring structure. The hydrogen atom of the ring is replaced by the acetoxy-mercuri group ($-HgOCOCH_3$). It is known that the mode of addition to ethylenic double bonds depends on the nature of the solvent used, but since numerous investigators (31) have shown the absence of ethylenic double bonds in lignin, this phase will not be considered.

Freudenberg (30) applied this reaction to an insoluble partially methylated lignin ($OCH_3$, 28%) and was able to introduce 33 per cent mercury. The product formed was insoluble in all solvents and possessed a methoxyl value which did not agree with the assumption that the only reaction was the replacement of H atoms by $-HgOCOCH_3$ groups. (The found value for the methoxyl content was 13.8 per cent, as compared with the value 16.0 per cent calculated on the mercury content.) The reaction was carried out in an absolute ethyl alcohol solution of mercuric acetate. Freudenberg's work is of no value in so far as proof of structure is concerned, except that it confirms the presence of aromatic rings.

Gray (31) applied the reaction to fully methylated

glycol lignin in a mixture of mercuric acetate, anhydrous ethyl alcohol and dioxane at the boiling temperature of ethyl alcohol. The products obtained were soluble in organic solvents and possessed methoxyl contents agreeing very closely with the assumption that the sole reaction was substitution of hydrogen by acetoxy-mercuri groups. The reaction took place slowly and the mercury content increased gradually to 42.7 per cent after 120 hours. On the basis of Gray's proposed formula for glycol lignin with a molecular weight 762, this value corresponded to the introduction of about four acetoxy-mercuri groups (theoretical mercury value, 43.2%). This work, like Freudenberg's, indicates the presence of aromatic rings and suggests the possibility of four rings, since in alcoholic solution generally only one acetoxy-mercuri group is introduced under these conditions.

The mercuration reaction was investigated by the author with the object of showing the presence of ring structures in lignin by comparing its behavior with that of certain type substances.

It is known that the manner in which mercury salts react with organic compounds varies with the experimental

conditions of the reacting medium (solvent used, acidity, temperature, and nature of the mercury salt employed) and the various anomalies arising in this connection have led to markedly different structural interpretations.

It has been stated (33) that mercury replaces the most acidic hydrogen atom and forms numerous double compounds having both bonds of mercury attached to carbon. For the aromatic series, Gilman (33) has suggested that if mercuration is a function of relative acidity, then the hydrogen atom replaced in a given reaction should be distinguished by most pronounced acidic function. This, however, is not the case with fluorene (34) which mercurates in the four or three position (depending on the solvent used) instead of the nine position, the latter characterized by a hydrogen atom which reacts readily with sodium and the Grignard Reagent. Other investigators (35) have shown that mercuric acetate mercurates abnormally in the aromatic series, generally in the position least expected.

However, since the author was not concerned with orientation, with complex reactions of ethylenic compounds, or with groups such as C=O, $NO_2$, $NH_2$, etc. which give ill-defined compounds, it was possible so to restrict the field

of application of the mercuration reaction that it could be regarded as of diagnostic value, especially where conditions could be found which would permit of specific mercuration of certain ring compounds and not of others.

## Types of Compounds Mercurated

It was deemed advisable to establish type substances representing possible constituents as such, or as radicals present in lignin. The classes calling for consideration were:-

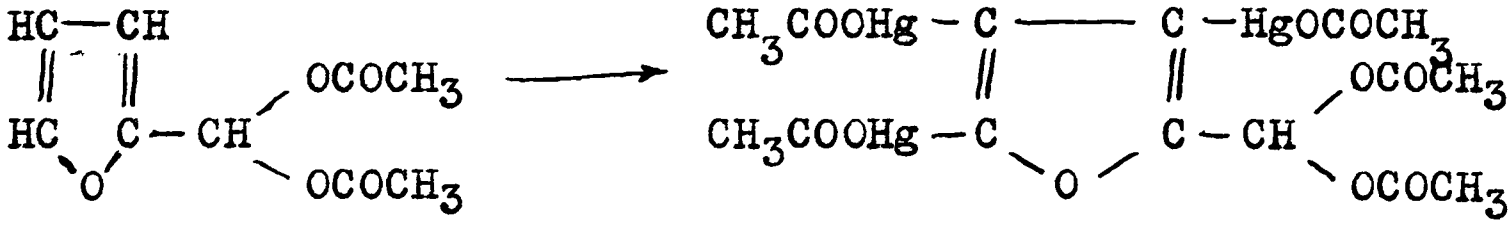
1) Unsaturated aliphatic groups.
2) Unsaturated aromatic compounds.
3) Ketones and aldehydes.
4) Furan derivatives.
5) Flavones and chromones.
6) Keto-enol derivatives.
7) Pyrones and other heterocyclic compounds.

As a result of numerous experiments, it was found that the mercuric acetate reagent used by Scheibler (36) appeared to offer most advantages. The important feature of this substance is that mercuration takes place at room

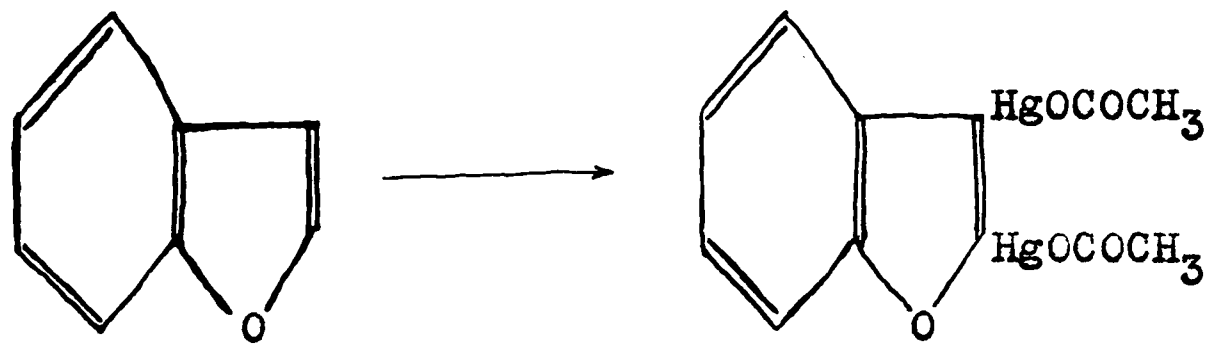
temperature and it could thus be applied to lignin studies without any possibility of rearrangements or decomposition. His procedure consists in dissolving the substance under investigation in a saturated solution of mercuric acetate in glacial acetic acid, and allowing the mixture to stand at room temperature for 48 hours. The reaction product is then evaporated to dryness, the acetoxy-mercuri product formed converted into its mercuri chlor derivatives by the action of hydrochloric acid. A preliminary investigation of the reaction showed that the acetoxy-mercuri compounds are soluble in chloroform and can be purified by precipitation into ether, while the inorganic mercury salts themselves are insoluble.

It was found that furan derivatives can be readily and completely mercurated under these conditions. Benzene derivatives such as anisaldehyde and benzalacetophenone do not react under these conditions, unless there is at least one phenol hydroxyl group present.

Furfural diacetate with excess of mercuric acetate yielded a product containing three acetoxymercuri groups



Similarly methyl-furfuraldiacetate was found to react with two moles of mercuric acetate forming a diacetoxymercuri-methyl-furfuraldiacetate; acetoxy-methyl-furfuraldiacetate yielded diacetoxymercuri-acetoxymethyl-furfuraldiacetate, and methyl-furfurol a triacetoxymercuri derivative. With furans and substituted furans all the unsubstituted nuclear hydrogen atoms are replaced by acetoxy-mercuri radicals. Coumaron also gave a well-defined diacetoxy-mercuri derivative.




Many different types of compounds containing the benzene ring were investigated, and it was found that compounds containing a free OH group, such as vanillin, quercetin, and p-hydroxybenzaldehyde, could be mercurated under these conditions yielding derivatives containing one or more acetoxy-mercuri groups. No reaction took place, however, when the hydroxyl group was replaced by methoxyl. Veratric aldehyde and anisaldehyde could not be mercurated, showing that neither the benzene ring nor

the aldehyde group was attacked by the reagent.

Attempts were made to mercurate ketones and unsaturated ethylenic compounds such as the simple chalcone, benzalaceto-phenone, and the substituted chalcone, 4-acetoxy-3-methoxy chalcone. In both cases the starting materials were recovered quantitatively. Evidently this reagent which mercurates furan derivatives so readily will not even add to open chain ethylenic linkages; however, this is of little value concerning the proof of lignin structure since no open chain ethylenic linkages exist in lignin.

Hydroxy-pyrones such as kojic acid could not be mercurated when the hydroxyl groups were protected by methoxyl or acetyl groups. When not protected, one acetoxy-mercuri group entered the molecule.

Fused pyrone-benzene systems such as quercetin (a compound found in nature as the glucoside quercetrin) when fully acetylated could not be mercurated under the conditions described above.

Another unsaturated compound, δ-methoxy-levulinaldehyde-dimethyl-acetal, was found to remain unchanged after treatment with the mercuration reagent.

Of the various types of compounds investigated—types which possibly exist in lignin—none could be

mercurated when the hydroxyl groups were protected, with the exception of furan derivatives.

## Mercuration of Different Types of Lignin

Fully methylated glycol lignin when treated in the usual manner with a saturated solution of mercuric acetate in glacial acetic acid at room temperature for two days yielded a mercurated derivative having 34 per cent mercury. Using the Brauns and Hibbert formula for glycol lignin, this corresponds to the entrance of three acetoxy-mercuri groups. $C_{42}H_{29}O_6(OCH_3)_9\ (OCH_2CH_2OCH_3)$-$(HgOCOCH_3)_3$ requires 34 per cent mercury.

This mercurated product was given a second treatment with the same reagent over a period of two weeks. The product gave the same percentage of mercury on analysis. Evidently no further mercuration took place.

These facts seem to indicate the presence of furan rings in lignin. Since all positions of the furan rings (not already substituted) can be mercurated, it cannot be said just how many rings are present, but it seems likely that there are two and possibly three.

The higher value (42.7% mercury) obtained by Gray for glycol lignin was perhaps due to the mercuration of

a benzene ring at the higher temperature employed (80°C.) and in an alcohol medium.

Fully methylated methyl alcohol lignin prepared by extracting methylated spruce wood meal in an autoclave slightly above 100°C. in the presence of 4 per cent methyl-alcoholic hydrogen chloride was mercurated under the same conditions at room temperature and a compound was obtained having a mercury value of 27.8 and methoxyl of 20.6 per cent. On the basis of the Brauns-Hibbert formula, this corresponds to the entrance of two acetoxy-mercuri groups. Calculated for $C_{42}H_{30}O_6(OCH_3)_{10}(HgOCOCH_3)_2$; %Hg, 27.5; $\%OCH_3$, 21.2. The fewer number of acetoxy-mercuri groups entering this lignin may be attributed to the possible scission of a furan ring in the preparation of the lignin. Furan rings in general are broken by the action of methyl alcohol and hydrochloric acid at high temperatures under pressure.

Methanol spruce lignin (20), $C_{42}H_{32}O_6(OCH_3)_6(OH)_4$, on the other hand, yielded a product containing 46 per cent mercury. This corresponds to the entrance of five acetoxy-mercuri groups calculated for $C_{42}H_{27}O_6(OCH_3)_6(OH)_4$-$(HgOCOCH_3)_5$; Hg, 45.0%. This high value may be explained by the presence of a phenolic hydroxyl group methylatable

with diazomethane, and also by a free hydroxyl group in some ring structure other than that of the furan or aromatic type (for example, an enol group such as that present in kojic acid).

Fully methylated native oak lignin, $C_{42}H_{32}O_5(OCH_3)_{12}$, was mercurated in the usual way over a period of two days using an excess of a saturated mercuric acetate glacial acetic acid solution. The resulting product showed, on analysis, Hg 34.2%, or the theoretical value for the addition of three acetoxy-mercuri groups (Theo. Hg, 34.0%).

It thus seems probable that in the expanded formula for oak lignin already discussed:

$$C_{29}H_{21}O_5(OCH_3)_5(>CH.C_6H_3.OCH_3,OH)(-CH=\overset{OH}{\dot{C}}-)(CHOH)_2(CH_2OH)_2$$

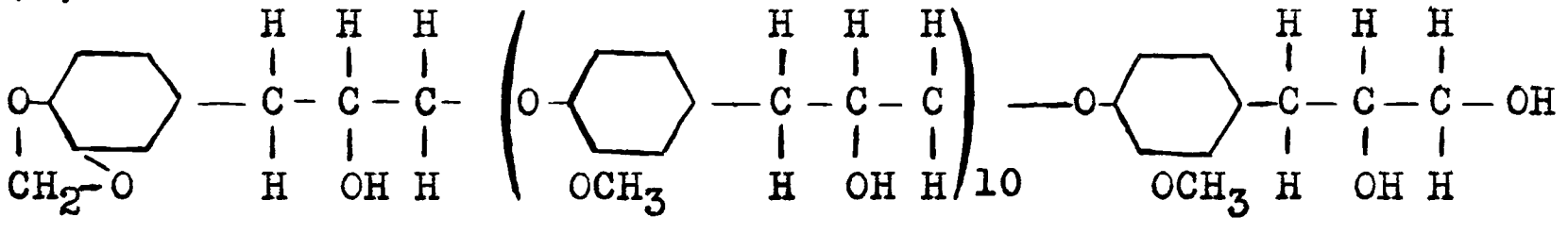
the residual unknown grouping $C_{29}H_{21}O_5$ is made up, at least in part, of furan rings, although the presence of other heterocyclic oxygen rings is not excluded.

## Criticism of Freudenberg's Formula

The results obtained by the writer indicate that Freudenberg's formula is incorrect for the following reasons:

### Structural Formula of Spruce Lignin (Freudenberg)(48)

(A)

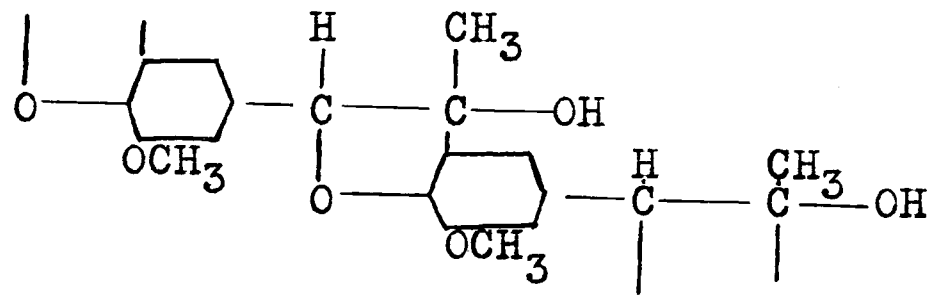


1. The formula shows the absence of free phenolic and enolic groups capable of methylation with diazomethane. It has been shown definitely by Hibbert and co-workers (20)(22)(23)(24) that the various types of spruce lignin contain one phenolic or enolic group capable of diazomethane methylation. The author has shown that oak wood lignin contains two such groups and has the same general structure as spruce wood lignin in so far as the building unit is concerned.

2. The ether linkage should undergo scission with hydriodic acid at 150°C. but this is not actually the case with spruce lignin, the reduction products of which have high molecular weights and resemble lignin. In a more recent publication (1933) Freudenberg (37) appears to have abandoned his former idea that the nuclei are joined

through ether linkages and now suggests that union may take place as indicated in the structure

(B)



3. Such a formula does not readily account for the production of vanillin in a yield of around 7-8 per cent from spruce lignin sulphonic acid.

4. Both spruce and oak lignin have been shown to contain two primary alcohol groups.

5. The lignin structures shown above, (A) and (B), would not undergo mercuration under the conditions employed by the author, using either the native or a methylated derivative.

Although Freudenberg has mustered more facts in support of his ideas than any other author of a tentative structure for lignin, his formula is not only inadequate to explain many known chemical facts regarding the properties of lignin, but is in actual conflict with many of these findings.

Criticism of Results Obtained by Hilpert and Co-workers (12)

It has long been known that a small amount of black material is formed from most sugars by treatment at a low temperature (5-10°C.) with 70-72 per cent sulphuric acid for about two days. This black residue has generally been termed "humic acid" and in most cases does not exceed 0.5 per cent of the total amount of carbohydrate used. The author has investigated the action of the same reagent on glucose, xylose and xylan at 5-10°C. for twelve hours. After dilution of the solution to a concentration of 2.5 per cent under conditions in which temperature increase was avoided, it was found that water-insoluble products had not formed in any of the three cases. Refluxing of the diluted solution for about six hours, however, gave 0.26 per cent of a flocculent black precipitate in the case of xylose, 0.47 per cent with xylan, and none with glucose. As no insoluble matter had been formed prior to refluxing, it is evident that the presence of such sugars could not interfere with the determinations of lignin. This finding is in marked contradiction with the results of Hilpert and Littmann who claim to have obtained a yield of 33 per cent "lignin" from xylan and of 35 per cent from xylose under similar conditions. The author has found,

however, that if either xylose or xylan is heated to 105°C. for 12 hours prior to the acid treatment a yield of humic acid or so-called "lignin" is obtained of 16.9 and 26.9 per cent respectively. On the other hand, it is remarkable that if the pre-drying is effected at a somewhat lower temperature (100°C. instead of 105°C.) for 12 hours the yield of water insoluble product ("humic acid") was 0.23% and 0.31% for xylose and xylan respectively. It should also be pointed out that the oak lignin isolated by the author as a fully acetylated product was obtained by acetylating oak wood meal at room temperature with acetic anhydride using concentrated sulphuric acid (0.5%) as a catalyst. The acetylated lignin was extracted, as such, by organic solvents (chloroform and acetone) without subjecting it to any further chemical action. The crude acetate thus obtained contained about 10% methoxyl and, in addition to the fully acetylated lignin, there were also present acetylated polysaccharides. The latter could be readily removed by treatment at 20-25°C. with dilute alkali. Under the above conditions, fructose undergoes acetylation forming fructose pentacetate which by hydrolysis with dilute alkali is converted into the original sugar without formation of humic acid or "lignin" products.*

* Hudson and Brauns have prepared fructose pentacetate by treatment of fructose with acetic anhydride containing 8% sulphuric acid (32).

Inasmuch as fructose is by far the most reactive of all hexose and pentose sugars, the fact that no water-insoluble humic acids or lignin products are formed would indicate the same behavior for the more stable sugars such as glucose, mannose, galactose, xylose, arabinose and the related polysaccharides, cellulose and pentosans. Furthermore, acetates formed simultaneously with the lignin acetate during the mild acetylation of the wood meal can be separated either by the use of organic solvents or dilute alkali. These reagents are incapable of bringing about decomposition to insoluble humic acids or "lignin".

## Criticism of Fuchs' Formula

The structural formula recently proposed by Fuchs (see p. 11) is entirely hypothetical, but according to him, it is capable of satisfying all the known facts brought to light in the investigation of spruce lignin. While it is in harmony with many of the experimental facts, it does not agree with some of the recent findings of other investigators.

Hibbert and co-workers (20)(22)(23) have shown that the building unit of spruce lignin contains five methoxyl and five hydroxyl groups and has a molecular weight of 872, as compared with the four methoxyl groups, four hydroxyl groups, and molecular weight of 782 postulated by Fuchs. They have also proven the presence of two primary alcohol groups, while the structure proposed by Fuchs contains only one.

Furthermore, the author's results on the mercuration of "type substances" related to lignin would indicate the improbability of any mercuration whatever occurring with such a structure under the conditions used. Methanol spruce lignin, on the other hand, reacts to give a penta-substituted acetoxy-mercuri compound, thus indicating five different points of attack within the building unit.

# EXPERIMENTAL PART

## I PREPARATION OF OAK LIGNIN

### (a) Preparation of the Wood Meal

The wood used for the preparation of the lignin was a section of a red oak log about eight inches in diameter from a freshly cut tree grown in the north-western part of New Brunswick. The wood was converted into shavings which were air-dried, then ground to a fine flour (300 mesh).

After drying the wood flour to constant weight at 50°C. in a vacuum oven, 800 gm. was placed in a cotton bag and extracted for 48 hours in a Soxhlet extracter with a mixture of benzene and 95% ethyl alcohol (1:1) in order to separate waxes, gums, resins, etc. The excess solvent was removed by filtration and suction on a Büchner funnel, followed by a period of air drying, the last traces of the solvent being drawn off at 50°C. in a vacuum oven. It was found that 3.03% of the wood was lost in the extraction process. When this extract was concentrated by removal of the alcohol and benzene a dark red viscous syrup was obtained, having a pleasant aromatic odor.

The wood flour was then extracted several times with hot water (about 50-60°C.) to remove soluble carbohydrates. After three days the mixture was filtered and the wood allowed to dry in the air, followed by drying at 50°C. in the vacuum oven. More than 10% by weight of the wood was dissolved out as soluble carbohydrates. A portion of this extracted wood flour was dried in an Abderhalden vacuum apparatus at 100°C. over phosphorus pentoxide and analyzed for methoxyl, lignin, cellulose, and pentosans.

| | 1 | 2 | Mean |
|---|---|---|---|
| Methoxyl (40) | 6.45% | 6.39% | 6.42% |
| Lignin (41) | 22.23% | 22.01% | 22.12% |
| Cellulose (42) | 58.50% | 59.06% | 58.78% |
| Pentosans (43) | 25.84% | 25.79% | 25.81% |

The extracted wood flour was now treated with dilute alkali to remove pentosans. The procedure followed was similar to that used by Suida and Titsch (44). 600 gm. extracted wood flour was stirred with 12 liters of dilute sodium hydroxide (5%) for 48 hours at room temperature in a nitrogen atmosphere. The reddish mixture was filtered by suction through a cloth filter and washed with hot

water (50-60°C.). This process was then repeated three times (eight days in all) to insure complete removal of soluble pentosans. The final product was washed with distilled water, acetic acid (1%), water, and methyl alcohol, and allowed to dry in the air, followed by drying in the vacuum oven at 50°C. The very pale yellow colored product so obtained represented about 60% of the original wood. A sample was dried in the Abderhalden at 100°C. over phosphorus pentoxide and analyzed for methoxyl, lignin, cellulose, and pentosans.

| | 1 | 2 | Mean |
|---|---|---|---|
| Methoxyl | 6.78% | 6.85% | 6.81% |
| Lignin | 31.03% | 30.93% | 30.98% |
| Cellulose | 66.91% | 66.20% | 66.56% |
| Pentosans | 13.37% | 13.25% | 13.31% |

(b) Acetylation of the Wood Flour and Isolation of Acetylated Oak Lignin.

50 gm. of the dry extracted oak wood was acetylated with a mixture of 450 gm. acetic anhydride, 250 gm. glacial acetic acid, and 3.5 gm. concentrated sulphuric acid (about 0.5%). The reaction was carried out in a bronze Werner-Pfleiderer mixer provided with a water

cooling system. The temperature was controlled carefully and allowed to rise gradually from 15°C. to 30°C. during the following successive time intervals:

1. Mixture stirred 2½ hrs. at 15°C.
2. " " ½ " " 15-20°C.
3. " " 2 " " 20-25°C.
4. " " 2 " " 25-30°C.

The heavy, viscous, dark-brown solution was then allowed to stand over night without stirring, in an atmosphere of nitrogen, after which it was poured into 2 liters of a vigorously stirred sodium acetate solution (20%). The viscous, acetylated mixture was converted into a pale-yellow stringy solid insoluble in the sodium acetate solution. The mixture was filtered, washed for 48 hours in a linen sack through which a stream of water flowed continuously to remove the last traces of acid, then allowed to dry in the air, after which it was dried in the vacuum oven at 50°C. for two days. Weight 90 gm. It was then mixed with a liter of chloroform and allowed to stand over night at room temperature. The viscous solution was filtered through a solid sodium chloride filter, leaving an insoluble residue amounting to about 10 gm. The chloroform filtrate was concentrated under reduced

pressure and allowed to dry in the air at room temperature. The residual hard glassy solid was crushed, pulverized, and extracted with acetone for five days. The acetone extract was concentrated to 200 cc., filtered, and poured into 2 liters of water to precipitate the acetylated lignin. The white precipitate was filtered, washed, and dried. Yield 27 gm.

(c) Purification of the Fully Acetylated Oak Lignin

The dry acetylated product (10 gm.) was dissolved in 50 cc. acetone, filtered, and precipitated by pouring in a fine stream into 500 cc. anhydrous ether. An almost white precipitate separated out. It was filtered, washed with ether, twice with petroleum ether, and dried in the Abderhalden at 80°C.

Analysis of the Acetylated Product

Methoxyl Determinations:

%$OCH_3$, 11.05 (11.1, 11.0)

(0.01712 gm. sample req'd 14.85 cc. 0.024728 N. $Na_2S_2O_3$)

(0.02354 " " " 20.21 " " " )

Acetyl Determinations:

%$CH_3CO$, 35.4 (35.2, 36.0, 35.0, 35.3)

(1) 0.04661 gm. sample req'd 18.8 cc. 0.02040 N. NaOH

(2) 0.05113 " " " 21.0 " " "

(3) 0.04258 " " " 17.0 " " "

(4) 0.05557 " " " 22.4 " " "

Lignin Determinations(41):

% Lignin, 75.2 (75.9, 74.6)

(1) 0.4000 gm. sample gave 0.3038 gm. residue lignin, 75.9%

(2) 0.4000 " " " 0.2986 " " " 74.6%

## Examination for Homogeneity

Since the methoxyl value of oak wood is much higher than that of spruce (6.42% compared with 4.88%) it was thought that the fully acetylated oak lignin would have a higher methoxyl value than the fully acetylated spruce lignin ($OCH_3$, 17.8%). However, as shown above, the value of the former was found to be 11.0%, thus indicating the probable non-homogeneous character of the acetylated oak lignin.

The crude acetylated oak lignin was therefore

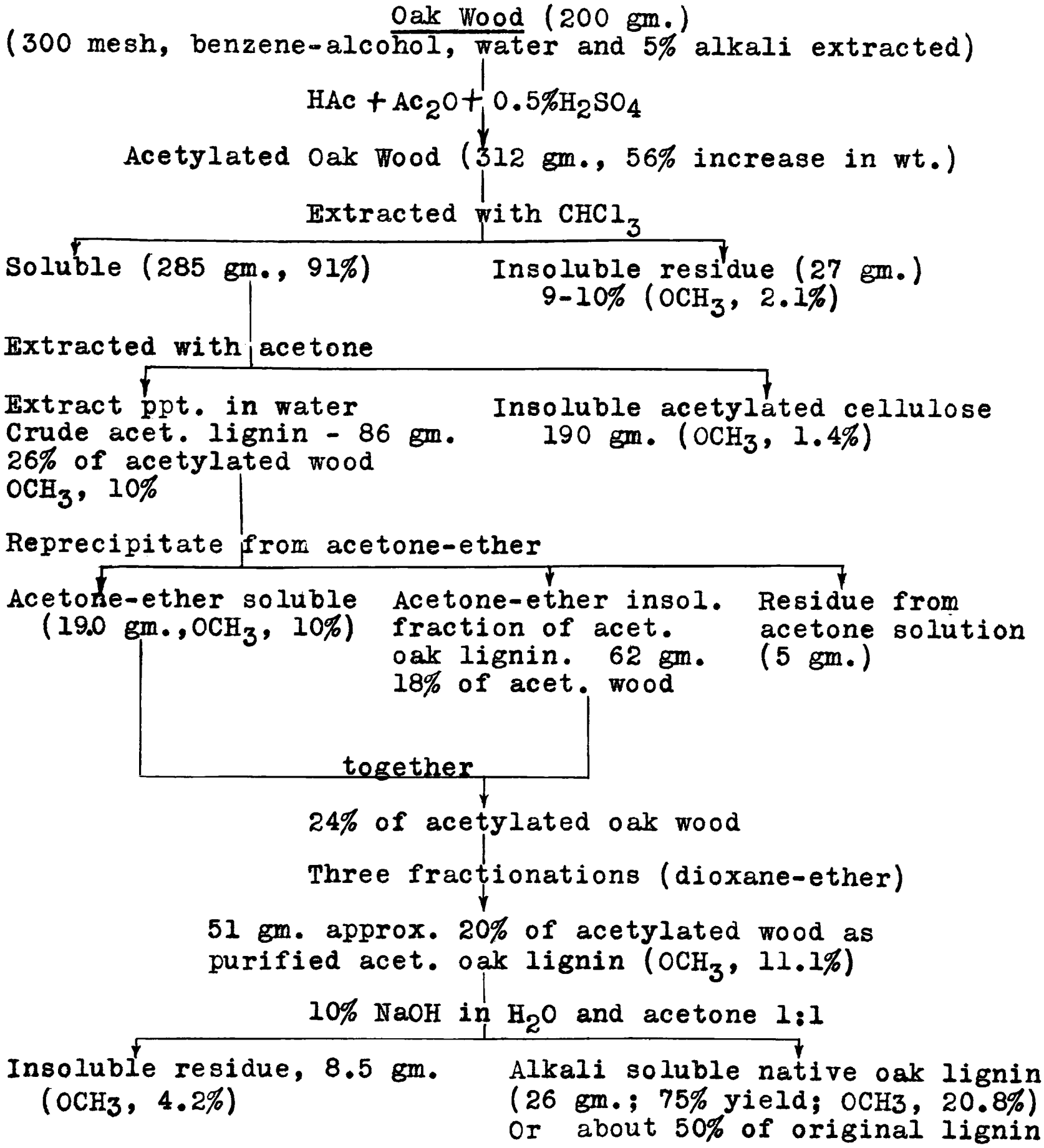
fractionated by precipitation of its dioxane solution into excess anhydrous ether. After three such precipitations the methoxyl content remained unchanged (11.1%). It was evident that any impurity such as cellulose or pentosans either must be attached chemically to the lignin molecule or have a solubility very similar to that of the lignin. An estimation for pentosans according to Doree's procedure (43) showed the presence of about 10% pentosans. As will be shown later, this non-lignin portion (pentosans, combined cellulose, or both) can be removed by deacetylating with dilute alkali.

Modification of the Above Procedure for Isolation of Fully Acetylated Oak Lignin

The procedure outlined above was modified somewhat without, however, changing the nature of the final fully acetylated product. 200 gm. dry oak wood meal was acetylated in a flask mechanically stirred at room temperature in an atmosphere of nitrogen for 48 hours. The sodium chloride filter was replaced by an anhydrous sodium sulphate filter. All reactions were carried out at 20-25°C. including the drying of the original oak wood flour. The final, crude, fully acetylated lignin had the same properties

as in the preliminary experiment. However, when the oak flour was insufficiently extracted with alkali (for only 2 days) the crude acetylated lignin obtained had a methoxyl value not much higher than that of the original oak wood.

(d) Brief Outline of the Process for Extraction of Lignin from Oak Wood



## II PREPARATION OF NATIVE OAK LIGNIN

Native oak lignin was prepared by the deacetylation of the crude acetylated oak lignin extracted with acetone from acetylated oak wood. Thirty grams acetylated oak lignin (11.1% $OCH_3$) was dissolved in a liter of acetone and to this a solution of 200 gm. sodium hydroxide in a liter of distilled water was added gradually at room temperature with stirring. The mixture was allowed to stand for 24 hours, the acetone removed under reduced pressure, and sufficient water added to dissolve the sodium salt of lignin which had precipitated, the total volume being kept at about 2 liters. The solution was then allowed to stand for 2 weeks at room temperature to insure complete hydrolysis. The reddish-colored solution was filtered to remove insoluble material (about 5 gm.) which was washed with water, dried at 80°C. in vacuum over phosphorus pentoxide* and analyzed for methoxyl. To the filtrate was added dilute sulphuric acid (5%) until the vigorously stirred solution reacted neutral or only slightly acidic. The hydrolyzed lignin which precipitated was then filtered through a blue ribbon filter. The light fawn-colored

*In this thesis all lignin samples that were analyzed were dried at 80°C. in an Abderhalden apparatus, unless otherwise specified.

residue was washed several times with distilled water until the filtrate no longer gave a test for sulphate. The dry product (15 gm., about 75% yield) was dissolved in 50 cc. dioxane and reprecipitated by adding in a fine stream to 500 cc. of ether, vigorously stirred. The precipitate was centrifuged, washed with ether, twice with petroleum ether (B.P. 30-50°C.), allowed to stand over night in petroleum ether, and dried thoroughly.

| Analysis for Methoxyl | 1 | 2 | Mean |
|---|---|---|---|
| Alkali Insoluble | 4.2% | 4.1% | 4.15% |
| Alkali Soluble | 20.8% | 20.8% | 20.8% |

Apparently the alkali-insoluble material contained the attached pentosans or cellulose, that could only be removed from the crude acetylated product by hydrolysis of the acetyl groups. The alkali-insoluble product was found to be insoluble in all the common organic solvents.

The deacetylated, alkali-soluble lignin ($OCH_3$, 20.8%) was reprecipitated from dioxane and ether. This was effected without change in methoxyl content. However, it was found that this product could be separated by the use of chloroform into two fractions, a chloroform soluble fraction ($OCH_3$, 23.4%) and a chloroform insoluble fraction ($OCH_3$, 20.6%), the soluble fraction amounting to about 10%

of the deacetylated lignin. As will be shown later, both the chloroform soluble fraction (A) and the insoluble fraction (B) gave the same derivative on methylation with diazomethane.

Elementary Analysis* (B)

(1) Sample 0.19657 gm.; $H_2O$ - 0.10870 gm.; $CO_2$ - 0.46091 gm.
% H - 6.19 % C - 63.94

(2) Sample 0.20340 gm.; $H_2O$ - 0.11197 gm.; $CO_2$ - 0.47742 gm.
% H - 6.16 % C - 64.02

Ash, 0.9%

Methoxyl (B)

$\%OCH_3$, 20.6 (20.6, 20.6)

(0.01658 gm. sample req'd 13.3 cc. 0.04987 N. $Na_2S_2O_3$)

(0.01894 " " " 15.1 " " " )

Methoxyl (A)

$\%OCH_3$, 23.45 (23.4, 23.5)

(0.01782 gm. sample req'd 15.9 cc. 0.05063 N. $Na_2S_2O_3$)

(0.01806 " " " 16.2 " " " )

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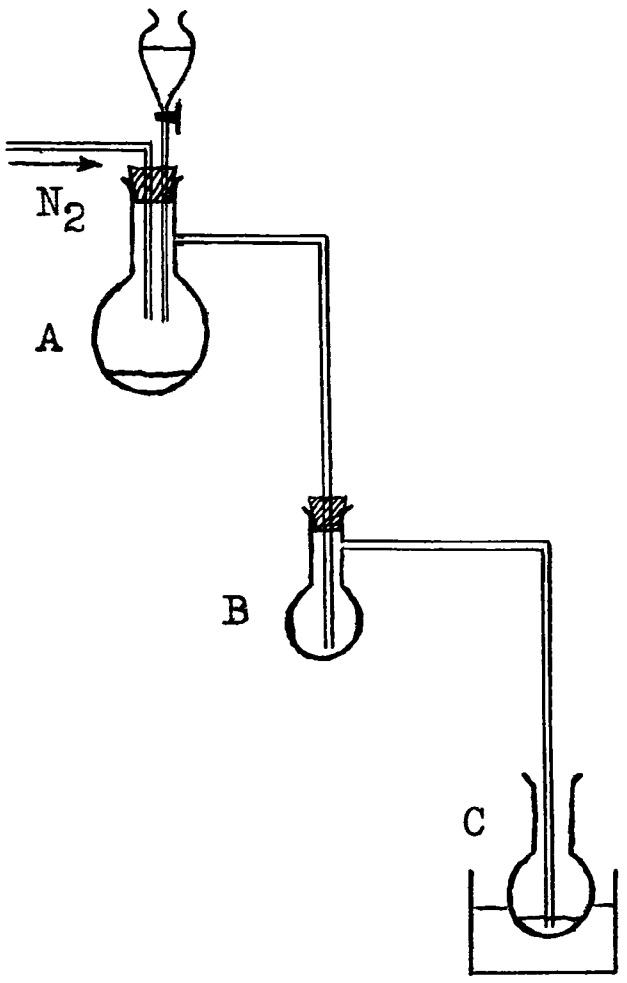
* All analyses are calculated on an ash-free basis throughout this thesis.

## III PREPARATION OF DIAZOMETHANE-METHYLATED NATIVE OAK LIGNIN

Native oak lignin ($OCH_3$, 20.6%) was methylated with diazomethane to determine the number of phenolic or enolic groups.




In flask "A" was placed 10 cc. nitrosomethylurethane. Flask "C" contained a solution of 1 gm. of oak lignin in 15 cc. of dioxane. Flask "B" served as a safety flask or trap to prevent material from distilling over into "C". 20 cc. of a 25% sodium glycolate solution in ethylene glycol was placed in the dropping funnel. After the air in the apparatus had been replaced by nitrogen about 5 drops of the sodium glycolate solution were dropped into the nitroso-methylurethane in flask "A". This operation was repeated about every 10 minutes, care being taken to insure that the sodium glycolate had reacted before adding more, since an accumulation of unchanged glycolate might product an uncontrollable reaction and in turn a violent explosion. To

prevent this an ice bath was kept near flask "A" to be used in an emergency. Flask "C" was cooled with ice water throughout the reaction. Flask "A" was heated to about 50°C. at the beginning of the reaction to insure a gentle evolution of diazomethane gas being given off evenly during the whole experiment, which generally takes about 3 hours. After the reaction was completed, flask "C" was placed in the refrigerator over night. The dioxane solution was then treated with a few drops of acetic acid to decompose any remaining diazomethane, after which the solution was filtered and poured into 200 cc. of vigorously-stirred anhydrous ether in a centrifuge jar. The lignin precipitate was centrifuged, washed with ether and twice with petroleum ether, and dried.

$\%OCH_3$, 25.5 (25.5, 25.45)

(0.01884 gm. sample req'd 18.6 cc. 0.049875 N. $Na_2S_2O_3$)

(0.01847 " " " 18.2 " " " )

A second methylation was made with diazomethane to insure complete methylation.

$\%OCH_3$, 26.65 (26.6, 26.7)

(0.02055 gm. sample req'd 21.30 cc. 0.049875 N. $Na_2S_2O_3$)

(0.02047 " " " 21.10 " " " )

As this did not insure complete methylation, the product was given a third treatment with diazomethane.

$\%OCH_3$, 26.6 (26.6, 26.6)

(0.02217 gm. sample req'd 22.5 cc. 0.05063 N. $Na_2S_2O_3$)

(0.02106 " " " 21.4 " " " )

Evidently the product was now completely methylated. The methoxyl value remained constant after careful fractionation from dioxane and ether.

Analysis (B)

(1) Sample 0.19710 gm.; $H_2O$ - 0.11410 gm.; $CO_2$ - 0.46470 gm.

% H - 6.48 % C - 64.30

(2) Sample 0.2026 gm.; $H_2O$ - 0.1176 gm.; $CO_2$ - 0.4794 gm.

% H - 6.43 % C - 63.97

Ash, 0.092%.

Diazomethane-methylation of Native Oak Lignin (Fraction A)

One gram native oak lignin ($OCH_3$, 23.4%) was methylated according to the above procedure.

$\%OCH_3$, 26.5

(0.02992 gm. sample req'd 30.3 cc. 0.05063 N. $Na_2S_2O_3$)

After a second methylation the product showed no appreciable increase in methoxyl content.

$\%OCH_3$, 26.6

(0.02251 gm. sample req'd 22.8 cc. 0.05063 N. $Na_2S_2O_3$)

Preparation of a Trityl Derivative of Native Oak Lignin Premethylated with Diazomethane.

One gram diazomethane-methylated native oak lignin ($OCH_3$, 26.6%) was dissolved in 10 cc. of dry pyridine and 2 gm. trityl chloride added to the solution. The trityl chloride dissolved rapidly without any appreciable generation of heat. The mixture was heated to about 50°C. in a water bath for one hour and then allowed to stand over night at room temperature. It was then poured into rapidly-stirred ice water, the mixture filtered, the residue washed thoroughly with water, then with alcohol and finally with ether on the suction filter. The tritylated product, after drying in a desiccator over sulphuric acid, was dissolved in dioxane and the solution added drop-wise to dry ether with vigorous stirring. The fine light fawn colored precipitate was centrifuged, washed with ether and petroleum ether, and dried. Yield 1.4 gm. The product, on analysis for methoxyl, showed that two hydroxyl atoms had been replaced by trityl groups.

$\%OCH_3$, 17.5 (17.5, 17.5)

(0.1827 gm. sample req'd 12.20 cc. 0.05063 N. $Na_2S_2O_3$)

(0.1377 " " " 9.20 " " " )

Theoretical for $C_{42}H_{32}O_5(OCH_3)_8(OC(C_6H_5)_3)_2(OH)_2$ is 17.53%.

Preparation of the Tosyl Ester of Tritylated Diazomethane-methylated Native Oak Lignin

Tritylated diazomethane-methylated native oak lignin, 0.2 gm. ($OCH_3$, 17.5%) was dissolved in 15 cc. pyridine and 0.5 gm. p-toluene sulphonyl chloride added. The solution was allowed to stand 24 hours at room temperature, and then poured into 150 cc. ice water, the ester separating as a finely-divided white precipitate. This was filtered, dried, dissolved in anhydrous dioxane, and reprecipitated as in the case of the trityl derivative. Yield 0.3 gm. The methoxyl analysis showed that the two remaining free hydroxyl groups were tosylated.

$\%OCH_3$, 14.85 (14.9, 14.8)

(0.01789 gm. sample req'd 10.2 cc. 0.05063 N. $Na_2S_2O_3$)

(0.01640 " " " 9.31 " " " )

Theoretical for $C_{42}H_{32}O_5(OCH_3)_8(OC(C_6H_5)_3)_2(OSO_2C_6H_4CH_3)_2$ is 14.39%.

A second tosylation showed no appreciable change in methoxyl value (14.7%).

## IV ACETYLATION OF NATIVE OAK LIGNIN

Ten grams oak lignin ($OCH_3$, 20.6%) was acetylated at room temperature with a mixture of 50 cc. of acetic anhydride and 100 cc. pyridine. After standing for 12 hours the solution was poured into a liter of ice water. The precipitate was filtered off, washed thoroughly and dried. The dry, acetylated product was reprecipitated from acetone into ether, washed with ether and finally twice with petroleum ether.

Analysis

$\%OCH_3$, 18.25 (18.1, 18.4)

(0.02358 gm. sample req'd 16.27 cc. 0.05063 N. $Na_2S_2O_3$)

(0.01369 " " " 9.6 " " " )

In order to insure complete acetylation, the acetylated product was reacetylated, in acetic anhydride and acetic acid, using sulphuric acid as a catalyst. 10 gm. of the acetylated product ($OCH_3$, 18.2%) was dissolved in 100 cc. of acetic anhydride and a mixture of sulphuric acid (0.7 gm.) and glacial acetic acid (50 gm.) was added slowly, the temperature being kept between 20° and 25°C. After 24 hours at room temperature the mixture was poured into a liter of 20% sodium acetate solution containing chopped ice. The

precipitate was filtered, washed thoroughly with water and dried over sodium hydroxide at room temperature. The dry product was purified by precipitating from acetone into ether. The precipitate was centrifuged, washed with ether and twice with petroleum ether. After the last washing it was allowed to stand over night in petroleum ether to insure complete removal of ether from the lignin. After a second precipitation from dioxane and ether the product was analyzed.

Methoxyl Analysis

%$OCH_3$, 16.85 (16.8, 16.9)

(0.01925 gm. sample req'd 12.4 cc. 0.05063 N. $Na_2S_2O_3$)

(0.02753 " " " 17.6 " " " )

Acetyl Analysis

%$CH_3CO$, 22.9 (22.5, 23.3)

(0.05376 gm. sample req'd 13.8 cc. 0.02040 N. NaOH)

(0.06022 " " " 16.0 " " " )

Elementary Analysis

(1) Sample 0.26040 gm.; $H_2O$ - 0.13740 gm.; $CO_2$ - 0.59555 gm.

% H - 5.94 % C - 62.35

(2) Sample 0.23338 gm.; $H_2O$ - 0.12322 gm.; $CO_2$ - 0.53471 gm.

% H - 5.91 % C - 62.47

Ash 0.7%

## V PREPARATION OF FULLY METHYLATED OAK LIGNIN

Fully methylated oak lignin was prepared by a simultaneous deacetylation and methylation of acetylated oak lignin ($OCH_3$, 11%) using dimethyl sulphate and an excess of sodium hydroxide. 30% caustic soda by weight was used (i.e. a solution formed by dissolving 30 gm. solid sodium hydroxide in 70 gm. water). The specific gravity of this solution was 1.333 or the same as that of dimethyl sulphate itself.

A simple calculation shows that 10 cc. of dimethyl-sulphate is equivalent to 10.55 cc. of the above 30% caustic soda solution, on the assumption that dimethyl-sulphate is converted into sodium methyl sulphate at the low temperature of methylation (20-25°C.) employed.

A slight excess of alkali (5%) was added so that 10 cc. of dimethylsulphate are taken to react with 11.1 cc. of 30% caustic soda.

The fully acetylated lignin (2 gm.) was dissolved in 40 cc. acetone in a 150 cc. three-necked round bottom flask equipped with a mechanical stirrer, and then 20 cc. dimethyl-sulphate and 22.5 cc. of sodium hydroxide were allowed to drop slowly at room temperature into the rapidly stirred

solution from separate burettes, the mixture being kept alkaline throughout by an excess of 1 cc. of the alkali. The addition occupied 5 hours, at the end of which the mixture was stirred for an additional two hours and then allowed to stand over night. About 50 cc. of water was added to the yellowish solution and the acetone distilled off under reduced pressure by means of a water suction pump. The precipitate separating out was filtered off, washed thoroughly with distilled water, dried, dissolved in 20 cc. dioxane, centrifuged, filtered, and precipitated in 200 cc. ether. The reprecipitated, almost white product was centrifuged, washed with ether, twice with petroleum ether, and dried.

Methoxyl Analysis

$\%OCH_3$, 37.6 (37.5, 37.7)

(0.02459 gm. sample req'd 35.8 cc. 0.04987 N. $Na_2S_2O_3$)

(0.02258 " " " 32.8 " " " )

A second methylation using the same procedure showed that the product was fully methylated.

$\%OCH_3$, 37.65 (37.6, 37.7)

(0.02829 gm. sample req'd 41.4 cc. 0.04987 N. $Na_2S_2O_3$)

(0.02308 " " " 33.7 " " " )

Elementary Analysis

(1) Sample 0.22180 gm.; $H_2O$ - 0.13460 gm.; $CO_2$ - 0.53125 gm.

% H - 6.79 % C - 65.30

(2) Sample 0.21838 gm.; $H_2O$ - 0.13271 gm.; $CO_2$ - 0.52201 gm.

% H - 6.81 % C - 65.19

Ash, 0.64%

## VI ACETYLATION OF DIAZOMETHANE-METHYLATED NATIVE OAK LIGNIN

Two grams methylated lignin ($OCH_3$, 26.6) was dissolved in 10 cc. of acetic anhydride and 20 cc. pyridine, and allowed to stand over night. The solution was then poured into 400 cc. of rapidly stirred distilled water. The milk-white precipitate was centrifuged, washed twice with distilled water, dried, and the product purified by precipitating twice from dioxane-ether.

Methoxyl Analysis

$\%OCH_3$, 21.0 (21.0, 21.0)

(0.01495 gm. sample req'd 12.0 cc. 0.05063 N. $Na_2S_2O_3$)

(0.01732 " " " 13.9 " " " )

Acetyl Analysis

$\%CH_3CO$, 16.75 (16.6, 16.9)

(0.05020 gm. sample req'd 9.5 cc. 0.02040 N. NaOH)

(0.05193 " " " 10.0 " " " )

Elementary Analysis

Sample 0.17484 gm.; $H_2O$ - 0.09501 gm.; $CO_2$ - 0.40290 gm.

% H - 6.10 % C - 62.83

Ash - 0.00016 gm. (0.009%)

## VII METHYLATION OF ACETYLATED DIAZOMETHANE-METHYLATED NATIVE OAK LIGNIN WITH DIMETHYLSULPHATE

One gram of acetylated-diazomethane-methylated native oak lignin (%$OCH_3$, 21.0) was dissolved in 20 cc. acetone and methylated according to the usual procedure, using 10 cc. dimethylsulphate and 11 cc. of 30% sodium hydroxide. After standing 16 hours at room temperature, about 20 cc. water was added and the acetone evaporated under reduced pressure at room temperature. The residue was filtered off, washed thoroughly, and dried. It was then reprecipitated twice from dioxane-ether. Methoxyl analysis indicated the probable identity with the fully methylated product obtained by the direct methylation of the crude acetylated lignin extracted from acetylated oak wood.

Methoxyl Analysis

%$OCH_3$, 37.65 (37.6, 37.7)

(0.02111 gm. sample req'd 30.30 cc. 0.05063 N. $Na_2S_2O_3$)

(0.02107 " " " 30.32 " " " )

A second methylation showed no increase in methoxyl value (37.6%).

## VIII METHANOL OAK LIGNIN

(a) Preparation

Oak wood flour (25 gm.), 300 mesh, which had been extracted with alcohol-benzene, water, and 5% sodium hydroxide, and dried to constant weight at 50°C. in vacuum over phosphorus pentoxide, was heated with 200 cc. of absolute methanol containing 2% of anhydrous hydrochloric acid in a sealed glass bomb tube in a tilting oven at 85-86°C. for 100 hours. The tube was cooled to -15°C. and, on opening, a pronounced evolution of gas, presumably methyl chloride or dimethyl ether, was observed. The reaction-product was centrifuged, the insoluble residue washed with a mixture of methanol and acetone (1:1) until no further red color was extracted, and dried in a desiccator over sulphuric acid. The weight was 17 gm., indicating that 32% of the original wood meal had been dissolved. The combined acetone and methanol solutions were concentrated under reduced pressure to a volume of about 30 cc. and then added in a very fine stream to 500 cc. of rapidly stirred distilled water. The methanol lignin was separated by centrifuging and washing with distilled water to remove soluble carbohydrates. The light brown product was dried

in vacuum at room temperature over solid sodium hydroxide flakes. Yield, 4.0 gm. or more than 50% of the total lignin present in the original oak wood. The methanol lignin was dissolved in 30 cc. of anhydrous dioxane, and then reprecipitated by adding the solution in a fine stream to 400 cc. of vigorously stirred anhydrous ether. The product was centrifuged, washed once with ether and three times with petroleum ether (30-50°C.). It was allowed to stand over night in the final petroleum ether wash liquor, centrifuged, and dried in an Abderhalden apparatus over phosphorus pentoxide at 10 mm. and 80°C. to constant weight (3 gm.).

Methoxyl Analysis

$\%OCH_3$, 29.5 (29.4, 29.6)

(0.01980 gm. sample req'd 22.3 cc. 0.05063 N. $Na_2S_2O_3$)

(0.01453 " " " 16.4 " " " )

Elementary Analysis

(1) Sample 0.1992 gm.; $H_2O$ - 0.1164 gm.; $CO_2$ - 0.4614 gm.

% H - 6.55 % C - 63.2

(2) Sample 0.1375 gm.; $H_2O$ - 0.0799 gm.; $CO_2$ - 0.3181 gm.

% H - 6.50 % C - 63.1

Ash - 0.0002 gm. (0.01%)

The dioxane-ether mother liquor obtained from the first purification of the methanol oak lignin had a pale reddish color. The solvent was removed by evaporation under reduced pressure, the brownish residue dissolved in about 4 cc. of dioxane and this solution added dropwise to well stirred petroleum ether. The precipitate was centrifuged, washed three times with petroleum ether and dried. Weight, 1.0 gm. The same methoxyl value was obtained as for the dioxane-ether insoluble product, indicating their identity.

Analysis

$\%OCH_3$, 29.6 (29.6, 29.6)

(0.02455 gm. sample req'd 27.7 cc. 0.05063 N. $Na_2S_2O_3$)

(0.02640 " " " 31.25 " " " )

(b) Diazomethane-methylation of Methanol Oak Lignin

Methanol oak lignin ($OCH_3$, 29.6%) was methylated with diazomethane to determine the number of phenolic or enolic groups. The procedure used was similar to that outlined for the methylation of native oak lignin. One gram of lignin was dissolved in 10 cc. dioxane and the diazomethane from 20 cc. nitrosomethylurethane bubbled into it at $5^{\circ}C$.

over a period of 4 hours. The solution was allowed to stand over night in a refrigerator, and a drop of glacial acetic then added to destroy any remaining diazomethane gas. The solution was then filtered, precipitated in anhydrous ether, centrifuged, washed three times with pteroleum ether and dried. During the final washing the lignin was allowed to stand over night in petroleum ether to remove any traces of dioxane or ether. The final product was dried in vacuum at 80°C. over phosphorus pentoxide for 24 hours and analyzed for methoxyl content.

Analysis

$\%OCH_3$, 32.45 (32.4, 32.5)

(0.01846 gm. sample req'd 22.85 cc. 0.05063 N. $Na_2S_2O_3$)

(0.02186 " " " 28.01 " " " )

Elementary Analysis

(1) Sample 0.1840 gm.; $H_2O$ - 0.1112 gm.; $CO_2$ - 0.4398 gm.
% H - 6.76 % C - 65.0

(2) Sample 0.1841 gm.; $H_2O$ - 0.1105 gm.; $CO_2$ - 0.4355 gm.
% H - 6.72 % C - 64.5

Ash - 0.0002 gm. (0.01%)

This product was given a second methylation with diazomethane.

Analysis

%$OCH_3$, 32.4 (32.4, 32.4)

(0.02102 gm. sample req'd 26.02 cc. 0.05063 N. $Na_2S_2O_3$)

(0.02011 " " " 24.88 " " " )

(c) Acetylation of Methanol Oak Lignin

Two grams of methanol oak lignin ($OCH_3$, 29.6%) was acetylated with a mixture of 6 cc. acetic anhydride and 10 cc. pyridine. After 24 hours at room temperature the mixture was poured into 500 cc. of rapidly stirred distilled water, the resulting fawn colored precipitate filtered, washed thoroughly with water to remove pyridine, and dried. It was then precipitated from dioxane-ether, washed, dried, and analyzed for methoxyl.

Analysis

%$OCH_3$, 25.6 (25.4, 25.7)

(0.01676 gm. sample req'd 16.3 cc. 0.05063 N. $Na_2S_2O_3$)

(0.01691 " " " 16.6 " " " )

(d) Dimethylsulphate Methylation of Acetylated Methanol Oak Lignin

A simultaneous deacetylation and methylation of acetylated methanol oak lignin was carried out in the presence of

dimethylsulphate and caustic soda. One gram of the lignin, suspended in 20 cc. acetone, was stirred vigorously at room temperature and methylated by the addition of 10 cc. dimethylsulphate and 11.2 cc. of 30% sodium hydroxide (by weight). The addition took place over a period of 48 hours, the mixture being kept basic throughout by an excess of 1 cc. alkali. At the end of this time about 50 cc. water was added and the acetone evaporated at room temperature in vacuum. The orange-yellow solid separating out was filtered off, washed thoroughly with water and dried. After purification in the usual manner from dioxane-ether, the methylated lignin was analyzed.

Analysis

%$OCH_3$, 37.9 (38.0, 37.8)

(0.01506 gm. sample req'd 21.85 cc. 0.05063 N. $Na_2S_2O_3$)

(0.02620 " " " 37.8 " " " )

A second similar methylation was carried out to insure complete methylation, and the product purified as above.

Analysis

%$OCH_3$, 37.8

(0.02061 gm. sample req'd 29.7 cc. 0.05063 N. $Na_2S_2O_3$)

Elementary Analysis

(1) Sample 0.2012 gm.; $H_2O$ - 0.1246 gm.; $CO_2$ - 0.4835 gm.

% H - 6.93 % C - 65.55

(2) Sample 0.2008 gm.; $H_2O$ - 0.1252 gm.; $CO_2$ - 0.4822 gm.

% H - 6.98 % C - 65.50

Ash - 0.0001 gm. (0.005%)

## IX PREPARATION OF FULLY METHYLATED SPRUCE WOOD LIGNIN

This was prepared by a prior complete methylation of the wood meal, hydrolysis with methanol-hydrogen chloride and subsequent remethylation of the lignin obtained.

### (a) Preparation of the Wood Meal

Spruce wood meal (about 100 mesh) was extracted with alcohol and benzene to remove resins and gums. This was followed first by hot water and then by 5% alkali extraction. The procedure followed was the same as that already given for the extraction of oak wood meal.

### (b) Methylation of the Wood Meal

The extracted spruce wood meal was methylated with dimethylsulphate and sodium hydroxide in order to bring about the complete methylation of all free hydroxyl groups present in the wood. The wood meal (300 gm.) was suspended in 1600 cc. water in a 12-liter bottle and placed in a large water bath kept at 20-25°C. To the mixture was added at a uniform rate during a period of 12 hours 2250 cc. of dimethylsulphate and 5400 cc. of 30% sodium hydroxide (by weight). The mixture was stirred rapidly and the dimethyl-sulphate and caustic soda run in from two individual burettes

at such a rate that the caustic soda added always remained in about 5% excess. The mixture was then stirred overnight at room temperature, filtered, washed thoroughly with water, and partially dried by suction in the Büchner funnel.

The product was given a second methylation identical with the first except that the partially methylated product was suspended in 2500 cc. acetone instead of water, in order to bring the dimethylsulphate and caustic soda into homogeneous reaction, and to bring about a more intimate surface reaction between the partially methylated wood meal and the dissolved dimethylsulphate and caustic soda.

A third and fourth methylation were carried out precisely as in the case of the second. The final product was washed thoroughly and dried in vacuum at 50°C. After the third methylation, the wood meal was found to have a methoxyl content of 36.4% which was increased during the fourth methylation to 38.7%, a value shown to be a maximum figure by the work of other investigators in these laboratories.

After third methylation:

$\%OCH_3$, 36.4 (36.5, 36.2)

(0.01930 gm. sample req'd 27.5 cc. 0.04954 N. $Na_2S_2O_3$)

(0.02412 " " " 34.0 " " " )

After fourth methylation:

%$OCH_3$, 38.7 (38.7, 38.6)

(0.02110 gm. sample req'd 31.8 cc. 0.04954 N. $Na_2S_2O_3$)

(0.01595 " " " 24.0 " " " )

(c) Hydrolysis of the Methylated Spruce Wood Meal

Fully methylated spruce wood meal (350 gm.) was placed in a glass-lined autoclave equipped with a mechanical stirrer and 4 liters of anhydrous methyl alcohol containing 170 gm. dry hydrogen chloride (approximately 4%) added. The mixture was heated to 100°C. (70 pounds pressure) for 3 days, after which it was filtered and the residue washed, first with dry methyl alcohol and then with acetone (weight insoluble lignin, 89 gm.). The filtrate and washings were united, concentrated to about 500 cc. and precipitated into 5 liters of distilled water. The precipitated lignin was allowed to settle and washed by decantation. It was then centrifuged, washed three times with water, dried over sodium hydroxide, then over phosphorus pentoxide, and purified by precipitation from dioxane-ether. The product was then methylated completely with dimethylsulphate and sodium hydroxide according to the procedure used for the methylation of oak lignin. On fractionation it was found to consist of several products having methoxyl values ranging from 32 to 35%.

## X PREPARATION OF SPRUCE WOOD LIGNIN BY ACETYLATION

### (a) Experiment I

Fifty grams spruce wood flour (300 mesh), previously extracted with alcohol-benzene (1:1), water, and 5% sodium hydroxide, was acetylated by shaking for 24 hours with a mixture of 450 gm. acetic anhydride and 250 gm. pyridine, and then allowing to stand for one month at room temperature with intermittent shaking. The yellowish-colored product was poured into ice water and the excess acetic anhydride neutralized by adding sodium carbonate. The mixture was filtered, the residual solid washed well, and dried. The product showed an increase of about 30% in weight. Treatment with acetone in a Soxhlet extracter for 2 days extracted only a trace of material. Evidently the lignin was either incompletely acetylated or the acetylating reagent was unable to cause a disruption of the linkage between acetylated polysaccharides and lignin.

### (b) Experiment II

Fifty grams wood flour, previously extracted as above, was acetylated with a mixture of 450 gm. acetic anhydride, 250 gm. glacial acetic acid, and 0.7 (0.1%) sulphuric acid.

The mixture was shaken mechanically for 2 weeks, after which it was allowed to stand for one month at room temperature. After 2 weeks shaking the mixture gradually turned dark-gray in appearance and the viscosity of the mixture had increased. At the end of one month, the solution was poured into 2 liters of a 20% solution of ice-cold sodium acetate. The acetylated wood was filtered, washed thoroughly, and dried in vacuum over sodium hydroxide (weight 87 gm., an increase of about 74% over that of the wood meal taken). The fine, buff-colored acetylated wood flour was now extracted with acetone for one week, leaving a residue weighing 66 gm. The acetone extract was concentrated to about 100 cc., filtered, and precipitated into 1000 cc. distilled water. The dried product was re-purified from acetone and ether. Yield 10 gm.

Analysis

%$OCH_3$, 4.33 (4.30, 4.36)

(0.01676 gm. sample req'd 5.63 cc. 0.02473 N. $Na_2S_2O_3$)

(0.02347 " " " 8.01 " " " )

%$CH_3CO$, 37.2 (37.1, 37.4)

(0.03687 gm. sample req'd 15.6 cc. 0.02040 N. NaOH)

(0.03210 " " " 13.7 " " " )

Experiment III

Extracted spruce wood meal (25 gm.) was acetylated by shaking for one week with a mixture of 225 acetic anhydride, 125 gm. acetic acid, and 1.0 gm. sulphuric acid (0.3%). The remaining part of the procedure was identical with that used in the second experiment. Weight of dry wood after acetylation, 41 gm. Weight of product extracted with acetone, 2.5 gm.

Methoxyl Analysis

$\%OCH_3$, 2.7

(0.01958 gm. sample req'd 4.20 cc. 0.02473 N. $Na_2S_2O_3$)

Experiment IV

Spruce wood meal (25 gm.), previously extracted with benzene-alcohol and water but not with alkali, was acetylated with a mixture of 225 gm. acetic anhydride, 125 gm. acetic acid, and 1.87 gm. sulphuric acid (0.5%) in the usual manner at room temperature for one week. Yield of acetone extract, 3 gm. (about 12%). After reprecipitation from acetone-ether it was analyzed.

$\%OCH_3$, 2.8 (2.7, 2.9)

(0.02037 gm. sample req'd 4.25 cc. 0.02473 N. $Na_2S_2O_3$)

(0.02207 " " " 5.00 " " " )

Acetyl Analysis

%$CH_3CO$, 42.5 (42.9, 42.2)

(0.04426 gm. sample req'd 21.9 cc. 0.02473 N. $Na_2S_2O_3$)

(0.05717 " " " 27.5 " " " )

Experiment V

This experiment was identical with the fourth except that the reaction was carried out at a higher temperature (30°C.) and over a period of 2 weeks. Five grams of crude product was obtained from 25 gm. wood meal (about 20%). This was repurified from acetone-ether.

Analysis

%$OCH_3$, 3.35 (3.3, 3.4)

(0.1980 gm. sample req'd 2.60 cc. 0.04987 N. $Na_2S_2O_3$)

(0.02071 " " " 2.65 " " " )

Since this product, like those of the four previous experiments, was much too low in methoxyl content and too high in acetyl content for spruce lignin, an attempt was made to remove the acetylated polysaccharides which were shown later to be present, by reacetylation. The same procedure was used. The methoxyl value increased only slightly.

%$OCH_3$, 3.55 (3.6, 3.5)

(0.02493 gm. sample req'd 3.51 cc. 0.04987 N. $Na_2S_2O_3$)

(0.02172 " " " 2.92 " " " )

Experiment VI

The procedure was similar to that used in the previous experiment (50 gm. wood meal, 450 gm. acetic anhydride, 250 gm. acetic acid, and 1.8 gm. sulphuric acid) except that in this case alkali extracted wood meal was used. The experiment was carried out over a period of 3 days. The methoxyl value of the purified product was lower (2.9% as compared with 3.3%) indicating that time of reaction was a more important factor than either the temperature of reaction or the concentration of acid used as a catalyst.

Analysis

$\%OCH_3$, 2.9 (2.9, 2.9)

(0.02157 gm. sample req'd 2.4 cc. 0.04987 N. $Na_2S_2O_3$)

(0.01622 " " " 1.85 " " " )

Experiment VII

Fifty grams spruce wood meal (extracted as in Exp. I) was acetylated in a solution of 450 gm. acetic anhydride, 250 gm. acetic acid, and 1.87 gm. sulphuric acid (0.5%). The acetylation was carried out in the usual way, with shaking at 30°C. for a period of 3 days. The viscous mixture was then poured into a rapidly stirred 20% sodium acetate solution containing crushed ice. The yellow

precipitate of acetylated wood (90 gm.) was filtered off, washed thoroughly with water, and dried at 40°C. under reduced pressure. The dry product was moistened with 10% by weight of anhydrous methyl alcohol and dissolved in 500 cc. chloroform. The solution was filtered through powdered anhydrous sodium sulphate, the residual mass washed with chloroform, and the united washings and solution evaporated to dryness. The hard, glassy solid, consisting of acetylated lignin and acetylated cellulose, was crushed to small fragments and extracted in a Soxhlet extracter for one week with acetone. The acetone solution was concentrated to about 50 cc. and precipitated into distilled water. The white precipitate was filtered, washed, dried, and re-purified by precipitation from acetone-ether. Yield, 8 gm.

Analysis

$\%OCH_3$, 2.95 (2.94, 2.96)

(0.02065 gm. sample req'd 2.35 cc. 0.04987 N. $Na_2S_2O_3$)

(0.01876 " " " 2.15 " " " )

The low methoxyl value indicates that the chloroform treatment is of no value for the removal of acetylated polysaccharides from the crude lignin product obtained.

Other experiments were carried out using as a catalyst p-toluene sulphonic acid, and trichloroacetic acid over a period of 4 weeks. Acetylation took place very slowly at room temperature in both cases. However, lignin could be extracted only from the former and it had the same methoxyl value as in the preceding experiments (about 3%).

Various attempts to remove spruce lignin from the extracted wood meal by treatment with the new commercial cellulose solvents, trimethyl-benzyl- and dimethyl-dibenzyl ammonium hydroxide, were unsuccessful.

(b) Deacetylation of Fully Acetylated Spruce Lignin Using Alkali

Five grams of fully acetylated spruce lignin ($OCH_3$, 3%) obtained by the extraction of acetylated spruce wood with acetone, was deacetylated by treatment with 200 cc. of 10% alcoholic potassium hydroxide for one week at room temperature. The solution was then diluted to twice its volume with water and the alcohol removed by evacuation at room temperature. The reddish solution was filtered, the filtrate neutralized with 5% hydrochloric acid, and the resulting precipitate centrifuged, washed with water several times and dried.

The material turned dark on drying, and became very hard and brittle, and was insoluble in most solvents. It was purified from dioxane-ether.

Analysis

%$OCH_3$, 13.05 (13.01, 13.0)

(0.02056 gm. sample req'd 10.40 cc. 0.04987 N. $Na_2S_2O_3$)

(0.02540 " " " 12.81 " " " )

(c) Deacetylation of Acetylated Spruce Lignin Using Ammonia

Two grams of crude acetylated spruce lignin ($OCH_3$, 3%) was dissolved in 25 cc. anhydrous methyl alcohol and cooled in a freezing mixture of ice and salt. A fairly rapid stream of dry ammonia was passed in for 45 minutes. The mixture was then allowed to stand in the refrigerator over night. At the end of that time it was filtered and concentrated to a syrup under reduced pressure at room temperature. This was dissolved in dry methyl alcohol, filtered and precipitated into ether. The product was centrifuged, washed, dried, and analyzed. A qualitative test showed that nitrogen was present. Possibly nitrogen had replaced oxygen in one of the heterocyclic oxygen rings.

Analysis

%$OCH_3$, 5.0 (5.02, 4.96)

(0.02089 gm. sample req'd 4.03 cc. 0.04987 N. $Na_2S_2O_3$)

(0.02431 " " " 4.66 " " " )

(d) Methylation

Five grams of the acetylated spruce lignin ($OCH_3$, 3%) was suspended in 100 cc. acetone and methylated with 50 cc. dimethylsulphate and 56 cc. of 30% sodium hydroxide (by weight). The temperature was kept between 20° and 25°C. throughout the experiment and the methylating reagents added over a period of 12 hours as indicated in a previous experiment on the methylation of acetylated oak lignin. The product (1.3 gm.), after purification by precipitation from dioxane-ether, was analyzed.

Analysis

%$OCH_3$, 32.7

(0.02013 gm. sample req'd 25.1 cc. 0.05063 N. $Na_2S_2O_3$)

A second methylation showed that the product was fully methylated.

%$OCH_3$, 32.8

(0.03093 gm. sample req'd 26.2 cc. 0.05063 N. $Na_2S_2O_3$)

This experiment is of considerable interest as it indicates the presence of acetylated pentosans rather than cellulose acetate in the crude acetylated lignin.

## XI MERCURATION OF SELECTED TYPE SUBSTANCES POSSIBLY RELATED TO LIGNIN

### (a) Mercuration of Furfural Diacetate

Furfural diacetate (63) was prepared by the acetylation of 50 gm. furfural with 100 gm. acetic anhydride and 0.6 gm. stannous chloride as catalyst at -3 C. Yield, 85 gm., m.p. 62°C.

It was mercurated by a procedure somewhat similar to that used by Scheibler (36). Five grams furfural diacetate (1/40 Mol.) was dissolved in 20 cc. glacial acetic acid and mixed with a solution of 24.6 gm. mercuric acetate (3/40 Mol.) dissolved in 180 cc. glacial acetic acid. The mixture was allowed to stand at room temperature for 48 hours, with occasional shaking. It was then evaporated to dryness under reduced pressure at room temperature. The dry, white, crystalline residue was dissolved in 50 cc. chloroform, filtered, and then added slowly to 500 cc. of rapidly-stirred ether. The white precipitate was filtered, washed with ether and dried in a vacuum desiccator over sodium hydroxide. After a second purification from chloroform-ether the product was analyzed for mercury. The purification depends on the fact that mercurous and mercuric acetates are insoluble in chloroform.

Mercury Analysis*

(1) 0.1523 gm. sample gave 0.1078 gm. HgS; %Hg, 61.1

(2) 0.1837 " " " 0.1279 " " " 61.6

This corresponds to the substitution of three acetoxy-mercuri groups.

(b) Mercuration of Methyl-furfuraldiacetate

Methyl-furfuraldiacetate (67) was prepared from methyl furfural (39) by acetylating with acetic anhydride using a trace of sulphuric acid as a catalyst. The diacetate (5.3 gm.) was dissolved in 20 cc. glacial acetic acid and mercurated by mixing with a solution of 24.6 gm. mercuric acetate (3/40 Mol.) dissolved in 180 cc. glacial acetic acid. After standing for 2 days at room temperature, the mixture was evaporated to dryness at room temperature, the residue was extracted with chloroform and the extract precipitated into ether. The white precipitate was filtered, washed, dried and analyzed.

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* The procedure used for mercury determinations was the following: 200 mg. sample is weighed out into a small beaker (150 cc.) and digested at about 100°C. with 5 cc. fuming $H_2SO_4$ for about 2 hours. Potassium persulphate (0.2-0.5 gm.) is then added until the solution becomes colorless. After the effervescence has ceased the solution is diluted to 50 cc. and boiled gently for a few minutes. About 50 cc. of a saturated $H_2S$ solution is then added and the black precipitate of HgS coagulated by further heating at about the boiling point. The HgS is then filtered, washed, dried at 110°C. for 4 hrs. and weighed.

Mercury Analysis

(1) 0.2457 gm. sample gave 0.1610 gm. HgS; %Hg, 55.3

(2) 0.2390 " " " 0.1460 " " " 53.1

Theoretical for $C_6H_4O(OCOCH_3)_2(HgOCOCH_3)_2$, Hg - 55.0%

(c) Mercuration of Vanillin

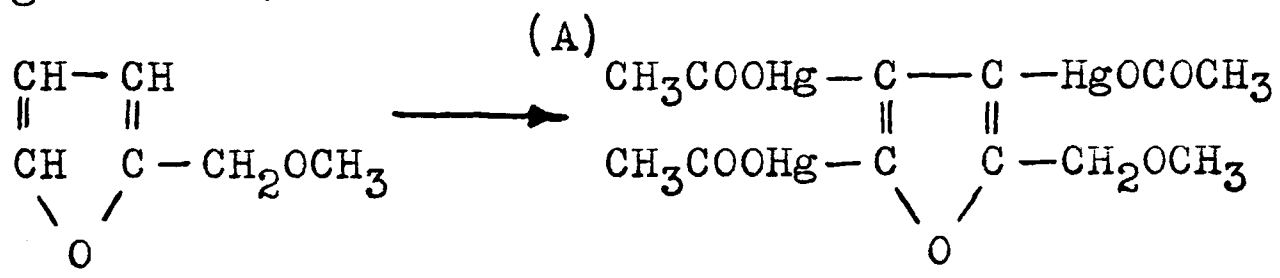
Five grams vanillin was mercurated according to the usual procedure at room temperature using a solution of 20 gm. mercuric acetate and 200 cc. glacial acetic acid. The mono-mercurated derivative (44) was formed. Analysis* showed 48.5% Hg (Theoretical - 48.4%). The methylated vanillin (veratric aldehyde), however, did not react when treated in a similar way. The original materials were recovered quantitatively.

(d) Mercuration of Furfuryl Methyl Ether

Furfuryl methyl ether (2.8 gm.), prepared by the methylation of furfurol with dimethylsulphate and caustic soda, was mercurated with a solution of 25 gm. mercuric acetate in 200 cc. glacial acetic acid at room temperature over a period of 2 days. The mixture was then evaporated to dryness under reduced pressure at room temperature, the

* Analysis of this compound was made by H.W. MacKinney

dry residue dissolved in chloroform, filtered, and precipitated in anhydrous ether. The precipitate was filtered, washed, and dried over sodium hydroxide in the usual manner. An analysis for mercury showed that three acetoxymercuri groups had replaced all the available hydrogen atoms.




Mercury Analysis

(1) 0.1820 gm. sample gave 0.1414 gm. HgS; %Hg, 67.0
(2) 0.1953 " " " 0.1522 " " " 67.2
Theoretical for (A), Hg - 67.7%

(e) Mercuration of Acetoxymethyl-furfuraldiacetate*

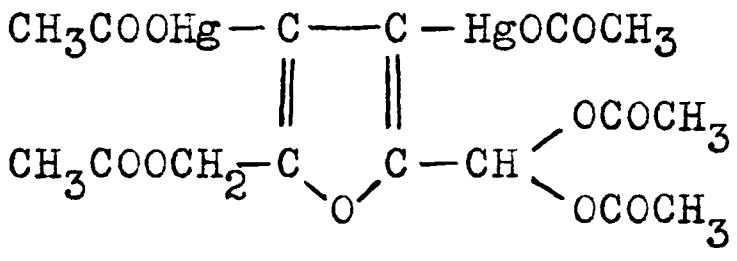
6.25 gm. acetoxymethyl-furfuraldiacetate (1/40 Mol.) was dissolved in 20 cc. glacial acetic acid and mercurated according to the usual procedure with a solution of 16 gm. (2/40 Mol.) mercuric acetate dissolved in 180 cc. glacial acetic acid. After evaporation to dryness the dry product was extracted with ether. No unchanged material was extracted. The residue was purified by dissolving in chloroform

* The writer wishes to thank S.M. Trister for the preparation of this compound.

and precipitating in ether. An analysis of the final, dry, purified product showed that each of the available hydrogens on the furan ring had been replaced with formation of acetoxymethyl-diacetoxymercuri-furfuraldiacetate.

Mercury Analysis

(1) 0.2046 gm. sample gave 0.1199 gm. HgS; %Hg, 50.4

Theoretical for  is 51.0%

(f) Mercuration of Coumaron

Three grams coumaron (68), prepared from coumarin, was dissolved in 20 cc. glacial acetic acid and mercurated according to the usual procedure with a solution of 25 gm. mercuric acetate in 180 cc. of glacial acetic acid at room temperature for 2 days. The final, dry product was analyzed for mercury and the analysis showed that two acetoxymercuri groups had replaced the two available hydrogen atoms, presumably on the furan ring.

Mercury Analysis

(1) 0.2036 gm. sample gave 0.1451 gm. HgS; %Hg, 61.5

(2) 0.2033 " " " 0.1455 " " " 61.7

Theoretical for Hg - 61.6%

Acetyl Analysis

(1) 0.07196 gm. sample req'd 15.1 cc. 0.02040 N. NaOH

$\%CH_3CO$, 18.4

(2) 0.05268 gm. sample req'd 11.0 cc. 0.02040 N. NaOH

$\%CH_3CO$, 18.3

Theoretical for $CH_3CO$, 18.5%

(g) Mercuration of Chalcones

(i) Benzalacetophenone

Five grams benzalacetophenone (69) was treated according to the usual procedure with a large excess of mercuric acetate (32 gm.) in glacial acetic acid (240 cc.). After 2 days the original materials were recovered quantitatively, no reaction having taken place. It is evident that ethylenic linkages of this type cannot be mercurated under these conditions.

(ii) 4-Acetoxy-3-methoxyl Chalcone

One gram of 4-acetoxy-3-methoxy chalcone (m.p. 117°C.) obtained by the acetylation of 4-hydroxy-3-methoxy chalcone (64) which was prepared from the condensation of acetophenone and vanillin was treated with a solution of 2.3 gm. mercuric acetate and 50 cc. glacial acetic acid for a period of 2 days. The chalcone was recovered quantitatively,

showing that no reaction took place and again confirming the non-reactivity of ethylenic linkates with mercuric acetate at room temperature.

(h) Mercuration of Pyrone Rings

(i) Pentacetylquercetin

Pentacetylquercetin (2.5 gm., m.p. 193°C.), prepared by the acetylation of quercetin (45), was treated in the usual way with an excess of mercuric acetate (3.2 gm.) in glacial acetic acid (40 cc.) at room temperature. The original material (m.p. 192°C.) was recovered quantitatively, showing that no reaction had taken place.

(ii) Diacetylkojic Acid

Five grams diacetylkojic acid m.p. 102°C. (65), prepared by acetylating kojic acid with acetic anhydride in pyridine solution, was treated with 25 gm. mercuric acetate in 200 cc. glacial acetic acid. The original product (diacetylkojic acid, m.p. 102°C.) was recovered quantitatively unchanged, indicating that fully acetylated and presumably fully methylated pyrone rings cannot be mercurated at room temperature in a glacial acetic acid medium.

(i) Miscellaneous Mercurations

(i) 5-Methoxylevulinaldehyde-dimethyl Acetal (66)

Three grams of the acetal (b.p. 88-90°C. at 18 mm.) was treated with a large excess of the mercurating reagent (13 gm. mercuric acetate in 150 cc. glacial acetic acid). No reaction took place. The original materials were recovered quantitatively.

(ii) Anisaldehyde

3.4 gm. anisaldehyde (1/40 Mol.) was treated with 24 gm. mercuric acetate in 200 cc. acetic acid. No reaction took place whatsoever. The anisaldehyde and mercuric acetate were recovered.

## Mercuration of Lignin

(a) Glycol Lignin (Fully Methylated)

One gram fully methylated glycol lignin (22)($OCH_3$, 31.8%), prepared by K.R. Gray, was dissolved in 10 cc. glacial acetic acid and the solution added to a mixture of 6 gm. mercuric acetate and 50 cc. glacial acetic acid. After standing 2 days at room temperature the solution was evaporated to dryness at room temperature under reduced pressure. The residue was dissolved in chloroform, filtered, and precipitated into anhydrous ether. The precipitate was filtered, washed

thoroughly with ether and petroleum ether, and dried. The mercury content (34%) corresponds to the entrance of three acetoxymercuri groups.

Mercury Analysis

(1) 0.1018 gm. sample gave 0.0402 gm. HgS; %Hg, 34.0

(2) 0.1096 " " " 0.0430 " " " 33.9

Theoretical mercury value for $C_{42}H_{29}O_6(OCH_3)_9(OCH_2CH_2OCH_3)$-$(HgOCOCH_3)_3$ is 34.1%.

(b) Mercuration of Methanol Spruce Lignin (20)

One gram of methanol lignin ($OCH_3$, 21.0%) was mercurated at room temperature, using a solution of 6 gm. mercuric acetate and 60 cc. glacial acetic acid. An analysis of the pure product showed the presence of 46% mercury. Using the formula proposed by Brauns and Hibbert (20) for methanol lignin $C_{42}H_{32}O_6(OCH_3)_6(OH)_4$, this corresponds to the replacement of five hydrogen atoms by five acetoxymercuri groups, as shown in the formula $C_{42}H_{27}O_6(OCH_3)_6(OH)_4(HgOCOCH_3)_5$. Theoretical value for Hg - 46.1%.

Mercury Analysis

(1) 0.1736 gm. sample gave 0.0930 gm. HgS; %Hg, 46.3

(2) 0.1715 " " " 0.0916 " " " 46.1

(c) Methylated Methanol Lignin

4.5 gm. methylated methanol lignin (%$OCH_3$, 32.5-33.2), prepared by the extraction of fully methylated wood meal (%$OCH_3$, 38.8) in an autoclave with anhydrous methyl alcohol containing about 4% dry hydrogen chloride (p. 42), was mercurated by means of a large excess of mercuric acetate in glacial acetic acid (28 gm. in 200 cc.). The procedure followed was identical with that used in the mercuration of glycol lignin. An analysis of the dry product showed that two acetoxymercuri groups had entered the molecule.

Mercury Analysis

(1) 0.1035 gm. sample gave 0.0334 gm. HgS; %Hg, 27.8

(2) 0.1032 " " " 0.0330 " " " 27.6

After second precipitation from chloroform-ether.

Mercury Analysis

(1) 0.1043 gm. sample gave 0.0333 gm. HgS; %Hg, 27.6

(2) 0.1104 " " " 0.0354 " " " 27.7

Methoxyl Analysis

%$OCH_3$, 20.6 (20.7, 20.5)

(0.02228 gm. sample req'd 17.6 cc. 0.05077 N. $Na_2S_2O_3$)

(0.02795 " " " 21.6 " " " )

Theoretical for $C_{42}H_{30}O_6(OCH_3)_{10}(HgOCOCH_3)_2$ is 27.5% Hg and 21.2% $OCH_3$.

(d) Mercuration of Fully Methylated Oak Lignin

Fully methylated native oak lignin (0.5 gm.), prepared as already described on page 77 (%$OCH_3$, 37.6), was mercurated with a mixture of 3 gm. mercuric acetate in 25 cc. glacial acetic acid during a period of 2 days at room temperature in the usual way. The mercurated product was purified and dried, then analyzed.

Mercury Analysis

(1) 0.1929 gm. sample gave 0.0771 gm. HgS; %Hg, 34.5

(2) 0.1991 " " " 0.0802 " " " 34.2

The mercury analysis shows that three acetoxymercuri groups have replaced three hydrogen atoms of the lignin molecule, forming the compound $C_{42}H_{29}O_5(OCH_3)_{12}(HgOCOCH_3)_3$ which has a calculated mercury percentage value of 34.04.

## S U M M A R Y

1. An investigation of the extraction of lignin from oak and spruce wood meal at room temperature by the use of mild reagents has been made. Wood meal was acetylated with a mixture of acetic anhydride and acetic acid, using 0.5% sulphuric acid as a catalyst, as suggested previously by Suida and Titsch. This brings about a complete acetylation of the polysaccharides and lignin, the latter being extracted with acetone.

2. A satisfactory method for isolation of the oak lignin has been developed whereby about 75% of the total quantity present can be extracted, and more than 50% isolated in the form of a deacetylated product ($OCH_3$, 20.6%).

3. The acetylated, diazomethane methylated, and fully methylated derivatives of oak lignin have been prepared, purified and analyzed. The

methoxyl contents of these are 16.9%, 26.6%, and 37.6% respectively.

4. Methanol oak lignin has been prepared from oak wood by heating with methanol and hydrogen chloride under pressure in yield greater than 50% ($OCH_3$, 29.6%). The diazomethane and dimethylsulphate methylated derivatives were prepared and their methoxyl contents shown to be 32.4% and 37.8% respectively.

5. On the assumption that methylation of <u>methanol</u> oak lignin with diazomethane results in the replacement of one hydroxyl group by a methoxyl group, and two in the case of native oak lignin, an empirical formula $C_{48}H_{56}O_{17}$ has been derived, which when expanded shows the presence of six methoxyl and six hydroxyl groups in native oak lignin, $C_{42}H_{32}O_5(OCH_3)_6(OH)_6$.

6. This new formula for oak lignin differs from that proposed by Hibbert and co-workers

for methanol spruce lignin, $C_{42}H_{32}O_6(OCH_3)_6(OH)_4$, in that it contains two more hydroxyl groups and one less oxygen atom. This suggests the possible existence of an additional oxygen ring in spruce as compared with oak lignin.

7. An investigation was made of a method for extracting methylated spruce lignin from methylated spruce wood in an autoclave, using 4% dry hydrogen chloride as a catalyst in absolute methyl alcohol.

8. The method of preparation of spruce lignin by acetylation and extraction was investigated and shown to be of little or no value as a means of preparing native spruce lignin, owing to the difficulty in separating the latter from adhering pentosans. Fully methylated spruce lignin was prepared, however, and shown to have a methoxyl value of 32.8%, the same as that for fully methylated methanol lignin prepared by Hibbert and Brauns.

9. The mercuration by mercuric acetate of numerous organic compounds having a possible relationship to lignin was investigated. The behavior of these when contrasted with that of various lignin derivatives points to the presence of furan rings as part of the lignin complex. With fully methylated oak lignin and fully methylated glycol lignin three hydrogen atoms are replaced by acetoxymercuri groups. Native lignin, however, contains five active hydrogen atoms replaceable by acetoxymercuri groups, two presumably arising from the presence of a free phenolic hydroxyl group in one of the benzene rings.

10. The data on acetylation; diazomethane and dimethylsulphate methylation; tritylation and tosylation are all in harmony with the following formula for oak lignin:

$$C_{29}H_{21}O_5(OCH_3)_5(>CH.C_6H_3.OCH_3,OH)(-CH=\overset{OH}{\overset{|}{C}}-)(CHOH)_2(CH_2OH)_2$$

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