

ATTEMPTED DEGRADATIONS OF SPRUCE  
FORMALDEHYDE - PERIODATE LIGNIN

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## GENERAL INTRODUCTION

Previous workers observed that wood lignin, when condensed with formaldehyde in 72% sulfuric acid, gave a formaldehyde lignin in which about one-fourth of the carbon atoms were derived from formaldehyde. Reactive sites in the lignin, which normally promoted condensation to insoluble Klason lignin in the strong acid, appeared to be preferentially substituted by formaldehyde in such a way that the formaldehyde lignin was soluble in water and other liquids.

The object of the present work was to degrade the formaldehyde lignin in the hope of identifying some molecular fragments indicative of its structure. Earlier results on the separation by sulfonation, of the formaldehyde lignin into a major insoluble fraction which, on reduction with constant-boiling hydriodic acid, gave a dialysable material, were confirmed and extended. After an unsuccessful attempt at degradation with sodium in liquid ammonia, the results of previous oxidations in alkaline medium either with nitrobenzene or with potassium permanganate were confirmed. But oxidations with cupric hydroxide in alkali gave traces of bivanillyl and a new compound,  $C_{11}H_{15}O_7(OCH_3)$ . Other attempts at degradation involved the use of neutral potassium permanganate to oxidize the formaldehyde lignin, its

methyated derivative, and the derivative produced by fusion with potassium hydroxide prior to methylation.

The results strengthened the view that formaldehyde lignin, although soluble, was still a large molecular aggregate held together by carbon-carbon methylene bridges derived from the formaldehyde.

## HISTORICAL INTRODUCTION

Comprehensive descriptions of lignin chemistry have appeared in three excellent books by Brauns<sup>(1)</sup>, Brauns and Brauns<sup>(2)</sup>, and by Huggland<sup>(3)</sup>. A number of recent theses from this laboratory intensively discussed various aspects of lignin chemistry, and therefore a general review does not seem necessary in the present work.

It has been well established that the main structural component present in a soft-wood lignin like spruce is an oxygenated phenyl propane unit united in an unknown way through carbon-carbon and carbon-oxygen-carbon bonds to produce an insoluble, slightly cross-linked polymeric substance. Although there seems to be no method of isolating lignin in a completely unchanged native state, the method discovered by Ritchie and Purves<sup>(4)</sup> offers the research chemist a lignin, called 'Periodate Lignin', which is completely void of holocelluloses and retains most of the physical and chemical properties of lignin in situ. Nevertheless, this lignin gets slightly oxidized during its isolation.

In the standard method of determining lignin present in wood, the wood meal is treated with 72% sulfuric acid near 20°, whereby a highly condensed lignin, called Klason lignin, is obtained as a dark brown, amorphous, insoluble material. A pre-treatment of the wood meal with

formaldehyde causes the dissolution of the meal completely in 72% sulfuric acid. This observation was made by Ross and Hill<sup>(5)</sup> and Ross and Potter<sup>(6)</sup>. The authors diluted the above sulfuric acid solution with water in order to precipitate a 'formaldehyde lignin' which was soluble in alcohol, acetone, or acetic acid containing a little water. The solubility of the formaldehyde lignin suggested that the active centres originally present in the lignin molecule, which condensed with each other in the presence of sulfuric acid to form insoluble Klason lignin, had been substituted by formaldehyde. Assuming lignin to behave as a phenol or phenolic ether, the reaction of lignin with formaldehyde in the presence of strong sulfuric acid might be a condensation of the general phenol-formaldehyde type. The first major work, by Eisenbraun and Purves<sup>(7)</sup>, on the subject was planned to investigate the possible course of the reaction of lignin with formaldehyde.

Eisenbraun and Purves<sup>(7)</sup> showed the spruce periodate lignin, when condensed with formaldehyde in the presence of sulfuric acid of concentration not less than 69% and near 40°, gave rise in about 105% yield by weight to a product insoluble in dilute acids, and also to 7 - 9% of a fraction soluble in dilute acids. By a direct estimation of the formaldehyde remaining after condensation, they showed that 100 g. of lignin reacted with 32 g. of formaldehyde. The true amount condensed might be as high as 35 g., since

most types of lignin were found to evolve 2 - 3% formaldehyde on treatment with sulfuric acid<sup>(8)</sup>. However, a total of 115% of formaldehyde lignin was the most that was recovered, against an expected yield of at least 132% by weight. They explained this discrepancy as originating in the condensation, with loss of the elements of water, of hydroxymethyl groups introduced initially. The yield of the formaldehyde lignin, as calculated from the respective carbon contents of periodate and formaldehyde lignin, viz. 61.7 and 62.5%, was 121%, indicating a loss of about 11% of water if no carbon content was lost. The formaldehyde lignin had a methoxyl content of 9.9% against that of 12.2% for periodate lignin. When it was assumed that methoxyl groups were neither lost nor gained, the calculated yield of the formaldehyde lignin should be 123% by weight of the periodate lignin. Eisenbraun and Purves<sup>(7)</sup> also carried out condensations of periodate lignin with radioactive  $C^{14}$ -formaldehyde to determine the proportion of carbon in formaldehyde periodate lignin contributed by the formaldehyde. The amount of  $C^{14}$  in  $C^{14}$ -formaldehyde periodate lignin appeared to be between 15% and 18% of the total carbon content. If it was assumed that the acid soluble and insoluble  $C^{14}$ -formaldehyde lignin had the same percentage of carbon and radio-carbon, then 100 g. of periodate lignin had condensed with 25 to 29 g. of the radioactive formaldehyde and the total yield of formaldehyde lignin was 117 - 121%

based on the radioactive carbon values. Table I shows that the agreement between these calculated values was close enough to support the above assumptions and to indicate that about 10 - 15% of the elements of water was lost during the condensation.

Table I  
Calculated and observed yields of  
formaldehyde periodate lignin

<u>Basis of Calculation</u>	<u>Yield by weight</u> <sup>a)</sup> %
Formaldehyde <sup>b)</sup>	121
OCH <sub>3</sub> in product <sup>c)</sup>	123
Radioactive method <sup>d)</sup>	117 - 121
Directly observed <sup>e)</sup>	110 - 114

a) Based on periodate lignin - Eisenbraun's data<sup>(9)</sup>.

b) Calculated from the direct estimation of formaldehyde consumption, 32%, and a loss of 11% of water deduced from the percentage of carbon in periodate and formaldehyde periodate lignin.

c) Assuming methoxyl groups neither lost nor gained.

d) Based on the assumption that the acid-soluble and insoluble lignin had the same percentage of carbon and radio-carbon.

e) Based on total yield observed.

Eisenbraun and Purves<sup>(7)</sup> also showed that a few phenolic hydroxyl groups had been formed in the formaldehyde lignin during the condensation, probably by the cleavage of some ether linkages with the 72% sulfuric acid used in the reaction. Their evidence was that the original periodate lignin failed to show the presence of any phenolic hydroxyl groups when methylated with diazomethane, whereas an increase of 1.8% was observed with formaldehyde periodate lignin. Methylation with dimethyl sulfate caused the same increase in the methoxyl contents of the two lignins, thus showing that both had the same total number of phenolic and aliphatic hydroxyl groups, and suggesting that the content of aliphatic hydroxyls in formaldehyde lignin was probably less than that in periodate lignin. The acetylations of both lignin also supported the above statement. Table II summarizes the results of methylations and acetylations with both types of lignin.

The complexity of the lignin macromolecule prevented the assignment of a structure to the formaldehyde derivative, which could only be surmised on the basis of some closely related data. Structure I in Fig. 1 represents the major part of the lignin molecule, in which the ether links in the phenyl propane side chain are arbitrarily assigned. Some of the ether links in such a structure would be cleaved by the action of sulfuric acid, but with the simultaneous effect of auto-condensation, an

Table II

Methoxyl and Acetyl Content of Spruce Periodate

Lignin and of its Formaldehyde Derivative<sup>a)</sup>

	Methylated % OCH <sub>3</sub> <sup>b)</sup>			
	% OCH <sub>3</sub>	CH <sub>2</sub> N <sub>2</sub>	Me <sub>2</sub> SO <sub>4</sub>	Acetylated % Acetyl
Periodate lignin	12.2	-	17.6	-
	12.5 <sup>c)</sup>	13.1 <sup>c)</sup>	17.8 <sup>c)</sup>	5.0 <sup>c)</sup>
	11.5 <sup>d)</sup>	11.2 <sup>d)</sup>	-	-
Formaldehyde periodate lignin <sup>e)</sup>	9.9	11.7	15.2	3.9 <sup>f)</sup>

a) Data from Eisenbraun and Purves<sup>(7)</sup>.

b) Diazomethane in dry ether; dimethyl sulfate in 30% sodium hydroxide.

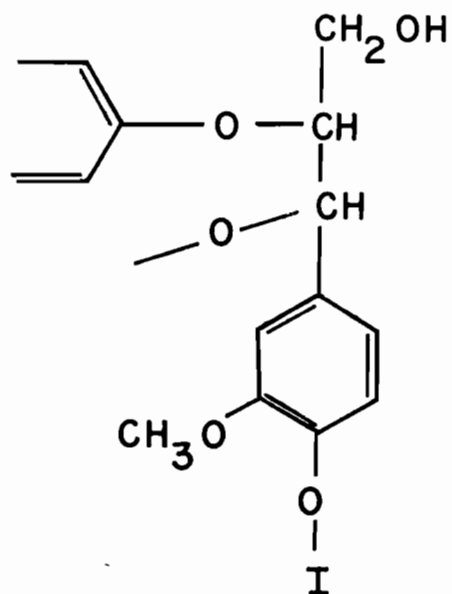
c) Data from Wieckowski<sup>(11)</sup>; acetylation in pyridine.

d) Data from Cabbot and Purves<sup>(12)</sup>.

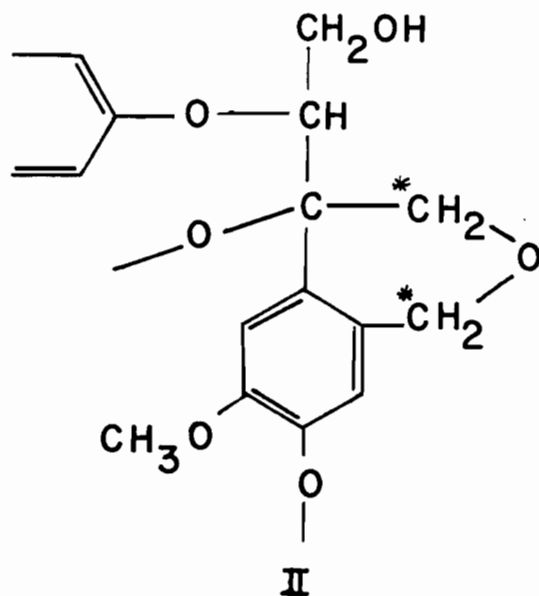
e) Major fraction, 105% by weight, uncorrected for sulfur.

f) Acetylated with anhydrous sodium acetate.





Base Molecular Weight  
~ 187



Base Molecular Weight  
~ 229

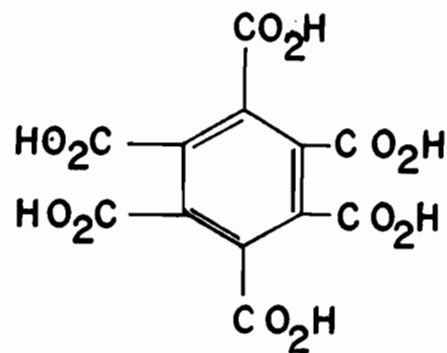
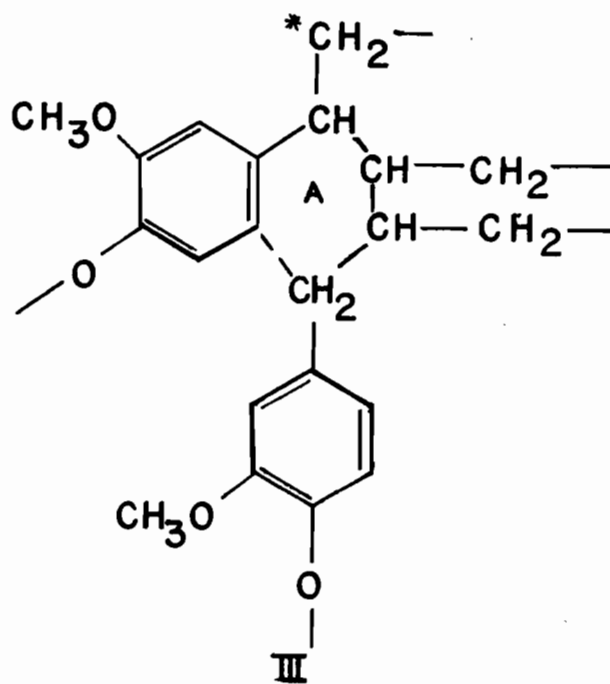


Figure 1

STRUCTURES FROM EISENBRAUN AND PURVES<sup>(7)</sup>

insoluble, more highly cross-linked lignin would result, with a probable loss of the elements of water<sup>(10)</sup>.

Lignin when heated in aqueous sodium bisulfite becomes sulfonated probably at the  $\alpha$ -position, and an alkaline nitrobenzene oxidation of a chlorinated lignin results in the isolation of 6-chloro vanillin. Hence both the  $\alpha$  and the 6-position in the structure I are probably susceptible to attack in substitution reactions. A preferential condensation of each of these two reactive sites with 1 mole of formaldehyde would result in an increase in the base molecular weight of the lignin from about 187 to about 247, or in the ratio 100:132 by weight, and the formaldehyde would contribute 16.7% of the carbon in the adduct, as Eisenbraun and Purves<sup>(7)</sup> found. The loss of a molecule of water in such condensation would result in a structure like (II) with a new ether linkage, and reduce the yield by weight in the ratio 132:122. This figure was close to that observed. Carbon atoms originating as formaldehyde are denoted by asterisks in this and subsequent structures.

The formaldehyde lignin was a soluble material, suggesting that the condensation reaction did not bring about any cross-linking. In structure II, the elimination of water has been shown to occur between the two hydroxymethyl groups, but the new ether group could also be formed between one hydroxymethyl group in the  $\alpha$ -position and the

primary hydroxyl group at the  $\gamma$ -position of the phenyl propane side chain; in any case, however, the new ether group would reduce the number of free hydroxyl groups to the original number in the periodate lignin, as was found<sup>(7)</sup>. In spite of the fact that both the original periodate and the formaldehyde periodate lignin did not show any difference in their infra-red and ultraviolet absorption spectra, the formaldehyde periodate lignin failed to produce even a trace of vanillin on oxidation with alkaline nitrobenzene or with alkaline cupric oxide. Hence there was a fundamental difference in structure between this lignin and other lignins, including Klason and periodate lignin, all of which yielded vanillin on such oxidations, presumably by oxidative cleavage at the  $\alpha$ -carbon atom in structure I. The above observation was found to fit well with the structure II, with its change in the status of the  $\alpha$ -carbon atom or with a new substituent in the nucleus.

Eisenbraun and Purves<sup>(7)</sup> oxidized the formaldehyde lignin with potassium permanganate in hot aqueous alkali to carbon dioxide, acetic acid, oxalic acid and a mixture of benzene polycarboxylic acids. The formation of a radioactive oxalic acid from such an oxidation of  $C^{14}$ -formaldehyde lignin showed that formaldehyde during the condensation had formed a direct C-C bond with the lignin, rather than a C-O-C bond, as shown in structure II. This structure and also structure I, however, would not produce

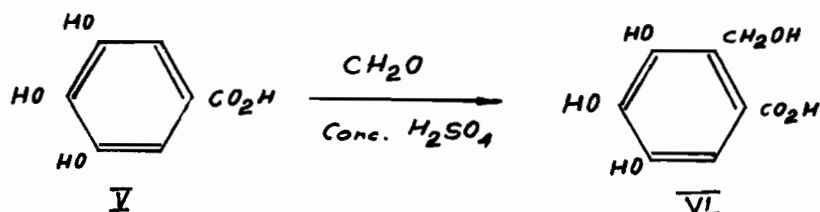
any polycarboxylic acids, since aromatic rings substituted directly by an oxygen atom would be completely destroyed on such oxidation. But the isolation of benzene penta- and 1,2,4,5-tetra carboxylic acids from periodate lignin by Read and Purves<sup>(13)</sup> and of benzene penta- and hexa carboxylic acid (mellitic acid) from formaldehyde periodate lignin by Eisenbraun and Purves<sup>(7)</sup> and then by Jain and Purves<sup>(14)</sup> pointed to the presence of a structure like III, Fig. 1, in periodate lignin which became substituted in the  $\alpha$ -position by a hydroxymethyl group<sup>(14)</sup>. A cyclohexene (tetrahydronaphthalene) ring A (structure III), as shown by the dotted line, could be formed by cyclisation; the ring A being completely substituted by carbon atoms could be oxidized to benzene hexacarboxylic acid and also penta-carboxylic acid by partial decarboxylation. The isolation of the radioactive mellitic acid (IV) and of benzene penta-carboxylic acid without any activity from a similar oxidation of C<sup>14</sup> formaldehyde periodate lignin by Eisenbraun and Purves<sup>(7)</sup> supported the above theory of substitution at the  $\alpha$ -position of the minor lignan structure by a hydroxymethyl group.

#### Condensation of Model Compounds with Formaldehyde

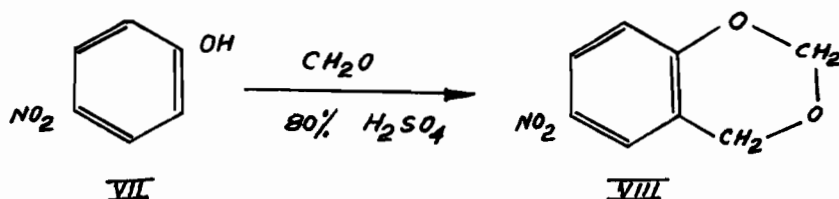
A good account of the condensation of phenols and other small molecules with formaldehyde was given in a book by Walker<sup>(15)</sup>, and the following discussion was restricted to

reactions of possible relevance to the condensation with wood lignin under acidic conditions.

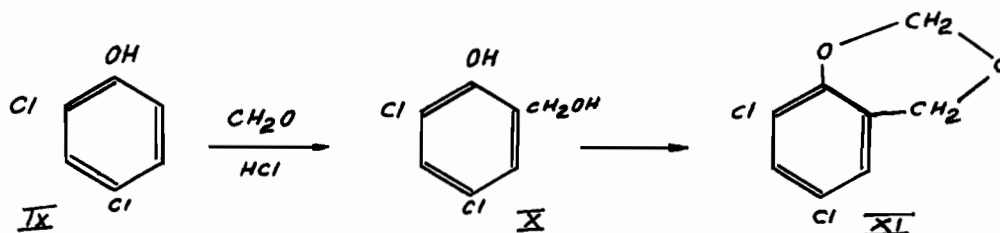
Alkaline conditions usually favoured the substitution of the formaldehyde as a hydroxymethyl group in the phenol but, in acidic conditions, it was very difficult to stop the reaction at that stage. However, Baeyer<sup>(16)</sup> isolated a monomethylol derivative (VI) by condensing gallic acid (V) with formaldehyde in the presence of concentrated sulfuric acid.



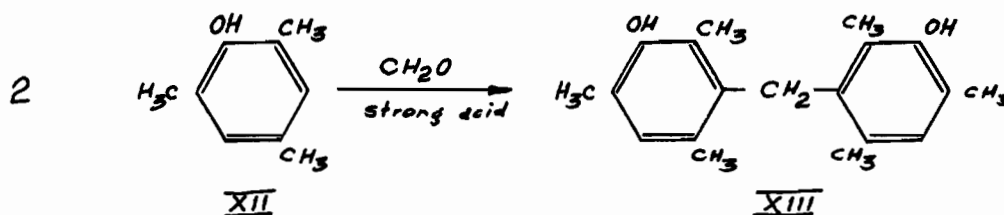
Borche and Berkhant<sup>(17)</sup> isolated a methylene ether or formal (VIII) by causing p-nitrophenol (VII) to react with two molecular proportions of formaldehyde in an excess of 80% sulfuric acid.



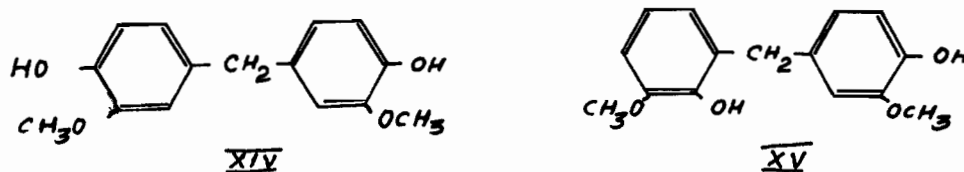
Ziegler and his co-workers<sup>(18)</sup> obtained a similar cyclic product (XI) by condensing 2,4-dichlorophenol (IX) with formaldehyde in the presence of cold hydrochloric acid. Dichlorosaligenin (X) was isolated as the intermediate product.



Tinn and Musty<sup>(19)</sup> showed that mesitol (XII), in which the three reactive positions were substituted by methyl groups, condensed with formaldehyde under strongly acidic conditions to produce a meta-linked diphenyl methane (XIII). This condensation did not occur in an alkaline medium.

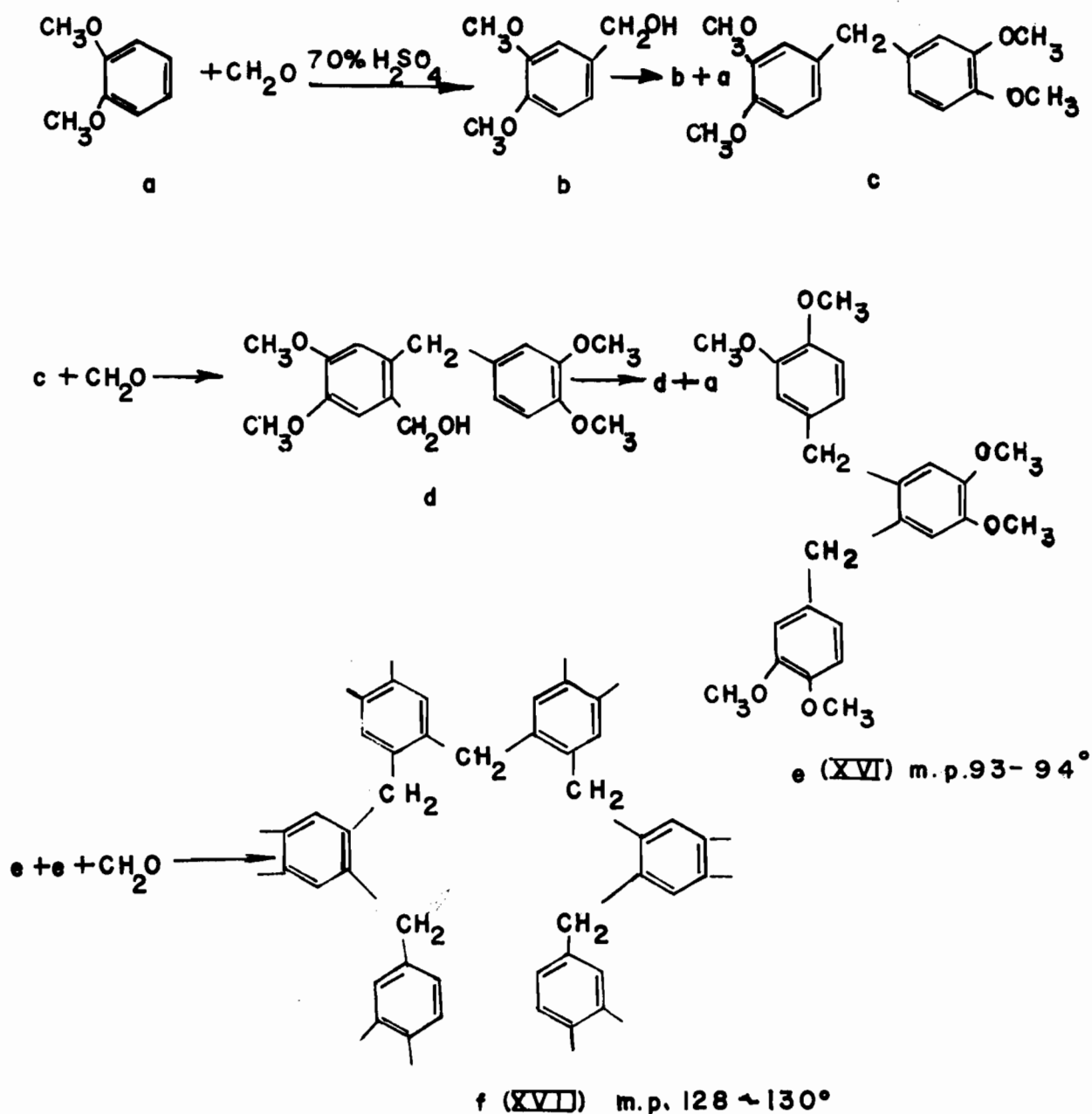


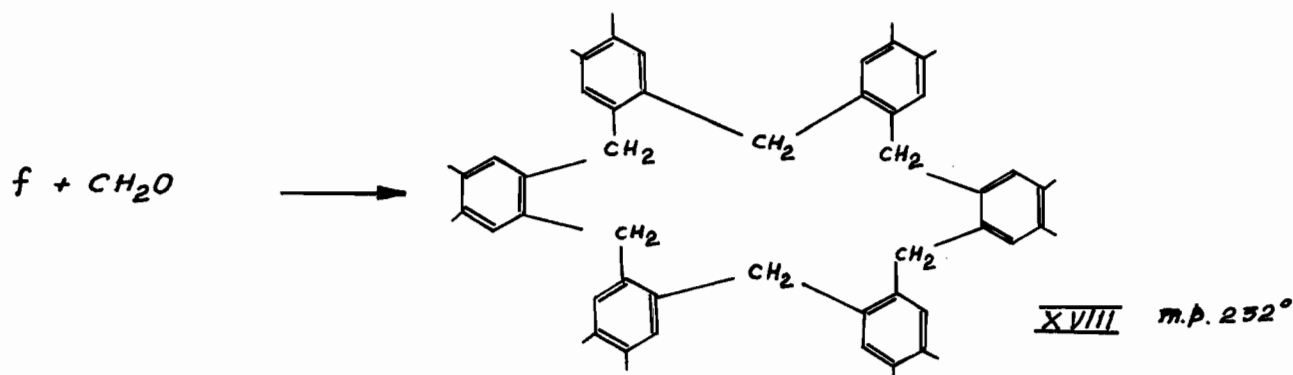
By condensing 2.2 mols of guaiacol with 1 mol of formaldehyde in concentrated hydrochloric acid, Euler, Adler and Friedman<sup>(20)</sup> isolated a dimer linked in the para-para (XIV) and in the ortho-para (XV) positions by methylene bridges, indicating a marked preference of the hydroxymethyl group for the para position, than ortho- or meta-positions.



Oliverio and Casinovi<sup>(21)</sup> added formaldehyde to veratrole kept

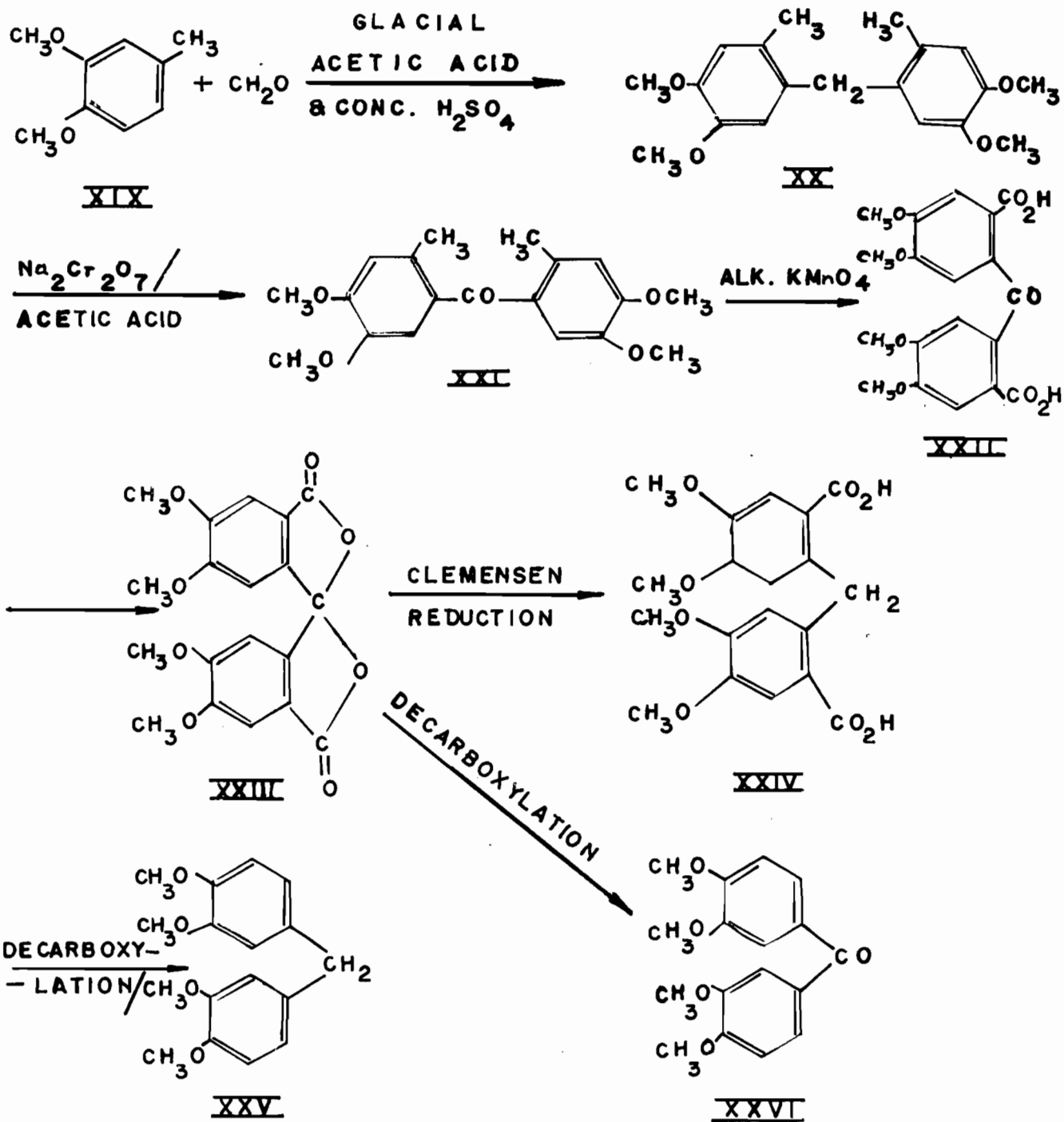
in 70% sulfuric acid at a temperature of about 10°. The resulting white viscous mass was fractionated from ether. Compounds (XVI), (XVII) were isolated from the ether-soluble fraction, and (XVIII) from the ether-insoluble part. Oliverio<sup>(22)</sup> also showed that the condensation of veratrole with acetaldehyde and propionaldehyde in the presence of 70% sulfuric acid resulted in diveratrylethane and diveratrylpropane respectively.





Since the guaiacylpropane was the most important structural element present in lignin, the condensation of 1-methyl-3,4-dimethoxy benzene (XIX) with formaldehyde in the presence of strong acid might be important in explaining the reactions leading to formaldehyde lignin. Oliverio and Boumis<sup>(23)</sup> used a mixture of glacial acetic acid and concentrated sulfuric acid and isolated 2,2'-dimethyl-4,5,4',5'-tetramethoxy diphenyl methane (XX) in quantitative yield from XIX. For purposes of identification, XX was oxidized by sodium bichromate in acetic acid to 2,2'-dimethyl-4,5,4',5'-diveratryl ketone (XXI), which when further oxidized with potassium permanganate in alkaline medium yielded 4,5,4',5'-tetramethoxy diphenyl ketone-2,2' dicarboxylic acid (XXII), isolated as the lactone (XXIII). A Clemensen reduction of the lactone (XXIII) to the acid, 4,5,4',5'-tetramethoxy diphenyl methane-2,2' dicarboxylic acid (XXIV) was followed by decarboxylation to diveratrylmethane (XXV). Decarboxylating the lactone (XXIII) directly in quinoline gave diveratryl ketone (XXVI).





The condensation of XIX to XX suggested the possibility of a similar methylene linkage between two aromatic nuclei in the formaldehyde periodate lignin, in

which case, the lignin might be extensively cross-linked. Nevertheless, the formaldehyde lignin was soluble in most organic liquids and showed no evidence of cross-linking. Solubility, however, might have been retained because of the hydrolysis of some of the ether linkages supposed to exist between the p-positions and the  $\beta$ -positions in the side chains of the original periodate lignin. In a word, attempts to guess the structure of formaldehyde periodate lignin were extremely difficult because the structure of the original periodate lignin is unknown. The only practical approach to the elucidation of the structure of formaldehyde lignin appeared to be the identification of structural fragments of low molecular weight. The remainder of this Introduction therefore deals with the various methods of degradation attempted by Jain and Purves<sup>(14)</sup>.

#### Attempted Degradations of Formaldehyde Periodate Lignin

Jain and Purves<sup>(14)</sup> studied various methods of degrading the formaldehyde lignin, using the mildest possible conditions in order to render less probable an inner condensation leading to a more complex and insoluble product. Since compounds whose molecular weight did not exceed a few thousands were known to pass in aqueous solution through a cellophane membrane, the percentage of the degradation products that could be dialysed was taken as a preliminary measure of degradation.

The degradation of lignin or wood meal by alcoholysis leading to 'Hibbert's monomers' was described in detail in the book by Brauns<sup>(1)</sup>, and in many theses from this laboratory<sup>(24,25,26)</sup>. The application of methanolysis or ethanolysis, using dry hydrogen chloride on the formaldehyde lignin, resulted in the isolation of about 20% of water-soluble material, out of which only 4% was dialysable, compared to about 19% obtained from the degradation of the original periodate lignin. The reduction in the time of methanolysis of the formaldehyde lignin from 24 hours to one hour did not alter the result. Jain and Purves<sup>(14)</sup> then extracted the dry formaldehyde lignin with boiling methanol, and the soluble fraction, of lower methoxyl content, on methanolysis showed an increased methoxyl content, but became insoluble in water. Since the dialysable fraction was not increased, alcoholysis did not appear to be an efficient method of degradation. The above dialysable and non-dialysable fractions from the methanolysis of the formaldehyde lignin failed to sulfonate when subjected to 16% sodium bisulfite solution at pH 5 for 20 hours at 98°, the conditions being adequate for the conversion of the periodate lignin to Stage I lignosulfonic acid (3% sulfur).

Jain<sup>(27)</sup> also subjected the formaldehyde periodate lignin to sulfonation using the above conditions, and isolated about 55% and 45% of soluble and insoluble Stage I lignosulfonate respectively, and about 21% of the soluble

fraction was dialysable. The insoluble stage I fraction on hydrolysis at pH 2 and at 100° for 24 hours gave only 6.5% of a soluble stage II lignosulfonic acid; under these conditions, the insoluble stage I lignosulfonate from periodate lignin could be hydrolysed to more than 90% of soluble stage II acid. Since the dialysates from the soluble lignosulfonate were heavily contaminated with bisulfite, Jain<sup>(27)</sup> attempted mild sulfonation of the formaldehyde lignin with 20 volumes of 1% sodium bisulfite at 98° for one hour and at pH 4.8 - 5.2, and the resulting black liquor on dialysis yielded about 15% of dialysable material with a sulfur content of 5.8%. This result suggested the presence of a lignosulfonic acid averaging 2 methoxyl and 1 sulfonic acid groups in a base molecular weight of about 500. The remaining 85% of the soluble, non-dialysable material was resistant to auto-hydrolysis to stage II lignosulfonic acid, and this light-brown powder was designated Fraction A in later degradation studies. The failure to sulfonate Fraction A was attributed by Jain and Purves<sup>(14)</sup> to the substitution of the sulfonatable sites by formaldehyde, rather than to the condensing action of sulfuric acid, since Klason lignin could be sulfonated under similar conditions.

Other attempts at degradation by Jain and Purves<sup>(14)</sup> employed a saturated solution of boron trifluoride in acetic anhydride, which Francis and Rudloff<sup>(28)</sup> used to cleave ether linkages. Formaldehyde fraction A, described in the previous

paragraph, produced a black mass completely soluble in water, in 200% yield by weight, out of which about 69% was dialysable. The dialysable fraction differed markedly from lignin in being transparent to ultraviolet light throughout the wave-length range 200 to 400  $m\mu$  and was not examined further.

Aluminium tribromide was another Lewis acid known to cleave aryl alkyl ethers, but not diaryl ethers<sup>(29,30,31)</sup>. Very little of the formaldehyde lignin fraction A could be degraded by this reagent which brought about extensive demethylation of the lignin. This demethylation did not take place with the original periodate lignin, and the suggestion was made that the stability of some of the methoxyl groups was diminished when lignin condensed with formaldehyde.

The same authors also employed the Schmidt reaction, sodium azide in concentrated sulfuric acid near 0°, with formaldehyde lignin A in the hope of breaking the carbon skeleton at any potential carbonyl groups<sup>(32,33)</sup>. A black gum insoluble in dilute sulfuric acid resulted in very large yield. The large dialysable fraction, about 25%, was not further examined, as it was heavily contaminated with salts.

The results of the above degradations attempted by Jain and Purves<sup>(14)</sup> were recorded in Table III. For purposes of comparison, the results with the original periodate lignin were also shown.

Table III

Conversion of Formaldehyde Lignin and Periodate  
Lignin to Dialysable Materials<sup>a)</sup>

	Formaldehyde Lignin %	Periodate Lignin %
Neutral Methanol		
Insol. H <sub>2</sub> O	70	100
Non-dialysable Sol. H <sub>2</sub> O	19	-
Dialysable Sol. H <sub>2</sub> O	5	-
Methanolysis <sup>b)</sup>		
Insol. H <sub>2</sub> O	80	70
Non-dialysable Sol. H <sub>2</sub> O	16	-
Dialysable Sol. H <sub>2</sub> O	4	19
Stage I Sodium Bisulfite Cooks <sup>c)</sup>		
Insol. 16% NaHSO <sub>3</sub>	44	80 <sup>d)</sup>
Non-dialysable Sol. NaHSO <sub>3</sub>	35	20 <sup>d)</sup>
Dialysable Sol. NaHSO <sub>3</sub>	21	
Hydrolysis to Stage II <sup>e)</sup>		
Insol. at pH 2.0	38	9
Non-dialysable Sol. H <sub>2</sub> O	42	
Dialysable Sol. H <sub>2</sub> O	20	91 <sup>d)</sup>
% of Fraction A <sup>f)</sup>		
Schmidt Reaction <sup>g)</sup>		
Crude yield	300	134
Insol. N NaOH	70	44
Non-dialysable Sol. Aq. NaOH	5	27
Dialysable Sol. Aq. NaOH	25 <sup>h)</sup>	29

Table III (Contd.)

	Formaldehyde Lignin %	Periodate Lignin %
Boron Trifluoride <sup>i)</sup>		
Crude yield	200	130
Insol. aq. Na <sub>2</sub> CO <sub>3</sub>	5	-
Non-dialysable Sol. Na <sub>2</sub> CO <sub>3</sub> aq.	132	-
Dialysable Sol. Na <sub>2</sub> CO <sub>3</sub> aq.	69	-
Aluminium Tribromide <sup>j)</sup>		
Crude yield	112	98
Insol. N NaOH	75	70
Non-dialysable Sol. aq. NaOH	18	15
Dialysable Sol. aq. NaOH	7	13

a) Quoted from Jain and Purves<sup>(14)</sup>.

b) In boiling 2% methanolic hydrogen chloride for 18 hours.

c) In 16% sodium bisulfite at 97° and pH 5 for 20 hours.

d) Ref. (34); soluble portion not dialysed.

e) In water at pH 2 and 98° for 24 hours. Insoluble stage I portion (44%) gave only 6% of soluble material which was included in the 42% quoted.

f) Fraction (85%) of formaldehyde lignin soluble in 1% bisulfite at 98°, but non-dialysable; remaining 13.5% dialysable.

g) Sodium azide in concentrated sulfuric acid near 0°.

h) Not isolated; yield obtained by difference.

i) In acetic anhydride at 122°. The products were acetylated.

j) An 8% solution in boiling benzene.

Wieckowski<sup>(11)</sup> showed for the first time that the action of 47% hydriodic acid at 50° for 8 hours on the periodate lignin resulted in over 90% by weight of a product, which was resistant to the further action of the acid, and retained over 80% of the original methoxyl groups. An oxygignin prepared by the exhaustive oxidation of periodate lignin with sodium hypochlorite at pH 12 was also subjected to the action of the acid under similar conditions. In contrast to the behaviour of the periodate lignin, the oxygignin dissolved extensively in the hydriodic acid, but the methoxyl groups were almost completely retained, and the acid caused a reduction of the apparent molecular weight of the oxygignin from about 2300 to 400-500.

The types of ether bonds which could be cleaved by the action of hydriodic acid under the above conditions were also investigated by Wieckowski<sup>(11)</sup> with the help of the synthetic model compounds recorded in Table IV. Only the benzyl ethers XXXI and XXXII were cleaved under the stated conditions, and both diaryl and aryl alkyl ethers were recovered unchanged.

Since the structure of formaldehyde periodate lignin (structure II, Fig. 1) visualized by Eisenbraun and Purves<sup>(7)</sup> contained a benzyl ether linkage, Jain<sup>(27)</sup> thought that the action of 47% hydriodic acid using Wieckowski's conditions might possibly open up a new method of degradation. He treated the formaldehyde lignin fraction A (Table III) with



Table IV

Model Substances for Possible Ether Links in Lignin

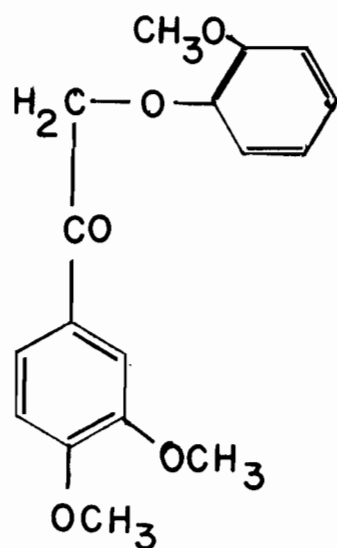
No.	Compound	Result <sup>a)b)</sup>
XXVII	w-(2-methoxy phenoxy)-acetoveratrone	Unchanged
XXVIII	2-(3:4 dimethoxy phenyl)-2-hydroxy-1-(2-methoxy phenoxy) ethane	Unchanged
XXIX	dehydro diisoeugenol	Unchanged
XXX	dehydro diisoeugenol methyl ether	Unchanged
XXXI	diveratryl ether	Cleaved
XXXII	p-methoxy benzyl guaiacyl ether	Cleaved

a) After heating with 47% hydriodic acid at 50° for 8 hours.

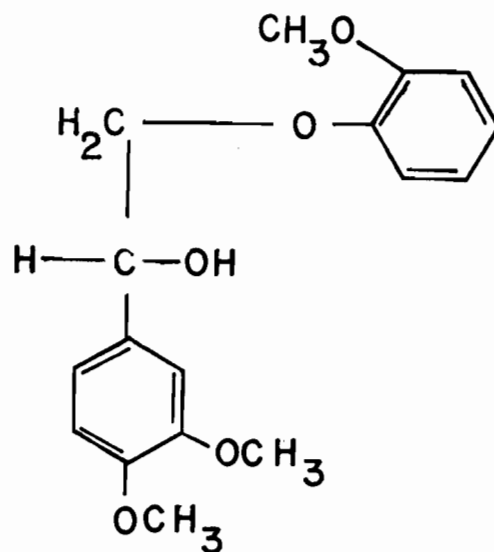
b) Data from Wieckowski<sup>(11)</sup>.

47% hydriodic acid for 8 hours at 50° and observed that about 92% of the starting material remained insoluble. This fraction retained all of the original methoxyl content and resisted further attack by the hydriodic acid. A small soluble portion showed extensive demethylation. Extraction of the insoluble product with 1 N sodium hydroxide left about 70% based on the formaldehyde lignin undissolved, and the alkali soluble fraction yielded 13% of the original formaldehyde lignin as a deep yellow dialysable portion, after purification on a cellulose column. This fraction gave about

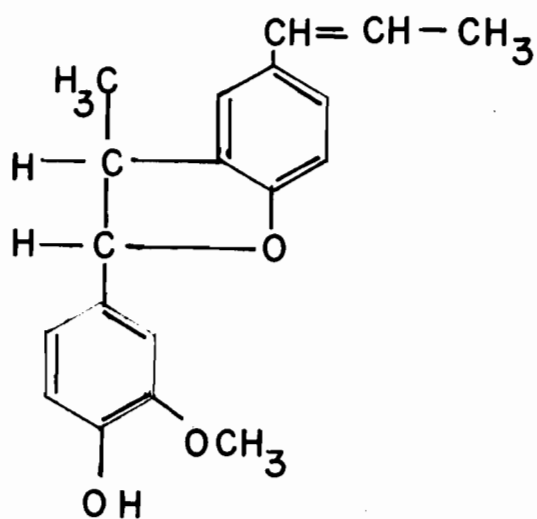
Appendix to Table IV



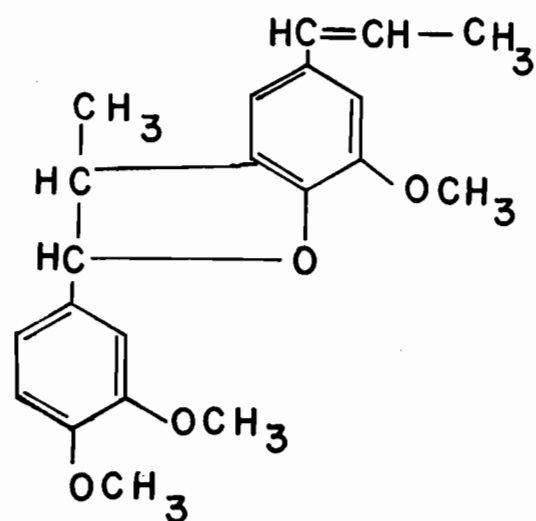
XXVII



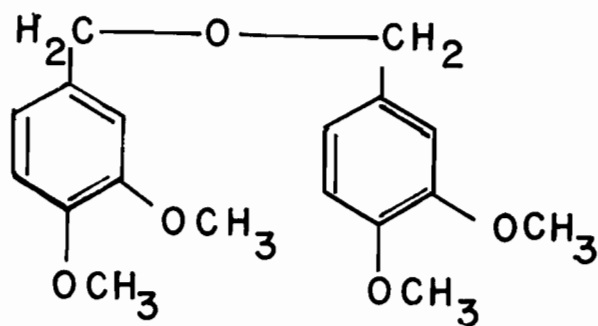
XXVIII



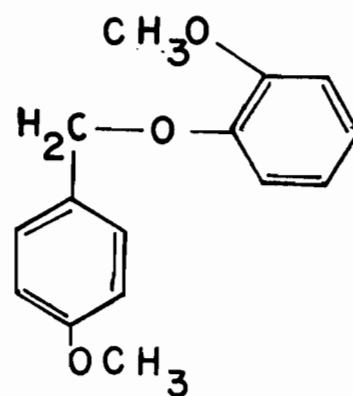
XXIX



XXX



XXXI



XXXII

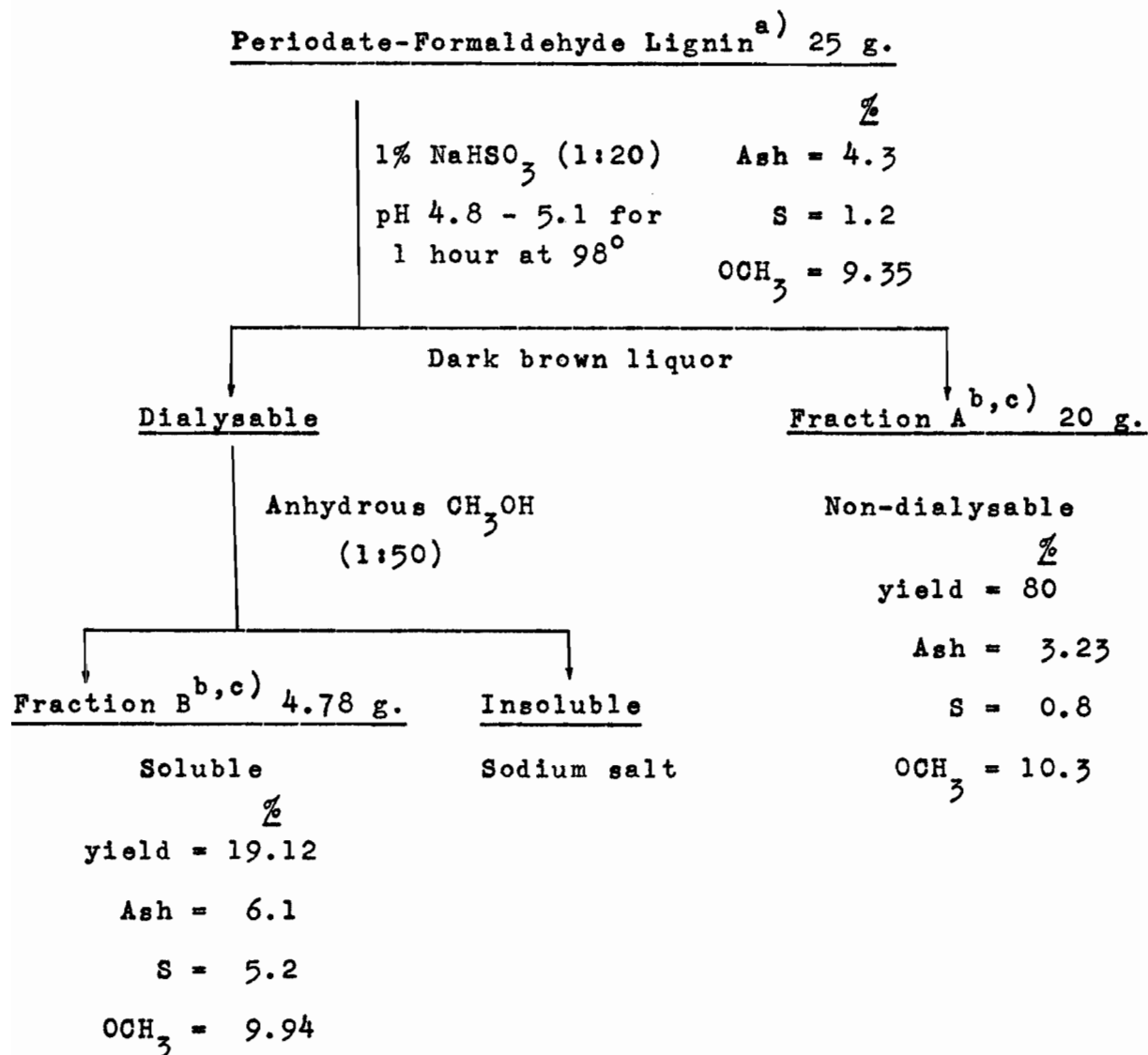
10% of a yellow, partly crystalline syrup which travelled as a unit with  $R_f$ , 0.26, on a paper chromatogram when butanol saturated with 2% aqueous ammonia was the solvent system and ferric chloride - potassium ferricyanide was the spray. Jain<sup>(27)</sup> concluded that the controlled action of hydriodic acid was the most effective and convenient degrading agent for formaldehyde periodate lignin that had been found. He made a number of fruitless attempts to separate the crystalline material from the syrup by paper chromatography; although he was able to separate a minute amount of white crystalline material between pads of filter paper, this product seemed to consist of ammonium sulfate mixed with a little sodium sulfate. The yellow amorphous material left showed the 280 m $\mu$  maximum in the ultraviolet absorption spectrum, characteristic of most lignin preparations and derivatives. The infra-red spectrum had the absorption maximum between 3200 - 3600 cm<sup>-1</sup> indicative of phenolic groups, an inference which was supported by the red colour observed when the sample was treated with ferric chloride solution. He failed to identify this dialysable, mobile fraction, but suggested that it was a phenol of molecular weight 250 to 300. Since a parallel hydrolysis of the spruce wood meal with hydriodic acid failed to yield the mobile fraction of  $R_f$  = 0.26, Jain<sup>(27)</sup> concluded that this mobile fraction might be a lignin dimer, and that its detailed study might constitute a structurally important research in its own right. Such a study was the initial object of the present research.

## RESULTS AND DISCUSSION

Periodate lignin was prepared from spruce wood meal by following the well-established method of Ritchie and Purves<sup>(4)</sup> as modified by Sacks<sup>(35)</sup>, and the air-dried material, with a methoxyl content of 12.1%, was used for the preparation of the formaldehyde derivative. The formaldehyde lignin was isolated by freeze drying in about 97.5% yield, which was lower by about 7 - 8% by weight than the yields reported by Eisenbraun<sup>(9)</sup> and Jain<sup>(27)</sup>. The methoxyl, 9.35%, and sulfur contents, 1.2%, were in good conformity with their findings.

In order to decide whether or not any periodate lignin had escaped the condensation with formaldehyde, the simplest way was to subject the product to micro oxidation in an alkaline medium with either nitrobenzene or cupric oxide, and to analyse the oxidation products by partition chromatography. Failure to isolate any vanillin by such oxidations of the formaldehyde lignin was a definite proof that none of the original periodate lignin had escaped the condensation with formaldehyde. This observation agreed well with that of Eisenbraun<sup>(9)</sup> and Jain<sup>(27)</sup>.

The next step was to check Jain's observation<sup>(14)</sup> that 1% aqueous sodium bisulphite near pH 5 at 98° for an hour separated the formaldehyde periodate lignin into a major insoluble and a minor soluble fraction. The present results were summarized in Fig. 2. A yield of 20% of soluble,



- a) Yields based on formaldehyde lignin, corrected for moisture and ash content.
- b) Yielded no vanillin on alkaline nitrobenzene oxidation.
- c) Yielded both hexa- and penta-carboxylic acids on alkaline permanganate oxidation.

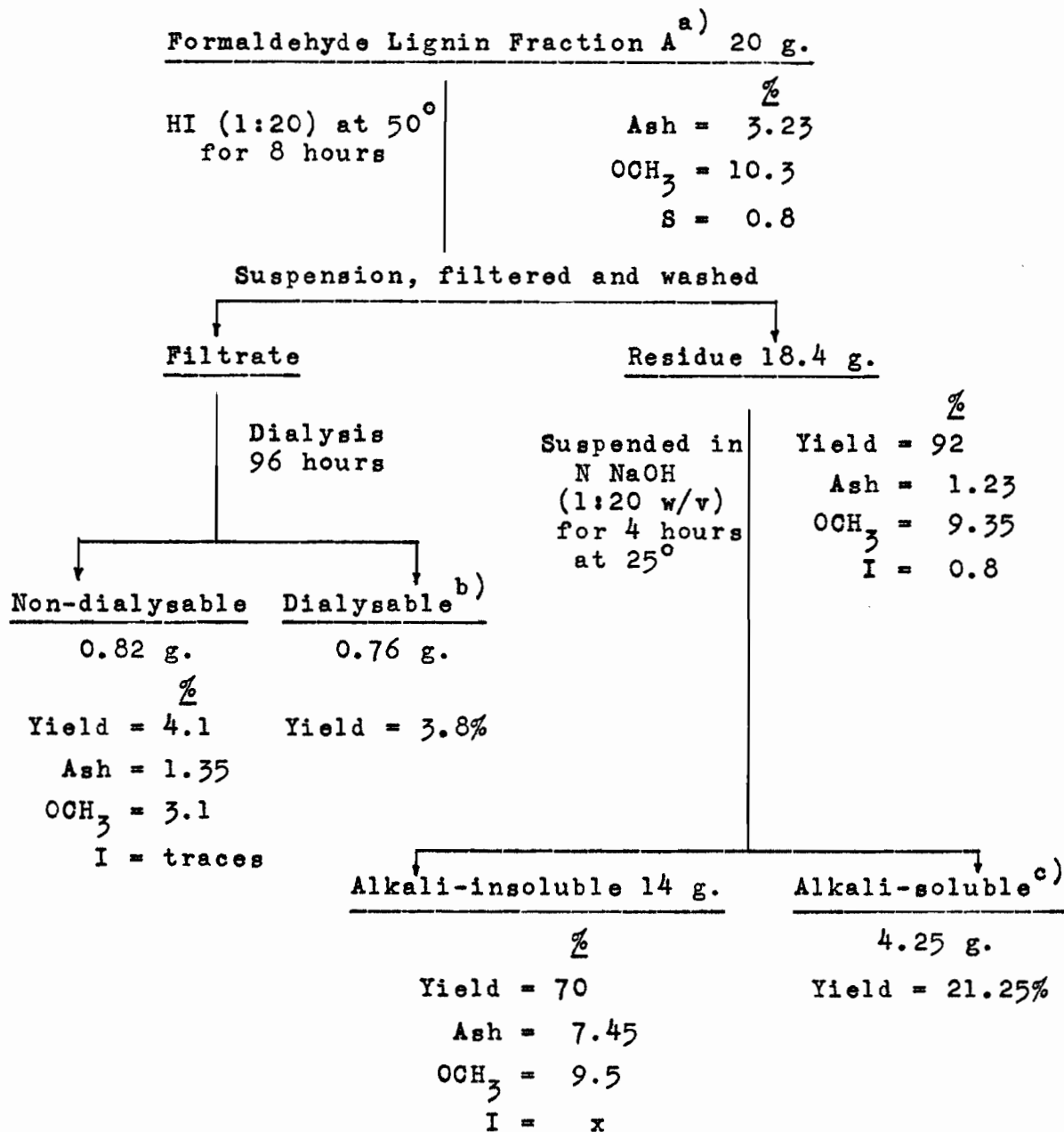
Figure 2

MILD SULFONATION OF FORMALDEHYDE PERIODATE LIGNIN

dialysable material with a sulfur content of 5.2%, and of 80% of a non-dialysable fraction with a sulfur content of 0.8%, was obtained. The latter sulfur content was close to that of the original formaldehyde periodate lignin. Jain's work was thus confirmed, and the formaldehyde lignin was not homogeneous in nature. The non-dialysable fraction (80%) represented the formaldehyde lignin A.

The bisulfite extracted formaldehyde-lignin fraction A (Fig. 2) was then subjected to 47% hydriodic acid at 50° for 8 hours. The work of Jain<sup>(27)</sup> was first confirmed with a 5 g. sample of the fraction A, and an experiment with 5 g. of formaldehyde lignin without any pretreatment with bisulfite was also performed for comparison purposes. The results indicated hardly any difference between the pretreated and the original formaldehyde lignin in the action of hydriodic acid. Finally, 20 g. of formaldehyde lignin fraction A (Fig. 2) was treated with the hydriodic acid; 92% of the starting material remained insoluble and resistant to the further action of hydriodic acid (Fig. 3). The insoluble material had a methoxyl content of 9.35%, showing that very little demethylation had taken place. An iodine content of 0.8% also indicated that very little iodination of the product had occurred.

The combined filtrate and washings were wine-red in colour, and half of the material was non-dialysable. The methoxyl content of this portion was about one-third that of



a) Yields based on lignin fraction A (Fig. 2) corrected for moisture and ash content.

b) Obtained by difference, and not actually isolated.

c) Obtained by difference, and not actually isolated.

Figure 3

ACTION OF HYDRIODIC ACID ON FORMALDEHYDE LIGNIN FRACTION A (Fig.2)

the original formaldehyde lignin fraction A, showing extensive demethylation had probably taken place. Extraction of the 92% residue with 1 N sodium hydroxide at room temperature left 70% of the formaldehyde lignin undissolved. There was no decrease in the methoxyl content of this portion and no iodine was present.

The alkali soluble fraction, 21.25%, after being freed from salts (Fig. 4) gave a non-dialysable fraction amounting to only 5.8% of the original lignin fraction A. This yellow powder had a methoxyl content of 8.66%, as compared to 10.3% for the original lignin fraction A (Fig. 2).

The deep yellow dialysable portion contained 15% of material which was very hygroscopic, neutral, soluble in ether and alcohol, and insoluble in benzene, chloroform and carbon tetrachloride. The infra-red spectrum (Fig. 5) indicated the presence of the phenolic hydroxyl group, and this evidence supplemented the deep violet colouration produced with 5% ferric chloride solution. The ultraviolet spectrum (Fig. 6) retained the 280 m $\mu$  maximum of the formaldehyde lignin, suggesting the material to be a degradation product from the formaldehyde lignin. Paper chromatography of the material, when developed with n-butanol saturated with 2% aqueous ammonia and with ferric chloride - potassium ferricyanide as the spray, revealed three spots of  $R_f$  = 0.00, 0.20 - 0.22 and 0.08 - 0.10, the last faint spot with slight tailing. Jain's results were confirmed in all respects, except that he observed only one spot of  $R_f$  = 0.26 under the same conditions.



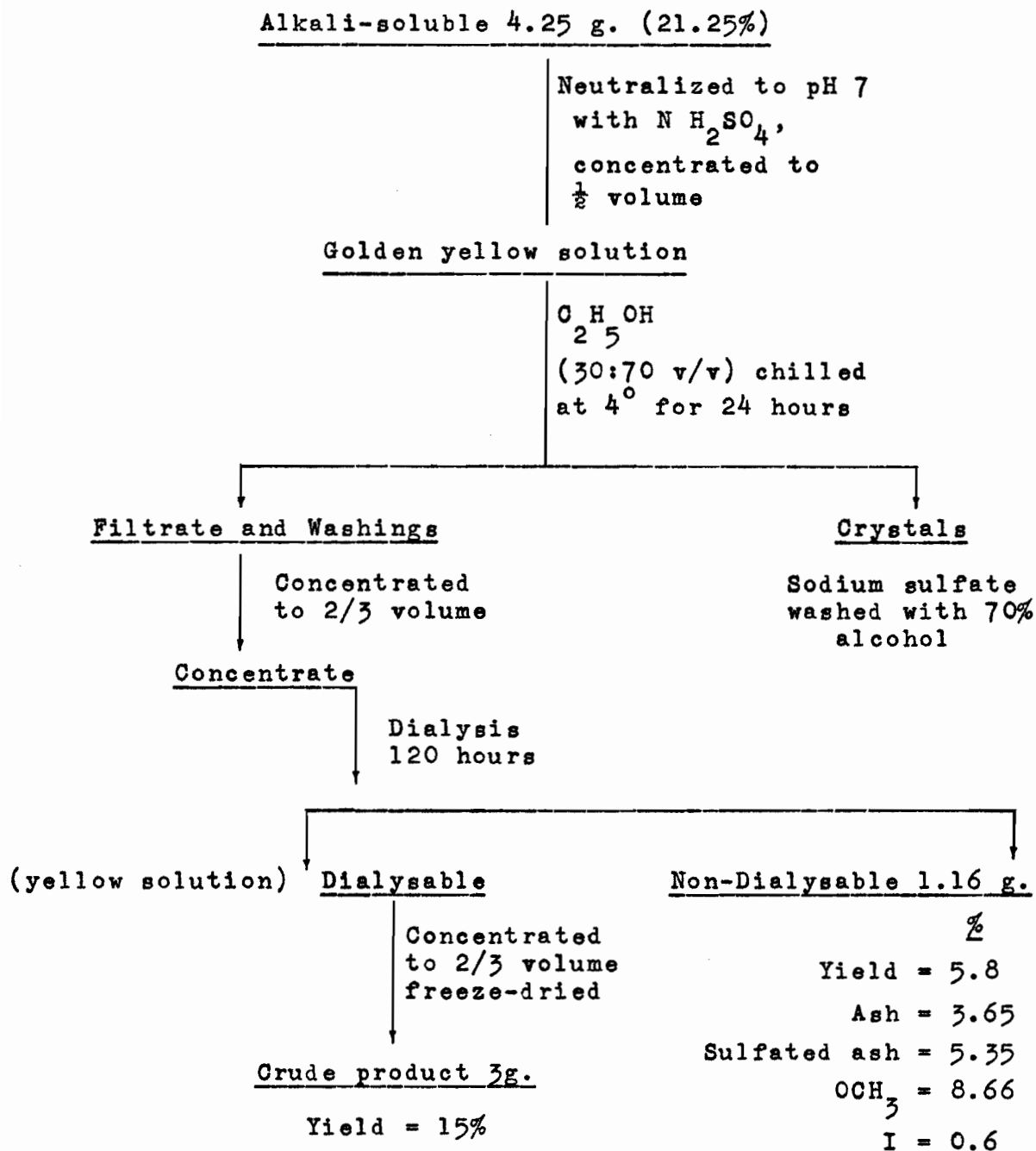


Figure 4

ISOLATION OF DIALYSABLE AND NON-DIALYSABLE FRACTIONS (see Fig. 3)

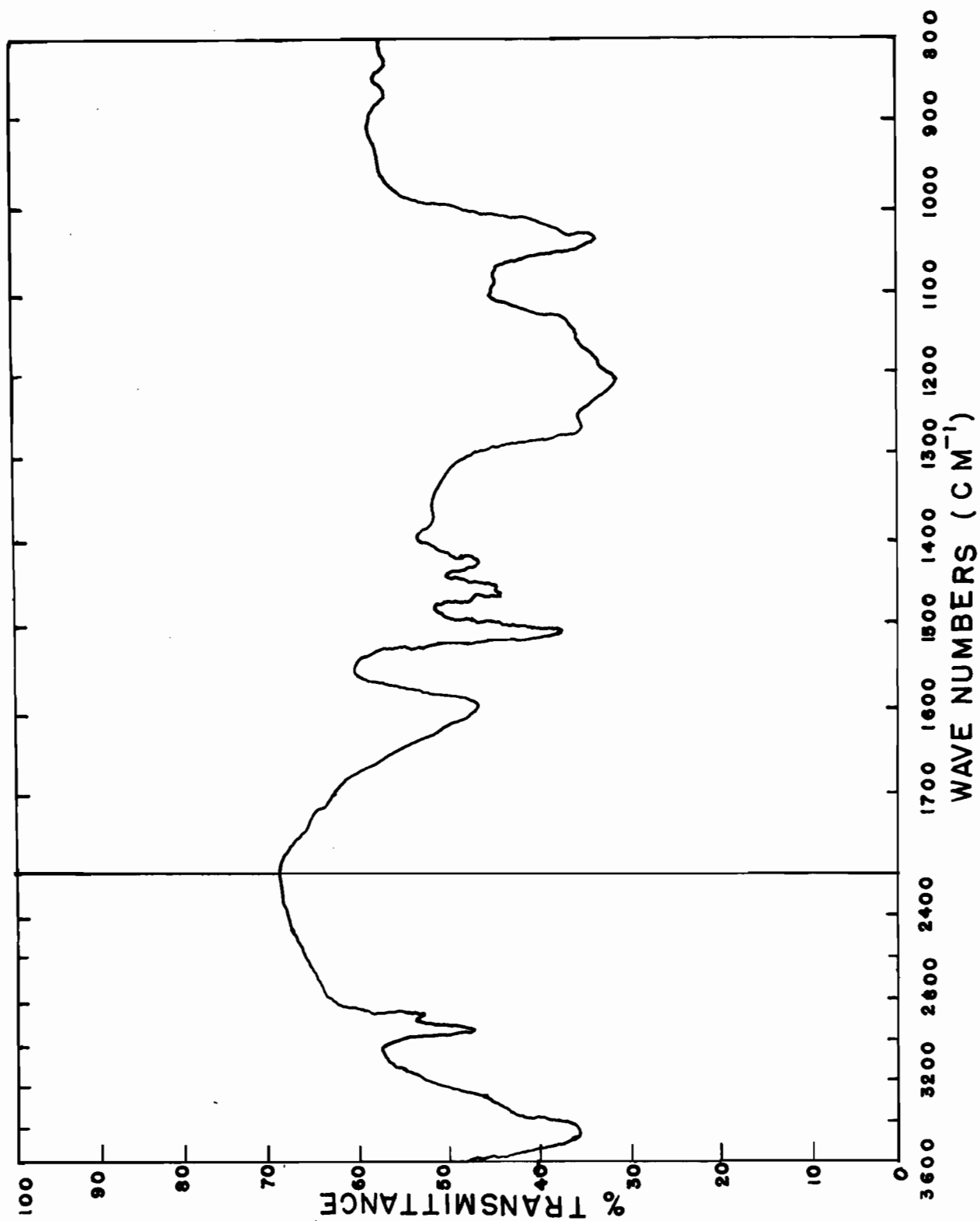


Figure 5

INFRA-RED SPECTRUM OF THE DIALYSABLE FRACTION (FIG. 4)

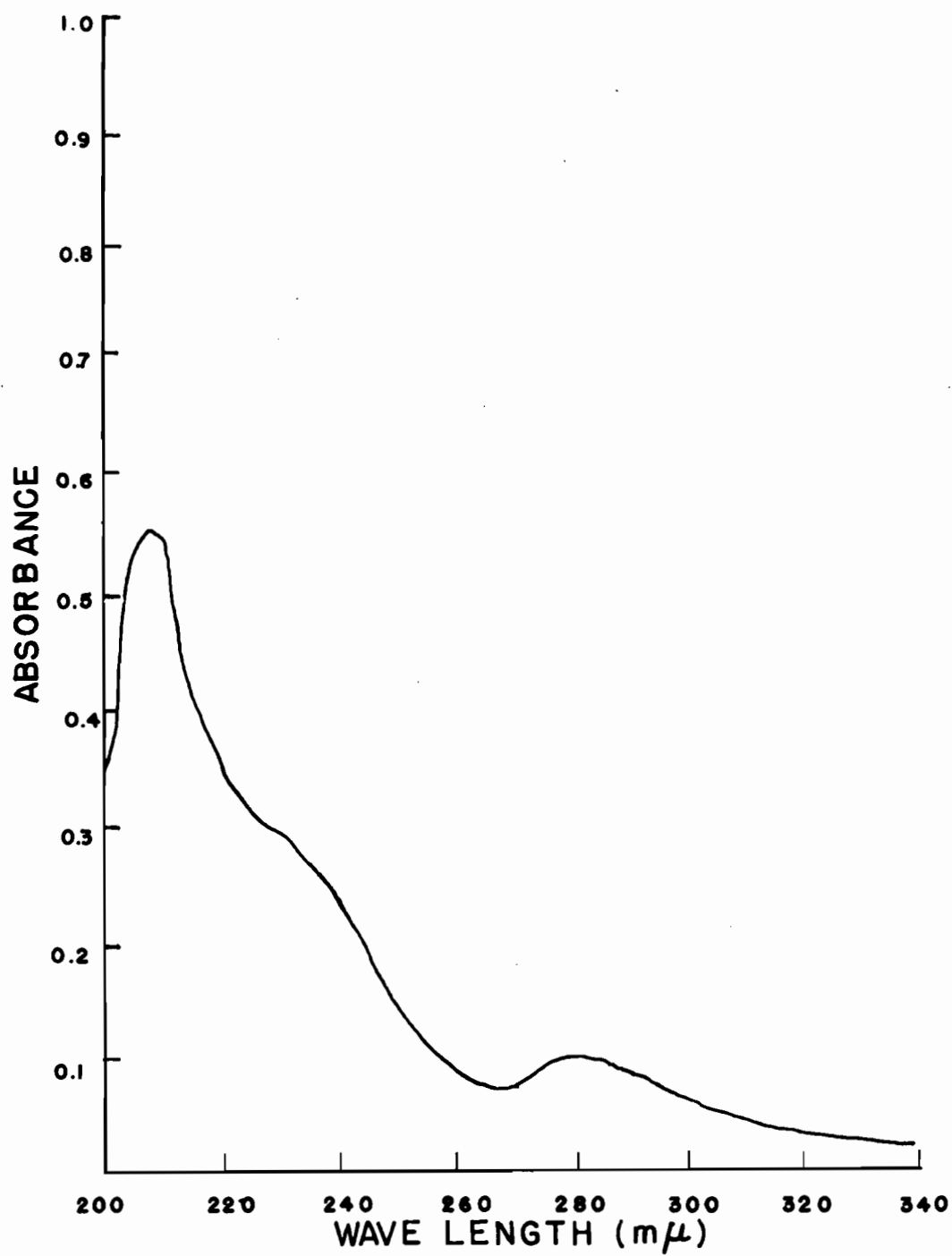


Figure 6

ULTRAVIOLET SPECTRUM OF THE DIALYSABLE FRACTION (FIG. 4,  
(in 95% ethanol)

The material when developed in n-butanol - acetic acid - water (4:1:5 v/v) and in n-butanol - pyridine - water (10:3:3 v/v) gave  $R_f$  values of 0.30 and 0.39 respectively, with a long streak from the starting line of the chromatogram to the solvent front in both cases.

Attempts were next made to find a better eluent adapted to the chromatographic identification of phenols and phenol derivatives. Of the systems described by Chromatographic Reviews, 1959<sup>(36)</sup>, the following were tried, but the material failed to move at all, thus excluding 450 different phenolic compounds listed in the Reviews.

- (a) methyl ethyl ketone - acetone - formic acid - water (40:2:1:6 v/v).
- (b) methyl ethyl ketone - diethylamine - water (921:2:77 v/v).
- (c) methylisobutyl ketone - formic acid - water (10 parts ketone saturated with 1 part of 4% formic acid).
- (d) chloroform - methanol - formic acid - water (10 parts chloroform saturated with a mixture of 1 part of methanol and 1 part of 4% formic acid).
- (e) benzene - methyl ethyl ketone - formic acid - water (9 parts benzene and 1 part ketone saturated with 1 part of 2% formic acid).
- (f) benzene - formic acid - water (10 parts benzene saturated with 1 part of 2% formic acid).

Finally, a cellulose column chromatographic separation of 2 g. of the dialysable yellow gum was made using n-butanol saturated with 2% aqueous ammonia which had been found to be the only eluent for effective separation. This

procedure yielded 1.3g. of a yellow amorphous powder of  $R_f$  value 0.22 and 0.39 - 0.41 in n-butanol - 2% aqueous ammonia and n-butanol - pyridine - water (10:3:3 v/v) respectively. A second fraction amounting to 0.7 g. was a dark brown resinous mass of  $R_f = 0.0$  in the above-mentioned solvent systems. The mobile material when examined under the microscope revealed crystals contaminated with a yellow amorphous substance. A solution of the material in water on decolorisation with decolorising carbon yielded a white crystalline substance which on prolonged drying again changed to yellow amorphous powder. This behaviour was rather strange, since any chemical change, e.g. oxidation, could not be conceived as the resulting yellow material could be decolorised again.

The material could be crystallized from several solvents, but on drying, the crystalline condition was lost and an amorphous yellow powder resulted. Finally, the observation that the neutral crystals, after being ion exchanged with Amberlite 120, gave an effluent of pH 2 led to their identification as ammonium iodide. This salt must have been formed during the elution of the reduced lignin chromatograms with n-butanol - ammonia - water. A reference to the literature<sup>(37)</sup> and control experiments with an authentic sample, showed that ammonium iodide, like the material under investigation, was very hygroscopic, formed colourless cubic crystals in ethanol, but fell to an amorphous powder when

dried; its solution in water turned yellow on standing, and the  $R_f$  values in paper chromatography were identical to those of the material under study. No less than 9.7% of the 15% of dialysable material obtained after degradation of formaldehyde lignin with 47% hydriodic acid was ammonium iodide. Jain's opinion<sup>(27)</sup> that this fraction must be a phenol was a natural one, because the extensive similarity in behaviour of ammonium iodide and the customary colour tests, and chromatographic behaviour of phenols was not realized prior to the present research.

The second fraction, isolated in 3.5% yield from the column chromatographic separation of the dialysable fraction (Fig. 4), was a dark brown immobile resinous mass with an ash content of 17.5%. After being freed from ash, which was a sulphate, the resin was recovered as an acidic dark brown transparent film which failed to move in paper chromatography with solvent systems generally used for aromatic acids<sup>(38)</sup>. Ethanol-ammonia-water, however, resolved the material, (0.30 g.), into three fractions of  $R_f$  values 0.00, 0.30 and 0.38, each fraction being isolated quantitatively on paper. Yields were 0.190 g., 0.084 g. and 0.025 g. respectively. The fractions, isolated in the form of ammonium salts, were again ion exchanged to liberate the free acids but none was obtained from the third fraction. The dark brown immobile resinous mass, (66 mg.), isolated from the first fraction failed to crystallize, and had the

same methoxyl content, (9.1%), infra-red and ultraviolet spectra as the original formaldehyde lignin. The second fraction, 0.084 g. of  $R_f = 0.30$ , after ion exchange yielded 0.032 g. of a yellow transparent film, with a methoxyl content of 8.93%, an ash content of 6.8%, and the same infra-red spectrum as the original lignin.

Even from 25 g. of formaldehyde periodate lignin, the percentage yields of the degradation products were so small that the quantity available for identification purposes was inadequate. The results were so indecisive that the study of the degradation with 47% hydriodic acid had to be stopped at this stage.

#### Degradations with Sodium in Liquid Ammonia

The next objective chosen for the research was to degrade the formaldehyde periodate lignin by the action of sodium in liquid ammonia with the hope of isolating phenolic substances of lower molecular weight. The application of this powerful reducing and ether cleaving agent on various types of lignin was reviewed in the works of Read<sup>(39)</sup>, Wieckowski<sup>(11)</sup>, Freudenberg<sup>(40)</sup> and Shorygina<sup>(41,42,43)</sup>.

The technique adopted (Fig. 7) was a slight modification of that used by Freudenberg<sup>(40)</sup> and other workers for the reductive cleavage of ether linkages in lignin. For purposes of comparison, the behaviour of the original periodate lignin was also examined for the first time. The experiments were carried out in an insulated Erlenmeyer flask

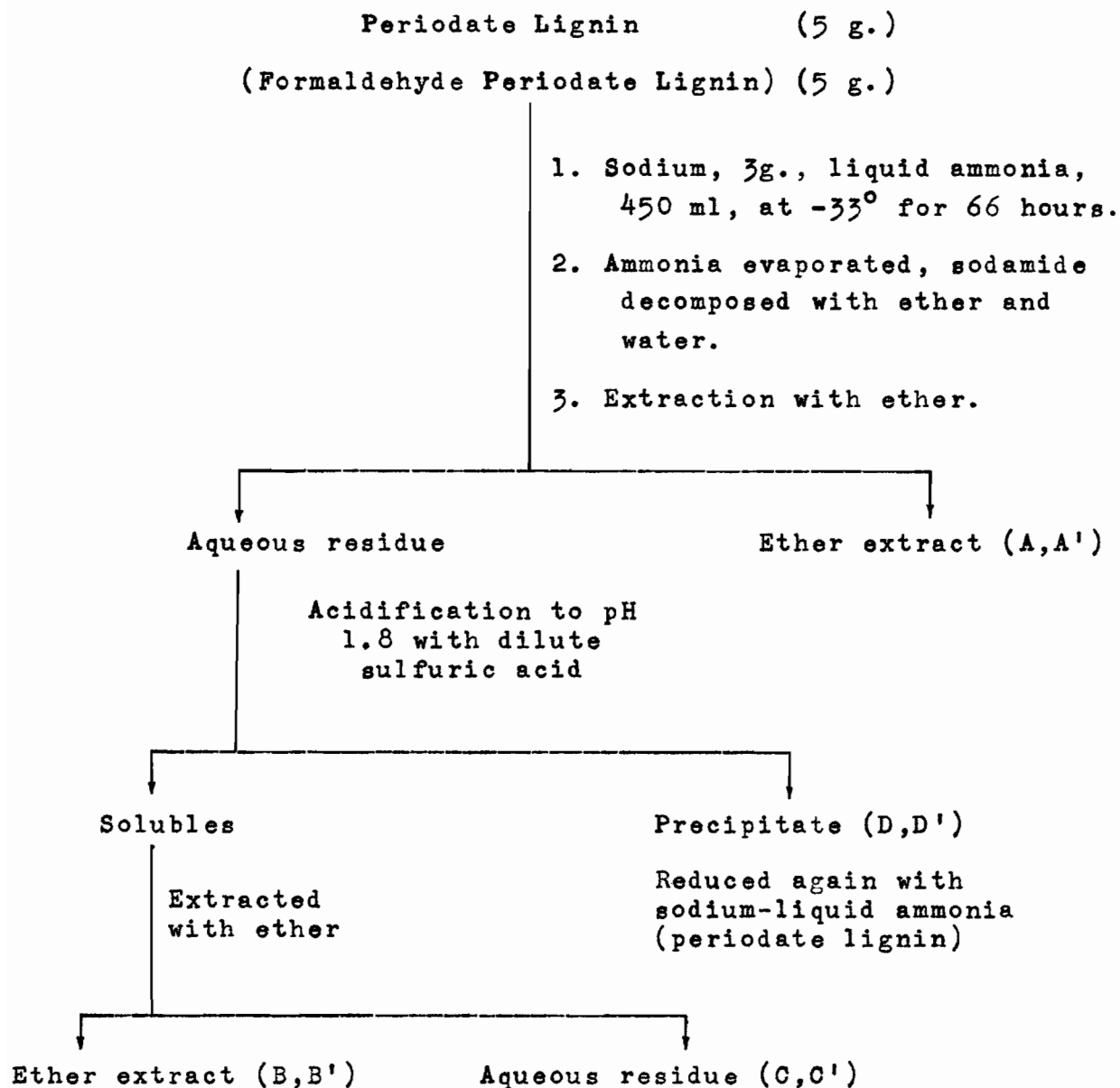


Figure 7

DEGRADATION OF PERIODATE LIGNIN AND FORMALDEHYDE  
PERIODATE LIGNIN WITH SODIUM IN LIQUID AMMONIA<sup>a)</sup>

<sup>a)</sup> Products A' to D' (with the superscript) were from the formaldehyde periodate lignin.



equipped with a guard tube to prevent the access of atmospheric moisture, oxygen, and carbon dioxide. At the end of the reaction, the products were eventually recovered in aqueous sodium hydroxide, and fractions, A,A', were extracted with ether. The aqueous residue, when acidified with dilute sulfuric acid, deposited apparently unreacted lignin (D,D'), which, only in the case of the periodate lignin was submitted to another similar degradation. The acidic mother liquor (pH 1.8) was once more extracted with ether to remove phenols and acids (B,B'). The aqueous residue was freed of the added sulfuric acid, and after evaporation was yielded the yellow acetone-soluble residues (C,C').

The results with the periodate lignin (Table V) showed that the four fractions differed markedly from each other in yield and composition. Both the ether-soluble fractions (A) and (B) contained almost the original percentage of methoxyl groups, but the water-soluble fraction (C) was low in methoxyl, perhaps because of demethylation. Fraction (D), insoluble in water, amounted to  $(55.2\% \times 93.2\% \times 97.1\%)$  or 49.8% of the original periodate lignin, after three degradation cycles of 66 hours each. This fraction was therefore resistant to further degradation and seemed to have a fairly high molecular weight.

Read<sup>(39)</sup>, in his chromatographic study of fractions from lignosulfonates degraded with sodium in liquid ammonia, investigated the solvent systems, n-hexane - dioxane - water

Table V

Degradation of Periodate Lignin with Sodium in  
Liquid Ammonia

Each cycle 66 hours (Fig. 7)

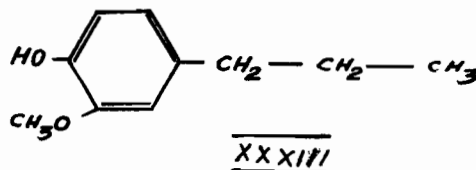
Starting Material  Periodate Lignin	Ether Extracts of Degraded Products		Ether Extracted Water Soluble Residue (C) %	Partially Degraded Acid Insoluble Material (D) %
	at pH 12(A)	at pH 1.8 (B)		
	%	%		
Yield	20.4	4.2	19.1	55.2
OCH <sub>3</sub>	9.23, 9.15	11.35, 11.38	5.32, 5.41	14.1, 13.9
Acid Insoluble Material (D) <sup>a)</sup>				
Yield	0.30	0.12	5.1	93.2
OCH <sub>3</sub>	-	-	5.01, 5.0	12.1, 12.25
Acid Insoluble <sup>b)</sup> Material (D)				
Yield	-	-	2.03	97.1
OCH <sub>3</sub>	-	-	4.79, 4.76	11.93, 11.92

a) From first cycle.

b) From second cycle (a).

(6:3:1 v/v) and n-hexane-pyridine-water (1:1:1 v/v). In the present case, the same systems gave the results shown in Table VI. Fraction (C) gave a blue streak from the starting line to the solvent front, and fraction (D) did not move at all. Both the fractions (C) and (D) when oxidized in alkaline medium with nitrobenzene gave rise to vanillin. They also yielded 1,2,4,5-tetra- and penta-benzene carboxylic acids on oxidation with potassium permanganate in alkaline medium, and showed infra-red and ultraviolet spectra similar to those of the original periodate lignin.

Fraction (A), from the ether extract at pH 12, was a brown oil amounting to 20.4% of the periodate lignin. This oil gave a single blue spot in both solvent systems, and on vacuum distillation in a micro distillation apparatus gave 10.5% of a distillate which was identified by the standard methods to be dihydroeugenol, (XXXIII)



This compound was obtained by Russian workers<sup>(41,42)</sup>, but in 6.7% instead of the present 20% yield, when they degraded cuoxam spruce lignin with sodium and liquid ammonia. Read<sup>(39)</sup> also reported that this substance resulted from spruce lignosulfonate degraded in the same way. Dihydroeugenol,

Table VI

Paper Chromatography of Various Fractions  
Isolated after Degradation of Periodate Lignin

Material	n-Hexane-dioxane-water <sup>b)</sup> (6 : 3 : 1 v/v) $R_f$ value <sup>a)</sup>	n-Hexane-pyridine-water (1 : 1 : 1 v/v) $R_f$ value
Extract A <sup>c)</sup>	0.73	0.76
Extract B <sup>c)</sup>	0.73 (faint)	0.75 (faint)
Water soluble residue C <sup>c)d)</sup>	streak	streak
Acid insoluble material D <sup>c)d)</sup>	-	-

a) Detection was with ferric chloride-potassium ferricyanide<sup>(44)</sup>.

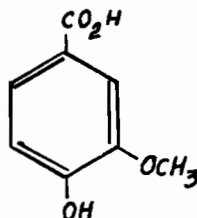
b) Organic phases were used.

c) Described in Table V.

d) Yielded vanillin on alkaline nitrobenzene oxidation, and 1,2,4,5 benzene tetra carboxylic acid plus benzene penta carboxylic acid on oxidation with alkaline potassium permanganate. Infra-red spectra were the same as that of the original periodate lignin.

being a phenol, was not expected to be extracted from such a strongly alkaline solution with ether. However, Stillson<sup>(45,46)</sup> observed that some phenols showed little acidic character because an electron donating para alkyl group depressed the ionization of the hydroxyl group.

Fraction(B), from the ether extract at pH 1.8, was a brown tar amounting to 4.2% of the periodate lignin. A paper chromatographic separation (Table VI) of this fraction, using n-hexane - pyridine - water (1:1:1 v/v) as the developer, yielded 0.26% of a crystalline material, identified by standard methods as vanillic acid. This acid was also obtained by Read<sup>(39)</sup> in traces on cleaving spruce ligno-sulfonate with sodium in liquid ammonia.



*Vanillic Acid (xxxiv)*

As mentioned before, the degradation of the formaldehyde periodate lignin was performed in the same way as that of periodate lignin and was outlined in Fig. 7. The fractions (A') and (B') (Table VII) were dark brown syrups and fractions (C') and (D') were yellow powders. All four fractions had a methoxyl content similar to that of the original formaldehyde periodate lignin, and the degradation did not bring about any demethylation in this case.

Table VII

Degradation of Formaldehyde Periodate Lignin  
with Sodium in Liquid Ammonia

Cycle : 66 hours

Starting Material	Ether Extracts of Degraded Products		Ether Extracted Water Soluble Residue (C')	Partially Degraded Acid Insoluble Material (D')
	at pH 12 (A')	at pH 1.8 (B')		
Formaldehyde Periodate Lignin $\text{OCH}_3$ - 9.35%	%	%	%	%
Yield	2.81	8.38	11.26	78.21
$\text{OCH}_3$	8.83, 8.91	9.12, 9.13	8.98, 9.0	9.35, 9.31

All attempts to move the four fractions in paper chromatography by using a number of solvent systems met with failure, except in one case. In n-hexane - pyridine - water (1:1:1 v/v), part of fraction (B') moved up to the solvent front, but most stayed on the starting line. The spot could be detected as a yellow streak before spraying, but became deep blue when sprayed with ferric chloride - ferricyanide. A paper chromatographic separation of the yellow spot, using the above developer, resulted in the isolation of 0.12 g. (2.4% based on the formaldehyde lignin) of a yellow gum, which could not be crystallized. Moreover, rechromatography of the isolated material did not show the spot near the solvent front,

but a long streak starting from  $R_f = 0.50$  to  $R_f = 0.95$ . This observation discouraged further work on the gum. None of the four fractions yielded vanillin or any substituted vanillin on alkaline nitrobenzene oxidation, showing thereby that any hydroxymethyl groups substituted in the vanillin nuclei of the formaldehyde lignin were neither present as such nor condensed to form an ether linkage. If they had been, the isolation of a carboxy vanillin could have been expected. The presence of a methylene bridge between two aromatic rings was a more probable explanation for the observed behaviour of formaldehyde lignin in this degradation.

#### Degradations by Oxidation

The degradation of the formaldehyde periodate lignin by various types of oxidation was next studied, the main objective being to recover, if possible, molecular fragments still substituted with formaldehyde residues. The following oxidants were chosen:

- (A) Nitrobenzene or cupric hydroxide in alkaline medium.
- (B) Potassium permanganate in alkaline medium.
- (C) Potassium permanganate in neutral medium.
- (D) Degradation in alkali, followed by methylation and oxidation with neutral potassium permanganate.

The first two types of oxidation were carried out by Eisenbraun<sup>(9)</sup> and Jain<sup>(27)</sup>, and were repeated in the present work on a larger scale. The last two represented new

attempts to degrade the original periodate lignin and also its formaldehyde derivative.

(A) Oxidation with Nitrobenzene or  
Cupric hydroxide in Alkali

The micro-oxidation method of Stone and Blundell<sup>(47)</sup> using nitrobenzene, when applied to the formaldehyde periodate lignin, substantiated the results of both Eisenbraun<sup>(9)</sup> and Jain<sup>(27)</sup>. Neither vanillin nor 6-carboxy vanillin were formed, but a new, mobile yellow spot was detected in paper chromatograms, developed with either n-butanol - 2% aqueous ammonia or n-butanol - pyridine - water (10:3:3 v/v). This spot had an  $R_f$  value of 0.88 and 0.82 respectively. The oxidation was conducted in a micro-bomb, made of 98% nickel, using 4 N sodium hydroxide at a temperature of 175° for three hours. Under these conditions, control experiments with other types of lignin - periodate spruce, periodate birch, wood meal and also vanillyl alcohol, produced the same yellow material on the paper, in addition to a spot for vanillin. Even benzyl alcohol and glucose were found to behave similarly. It became clear that this spot was an artifact, which had been occasionally noted, without comment, by one or two previous workers.

The material was isolated by paper chromatography as yellow needles melting at 112°, and was identified as p-nitrophenol by determining a mixed melting point which was not depressed. Since this substance was not produced when a



micro-bomb made of stainless steel was used, the catalytic effect of nickel on the reaction of nitrobenzene with alkali was probably the cause of its formation.

The same method of micro-oxidation was tried with 50 mg of the formaldehyde lignin, but using cupric hydroxide in place of the nitrobenzene. The oxidation products were directly spotted on the chromatograph which was developed in n-butanol - 2% aqueous ammonia or in n-butanol - pyridine - water (10:3:3 v/v) with diazotised p-nitroaniline as the spray. Since the resolution was very unsatisfactory, the oxidation was repeated with 250 mg of the formaldehyde periodate lignin, the alkaline liquor was acidified to pH 2 and the fraction extracted by ether was 'spotted' in high concentration on the paper. This chromatogram showed five distinct spots at  $R_f = 0.015, 0.037, 0.07, 0.12$  and  $0.82$  in n-butanol - 2% aqueous ammonia, with different colour and intensity when sprayed with diazotised p-nitroaniline. The spots at  $R_f 0.07$  and  $0.82$ , rounded and very deep violet in colour, were not observed in control experiments with the original periodate lignin and were of great interest because they might refer to fragments in which formaldehyde residues were substituted. The oxidation was therefore repeated with six grams of the formaldehyde lignin. The ether extract was recovered as a dark brown oil in about 13.3% yield, and the results of paper chromatography were in conformity with those mentioned above. Chromatographic separation of the oil, using three columns of

different dimensions packed with cellulose powder by wet methods, with n-butanol - 2% aqueous ammonia as the eluent, failed, probably because of the difficulty of achieving a compact column by the method used. Finally a paper chromatographic separation of the oil recovered from the columns isolated three fractions of  $R_f = 0.00, 0.15$  and  $0.86$  (n-butanol - 2% aqueous ammonia as eluent) in 3.3%, 0.5% and 0.3% yield respectively. The increase of the  $R_f$  values from 0.07 to 0.15, and from 0.82 to 0.86, in rechromatography was probably due to the prior elimination of impurities present in the original ether extracts. This explanation was also given by Pearl and Beyer<sup>(48)</sup> who showed in their studies of lignin oxidation mixtures that simultaneous paper chromatography of an oxidation fraction and of known pure compounds would give spots for identical compounds at different  $R_f$  values. Of course, they suggested two-dimensional chromatography to obviate the discrepancies in  $R_f$  values.

Since the above fractions were isolated in very low percentage yield, it seemed necessary to repeat the work on a larger scale with the hope of obtaining the pure fractions in sufficient quantities for identification. The flow-chart (Fig. 8) shows the processing of thirty grams of the formaldehyde periodate lignin, and Table VIII describes the chromatography of the three extracts (A), (B) and (C). The extracts (B) and (C) either did not move or streaked up to solvent front, in the two solvent systems used. After

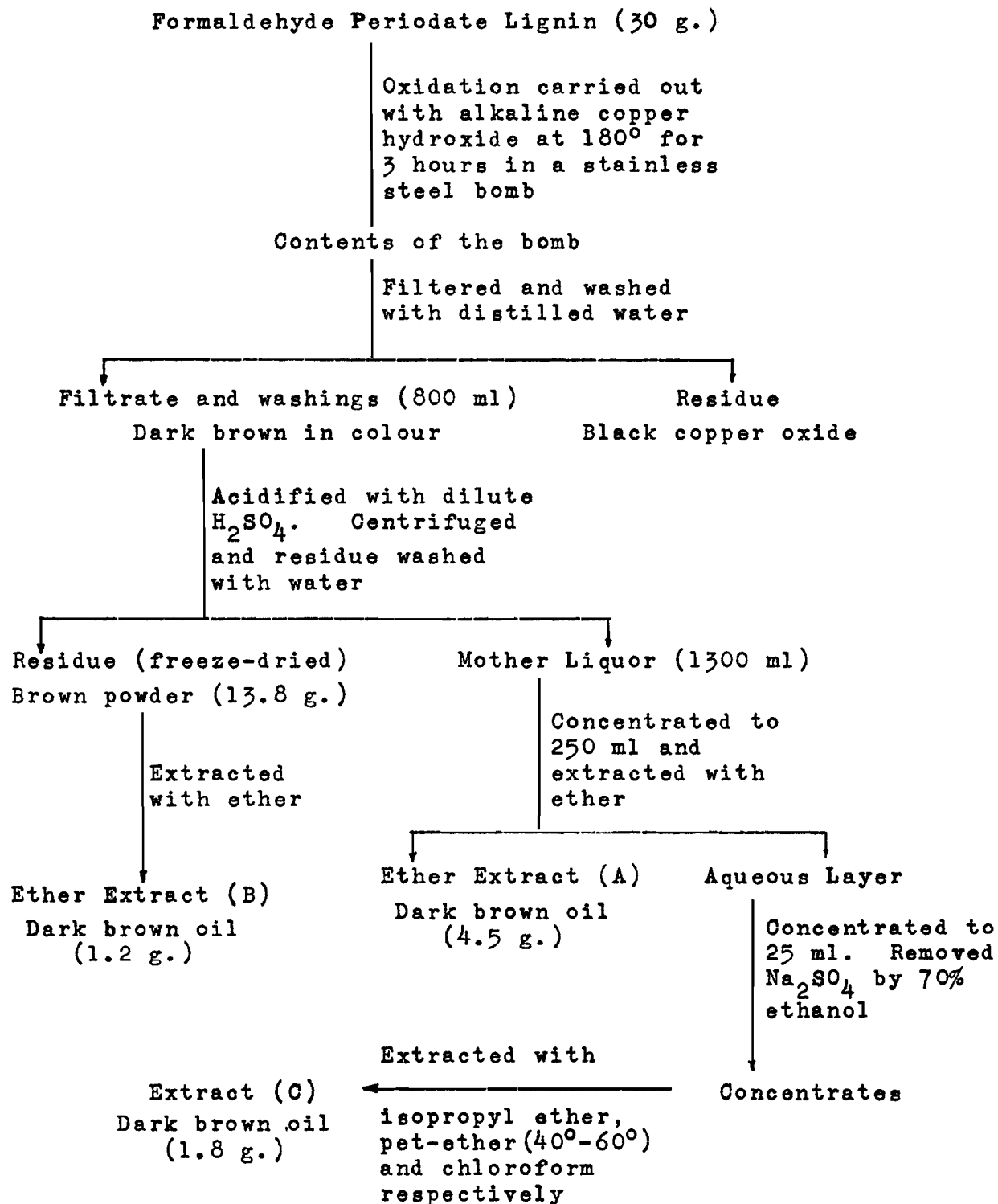


Figure 8

LARGE SCALE OXIDATION OF FORMALDEHYDE LIGNIN WITH  
ALKALINE CUPRIC HYDROXIDE

Table VIII

Paper Chromatography of Extracts (Fig. 8)

Extract <sup>a)</sup>	Solvent system <sup>b)c)</sup>	
	n-butanol-2% aqueous ammonia $R_f$	n-butanol-pyridine-water (10:3:3 v/v) $R_f$
Extract (A)	0.00 (violet) 0.07 (violet) 0.82 (yellow)	0.00 (violet) 0.70 (violet) 0.85 (yellow)
Extract (B)	0.00 (violet)	violet streak
Extract (C)	0.00 (violet)	violet streak

<sup>a)</sup> Described in Fig. 8.

<sup>b)</sup> Whatman No. 3 paper used for descending chromatography.

<sup>c)</sup> Diazotised p-nitroaniline was the spray.

failing to achieve a chromatographic separation of the extract (A) on a small cellulose column, the resolution of the extract into the following fractions was effected by laborious paper chromatography, using Whatman No. 3 paper with n-butanol - 2% aqueous ammonia as eluent.

(a) A fraction, yield of 1.33%, was a dark brown resinous

substance with  $R_f = 0.15$  and  $0.81$  in n-butanol - 2% aqueous ammonia, and n-butanol - pyridine - water (10:3:3 v/v) respectively. All attempts to crystallize the material from a number of solvent systems failed, and greatly reduced the amount available. The ultraviolet spectrum (Fig. 9) showed the usual maxima at  $210\text{ m}\mu$  and  $280\text{ m}\mu$ , observed with most lignin preparations, including formaldehyde lignin. In spite of the fact that the material showed a strong carbonyl peak at  $1675\text{ cm}^{-1}$  in the infra-red spectrum (Fig. 10), it formed neither a bisulfite derivative nor a 2,4-dinitrophenyl hydrazone, indicating the possibility of a hindered ketone. Since the material showed a positive test for phenol, fruitless attempts were also made to prepare an acetate. The elementary and group analyses corresponded to  $\text{C}_{11}\text{H}_{15}\text{O}_7(\text{OCH}_3)$ , but lack of material made it necessary to discontinue the investigation.

(b) Another fraction, isolated in 1.25% yield as a yellow shining powder from the paper chromatography of extract A (Fig. 8), crystallized as yellow needles from hot ethanol. The pure crystals had  $R_f$  value,  $0.86$  and  $0.84$ , in n-butanol - 2% aqueous ammonia and n-butanol - pyridine - water (10:3:3 v/v) respectively. Spraying reagents indicated the presence of the phenolic groups, which was confirmed by the infra-red spectrum. The usual  $280\text{ m}\mu$  maximum in the ultraviolet spectrum indicated the presence of an aromatic ring substituted with phenolic or phenolic ether groups. The

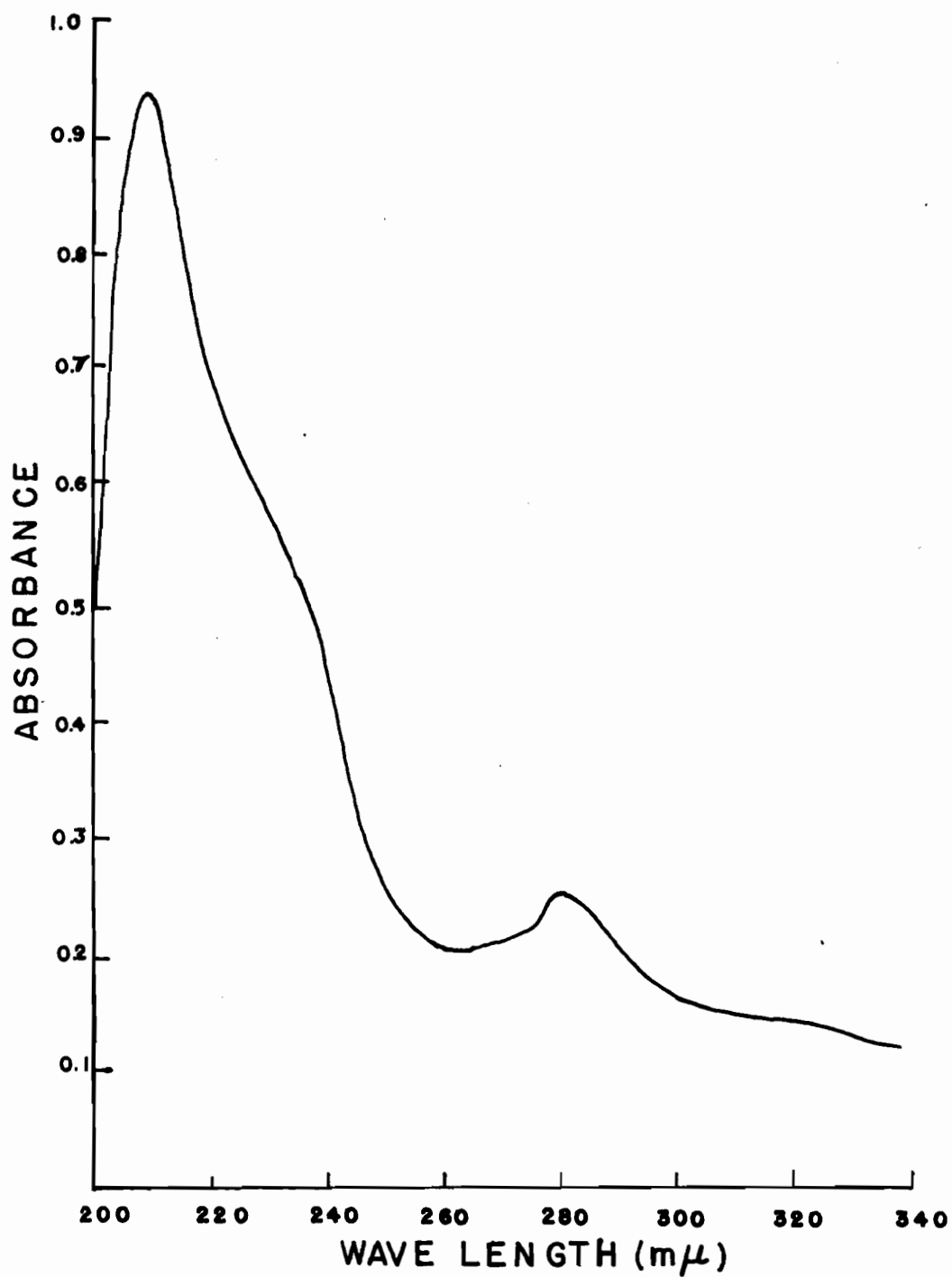


Figure 9

ULTRAVIOLET SPECTRUM OF PURE FRACTION ( $R_f = 0.15$  in  
butanol-2% aqueous ammonia) FROM EXTRACT (A) (Fig. 8)  
(in 95% ethanol)

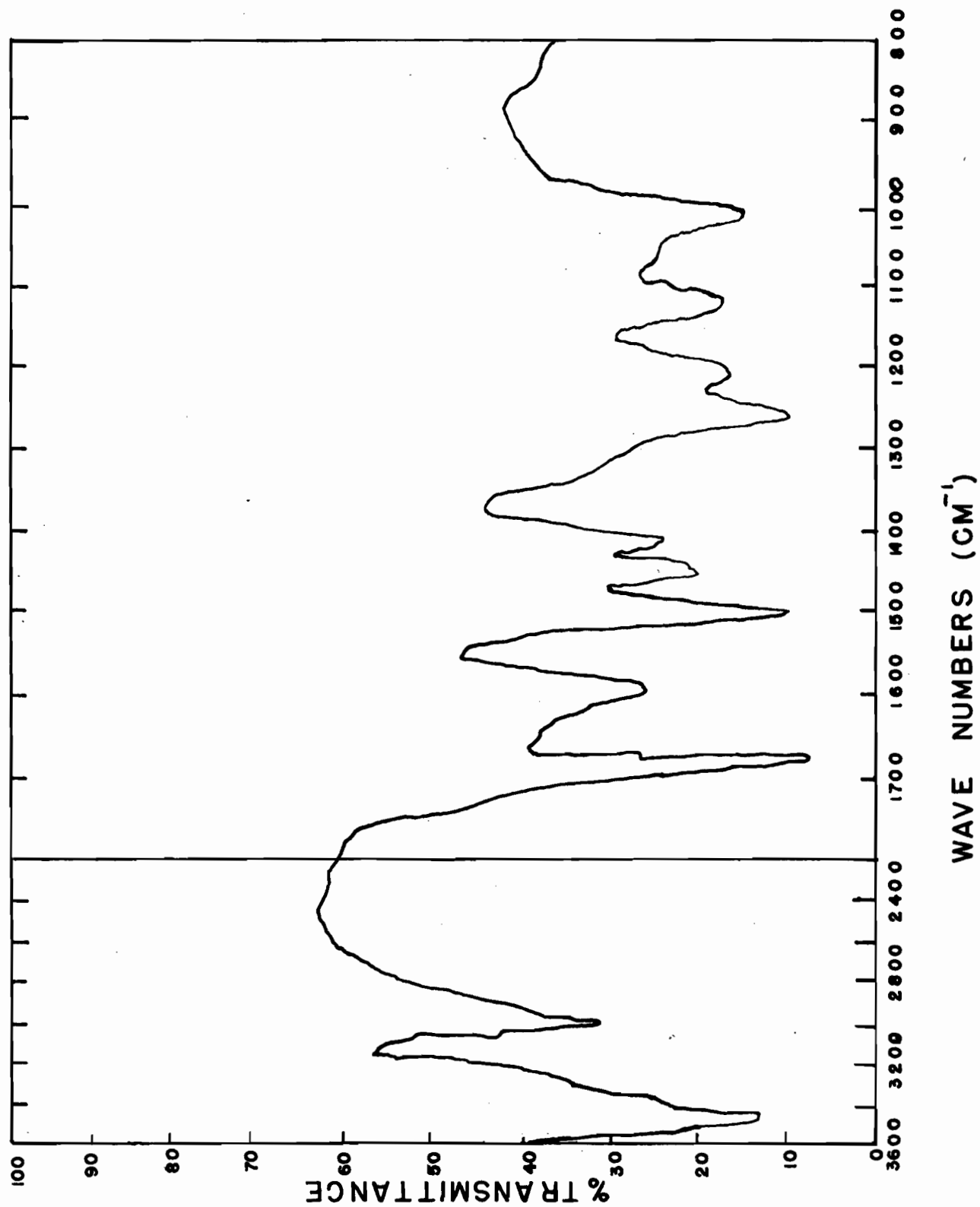
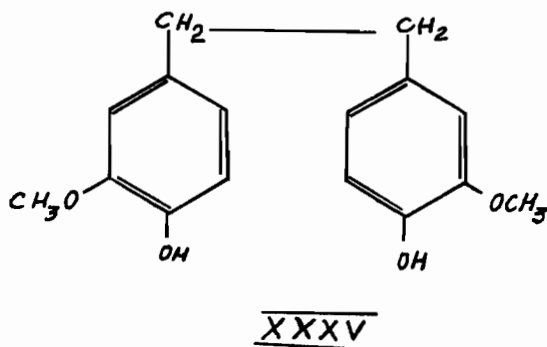


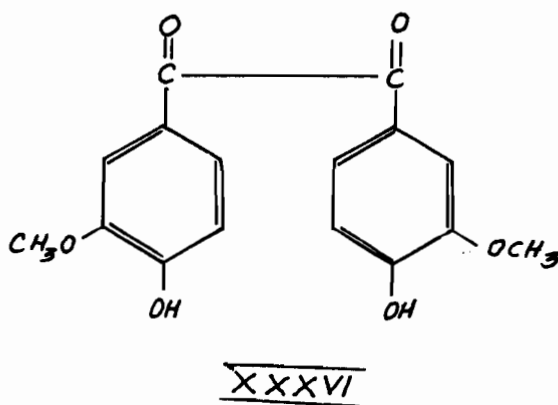
Figure 10

INFRA-RED SPECTRUM OF THE PURE FRACTION ( $R_f = 0.15$  in  
butanol-2% aqueous ammonia) FROM EXTRACT (A) (Fig. 8)

substance was identified as bivanillyl (XXXV) by a mixed melting point with an authentic sample, by identical  $R_f$  values, U.V and I.R spectra, and by a mixed melting point with the diacetates.



The formation of bivanillyl was difficult to explain, since one should expect that it would give rise to vanillil (XXXVI),



on oxidation with cupric oxide, which could be easily oxidized further to vanillic acid or vanillin. Although there was a faint spot of vanillil (XXXVI) when the ether extract (A)



(Fig. 8) was chromatographed, there was no indication of the formation of even a trace of vanillin.

(c) The third fraction separated by chromatography from the ether extract (A) was an immobile, dark brown oil, with spectra similar to those of the original formaldehyde lignin. The ether extracts (B) and (C), (Fig. 8), were also immobile oils, and were combined with the above immobile fraction from the ether extract (A) to give a total yield of about 18% of the formaldehyde lignin. The oil could not be distilled, since it started decomposing above  $140^{\circ}$ . All attempts to chromatograph the oil in a large number of solvent systems were fruitless, showing either a  $R_f$  value of 0.00 or long streaks. The oil retained the methoxyl content of the formaldehyde lignin, and failed to yield vanillin on further oxidation by nitrobenzene or cupric hydroxide in alkali. Although its physical state was different, this fraction thus resembled the parent lignin.

(B) Oxidation with Alkaline Potassium  
Permanganate

The present work had the purpose of confirming the results of both Eisenbraun<sup>(9)</sup> and Jain<sup>(27)</sup> as described in the Historical Introduction.

Thorough oxidations of both the formaldehyde periodate and the periodate lignin were carried out with hot aqueous potassium permanganate near pH 12, and the mixture of acids originally isolated were boiled with nitric acid to

destroy oxalic acid and any other  $\alpha$ -keto acid. Paper chromatography of the residual benzene carboxylic acids with methyl isobutyl ketone - formic acid - water (10:1:1 v/v) as eluent removed benzene pentacarboxylic acid,  $R_F = 0.08$  from the mixture, but left the hexa acid on the starting line. The hexa acid required a subsequent elution with 5% formic acid. The original periodate lignin yielded a mixture of benzene polycarboxylic acids, 3.0% by weight, thus confirming earlier data<sup>(13,14)</sup>. Paper chromatography in this case showed two spots with  $R_F$  values of 0.08 and 0.32, corresponding to penta- and 1,2,4,5-tetracarboxylic acid respectively. The bulk of the crude polycarboxylic acids were separated according to the method of Jain<sup>(27)</sup>, on a cellulose column using methylisobutyl ketone - water (10:1 v/v) as eluent. All the acids were isolated in the pure, crystalline form, the yields being shown in Table IX. Eisenbraun's<sup>(9)</sup> explanation for the formation of these acids was discussed in the Historical Introduction.

(C) Oxidations with Potassium Permanganate  
in Neutral Solution

Although an oxidation with permanganate in neutral solution was expected to destroy phenolic groups not protected as ethers, both the formaldehyde periodate and the original periodate lignin were oxidized without prior methylation. According to Eisenbraun<sup>(9)</sup>, the periodate lignin had aliphatic hydroxyl but no free phenolic groups, and the formaldehyde

Table IX

Recoveries of Benzene Polycarboxylic Acids

A. From Formaldehyde  
Periodate Lignin

Product	Recoveries in g. <sup>a)</sup>	Yield % <sup>b)</sup>	Yield % <sup>e)</sup>
Hexacarboxylic acid	0.044	0.9	1.18
Pentacarboxylic acid	0.121	2.5	3.50

B. From Periodate Lignin

Pentacarboxylic acid	0.115 <sup>c)</sup>	2.36 <sup>d)</sup>	2.90
1,2,4,5-tetra- carboxylic acid	0.032	0.66	0.82

a) Weight of penta- and hexa-carboxylic acids obtained from 0.17 g. of mixture.

b) Percent yields based on 3.4% of the mixture obtained by oxidizing 5 g. of formaldehyde lignin with alkaline permanganate.

c) Weight of penta- and tetra-carboxylic acids obtained from 0.150 g. of mixture.

d) Percent yield based on 3.0% of the mixture obtained by oxidizing 5 g. of periodate lignin with alkaline permanganate.

e) Jain's data (27).

derivative only a few (Table II). Oxidations of the non-methylated lignins were carried out by slightly modifying the method of Richtzenhain<sup>(49)</sup>, and were at 90° and pH 7. The periodate lignin failed to yield any substances that could be identified, but the formaldehyde derivative yielded about 0.25% of benzene hexacarboxylic acid, isolated quantitatively by paper chromatography. The mellitic acid was identified by a mixed melting point with the standard sample.

A neutral permanganate oxidation of the completely methylated lignins was then undertaken, as there existed some possibility of isolating aromatic carboxylic acids with the methoxyl substituents at the 3- and 4-positions of the benzene nucleus, particularly acids like metahemipinic acid, if the assumption that some hydroxymethyl groups had entered the 6-position of the nucleus during the condensation was correct.

Although Richtzenhain<sup>(49)</sup> isolated veratric acid, isohemipinic acid, metahemipinic acid and dehydrodiveratric acid by the neutral permanganate oxidation of methylated hydrochloric acid lignin, the present work would be the first attempt with the methylated periodate lignin. The lignin (OCH<sub>3</sub> 12.1%) was methylated by dimethyl sulfate and alkali to a constant methoxyl value of 17.35% according to a procedure of Brauns and Hibbert<sup>(50)</sup>. The methylated product, 8.5 g, was oxidized by neutral aqueous potassium permanganate, following a procedure by Freudenberg<sup>(51)</sup>. The flow-chart in

Fig. 11 showed the processing of the yellow mother liquor after the removal of the black manganese dioxide. Ether extract (A) and residue (C) were both yellow brittle powders, which failed to move in paper chromatography in a number of solvent systems tried, and were not further investigated.

The ether extract (B), removed at pH 1.8, was a yellow oil recovered in 15.3% yield. When chromatographed on paper with the solvent systems, benzene - acetic acid - water (8:11:4 v/v) and butanol - morpholine - water (5:1:4 v/v), suggested by Freudenberg<sup>(51)</sup> for the chromatographic identification of various aromatic acids, this oil showed only long streaks and no distinct spots (Table X). The difference between Freudenberg's results with hydrolysed pine wood lignin and those of the present work was striking. In particular, the absence of even a trace of veratric acid agreed with the absence of the free phenolic hydroxyl group in periodate lignin. Other attempts at fractionating the oil by partition chromatography were summarized in Table XI, the solvent system, butanol - acetic acid - water (4:1:1 v/v) being the most satisfactory. This system revealed two adjacent elongated spots at  $R_F = 0.75 - 0.80$ , although most of the material stayed on the starting line. The developed paper responded only to reagents, used for the detection of carboxylic acids. Slight variations in the relative amounts of the components in the solvent system, and also the replacement of butanol by tert-butyl alcohol, and of acetic acid by propionic

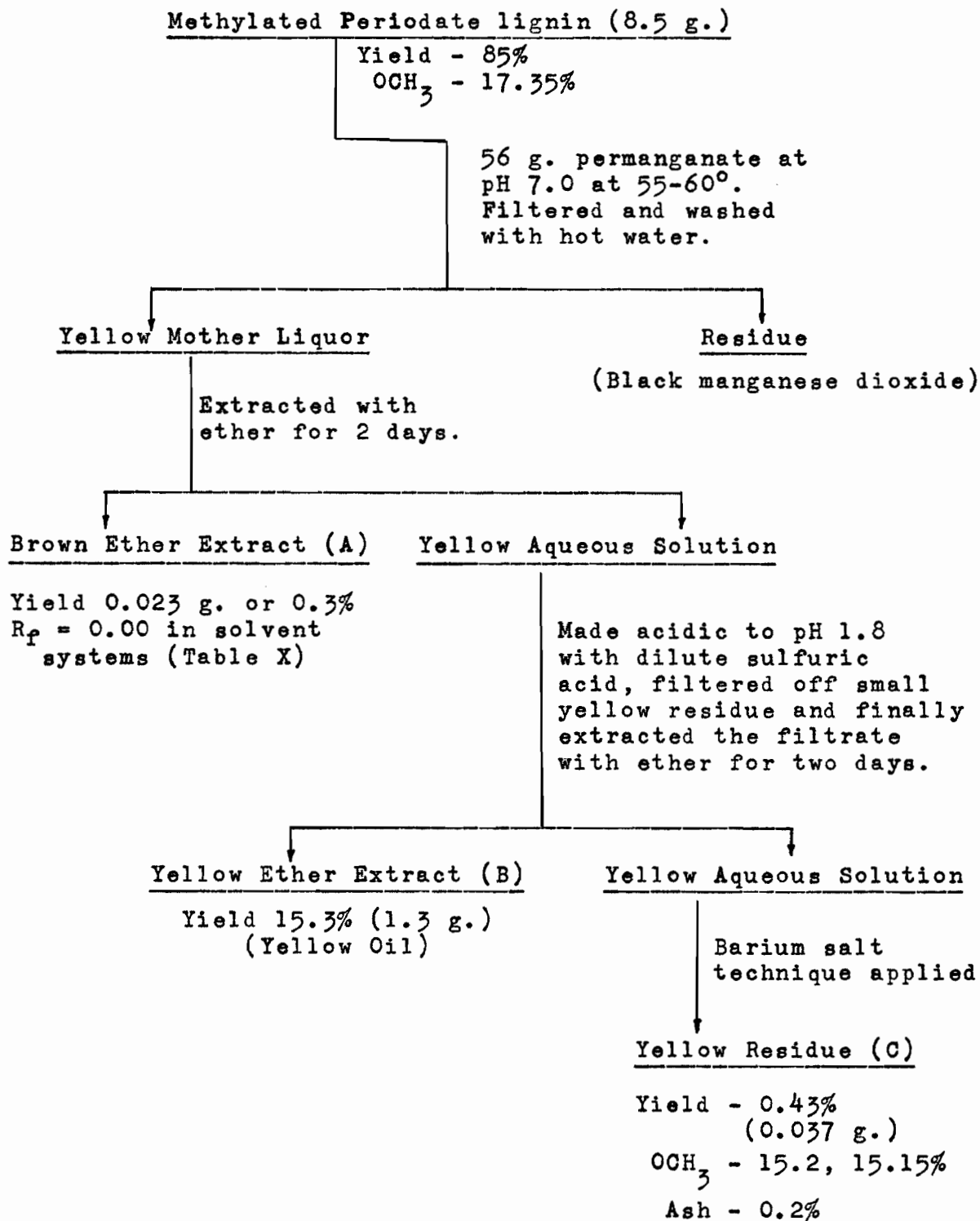


Figure 11

FLOW CHART SHOWING OXIDATION OF METHYLATED PERIODATE LIGNIN

Table X

Paper Chromatography of Hydrolysed Methylated  
Pine Wood Meal and Methylated Periodate Lignin  
Oxidized with Neutral Potassium Permanganate

Acids <sup>a)b)</sup>	Benzene- Acetic Acid-Water (8:11:4 v/v)	Butanol- Morpholine-Water (5:1:4 v/v)
	R <sub>F</sub>	R <sub>F</sub>
Mellitic acid	0.00	0.00
Dehydrodiveratric acid	-	0.27
-	0.06	-
Methoxy trimesic acid	0.11	0.02
-	elongated spot	0.05
Succinic acid	0.25	0.14
Metahemipinic acid	0.33	0.15
Hemipinic acid	0.42	0.18
4-methoxy isophthalic acid	0.50	0.08
Isohemipinic acid	0.58	0.12
-	elongated spot	-
Veratric acid	0.85	0.43
Ether Extract (B) <sup>b)c)</sup> (Fig. 11)	streaking from starting line to solvent front.	0.00 with slight streaking

a) Acids isolated and identified by Freudenberg<sup>(51)</sup>.

b) Spray was Rhodamine B in alcohol, followed by ultraviolet light.

c) Spray was bromophenol blue at pH 7.

Table XI

Paper Chromatography of the Ethereal Extract (B)

(Figure 11)

Solvent systems (v/v)	$R_f$ value <sup>a)</sup>
(1) Benzene-acetic acid-water (8:11:4)	Streaking from starting line to solvent front
(2) Butanol-morpholine-water (5:1:4)	0.00
(3) Methyl Isobutyl Ketone- formic acid-water (10:1:1)	Streaking from starting line to solvent front
(4) Methyl Isobutyl Ketone- water (10:1)	Streaking from starting line to solvent front
(5) Ethanol-ammonia-water (80:4:16)	Streaking from starting line to $R_f = 0.50$
(6) Butanol-2% aq. ammonia	Streaking from starting line to $R_f = 0.45$
(7) Butanol-1.5 N $\text{NH}_4\text{OH}$	Streaking from starting line to $R_f = 0.35$
(8) 2% acetic acid	0.00
(9) Xylene-dimethylformamide (9:2)	Long streak
(10) Butanol-acetic acid-water (4:1:1)	a) Two elongated close spots approximately at $R_f = 0.75 - 0.80$ $R_f = 0.00$

a) Bromophenol blue in ethanol at pH 7.0 was the spray.



acid, did not appreciably improve the separation. Attempts to separate the two close spots at  $R_f = 0.75 - 0.80$  by paper electrophoresis in 3 N ammonium hydroxide at 600 volts for 90 minutes met with no success, most of the sample forming streaks on the anode side.

The rest of the oil, 1.23 g, was then fractionated from benzene to yield an insoluble fraction (about 7.06% of methylated periodate lignin) which chromatographed as an elongated spot at  $R_f = 0.75 - 0.80$ , with bulging in between. The benzene-soluble portion had  $R_f$  0.00 and the separation appeared clean cut.

At this stage, another 23.5 g. of methylated periodate lignin was oxidized under similar conditions, yielding 1.65 g. (7.0%) of the same benzene-insoluble fraction.

An attempt was made to crystallize the benzene-insoluble fraction in the form of its brucine salt. Salts of this base often crystallize well and are sometimes used to separate closely related (stereoisomeric) acids<sup>(52,53,54,55)</sup>. There was, of course, no reason to think that stereoisomeric acids constituted the benzene-insoluble fraction. The salt was made by boiling a sample of the fraction with an excess of brucine in 95% ethanol, according to the procedure used by Bell and Morgan<sup>(52)</sup> for the resolution of 1:1'-dinaphthyl-5:5'-dicarboxylic acid. After removing the excess brucine, an aqueous solution of the product deposited a yellow amorphous salt (A) in small amount, and a similar,

more soluble salt (B) was recovered in 83% yield from the mother liquor. When attempts to crystallize these brucine salts failed, they were converted to the free acids via the barium salts by standard procedures. When chromatographed, the acid from (A) gave a single, very faint spot of  $R_f$  0.65, while that from (B) yielded the two spots of  $R_f$  0.75 - 0.80 possessed by the original benzene-insoluble fraction. Hence the separation attempted by means of the brucine salt had failed. Flow charts, 12, 13, and 14 summarized the series of operations involved.

The immobile benzene-soluble fraction did not form a well-defined brucine salt when submitted to the above procedure, and the investigation was abandoned.

Although the benzene-insoluble fraction, a yellow powder, had not been crystallized, and paper chromatography suggested that it consisted of at least two substances of  $R_f$  0.75 - 0.80, the infra-red spectrum shown in Fig. 15 was a very sharp one, suggesting that the substance was pure and homogeneous. This spectrum had the strong peak at frequency  $1700\text{ cm}^{-1}$  for the carbonyl or carboxyl group. Three maxima in the ultraviolet spectrum (Fig. 16) at  $218\text{ m}\mu$ ,  $253\text{ m}\mu$  and  $295\text{ m}\mu$ , were not in positions characteristic of lignin derivatives, which usually showed maxima at  $210\text{ m}\mu$  and  $280\text{ m}\mu$ . No closely similar ultraviolet spectra were found in the literature.

The nuclear magnetic resonance spectrum of the

Benzene Insoluble Fraction of  
Ether Extract (B)

(0.500 g.)

$\text{OCH}_3$  - 16.52, 16.49%

Dissolved in 85 ml of 95% ethanol; heated on a water bath; added a solution of 2 g. of brucine in 15 ml of 95% ethanol. Refluxed on a hot water bath for 2 hours, cooled slowly and filtered; washed solids with 95% ethanol.

Yellow-white amorphous Residue  
(0.135 g.)

Dissolved in hot water, cooled and kept for crystallization for 7 days. Failed to crystallize. Evaporated to approximately 10 ml and allowed to stand for crystallization, but again failed. The aqueous solution was extracted with chloroform.

Chloroform Extract (A)

Yellow-white amorphous powder extracted with hot alcohol.  
Yield = 0.080 g.  
No sharp melting point ( $180^\circ$  decomposes).  
Chromatographed in n-butanol-acetic acid-water (4:1:1).  
No spot

Aqueous Solution

Dried yellow powder.  
Yield = 0.042 g.  
(Not examined)

Yellow Mother Liquor

Concentrated almost to dryness, then added hot water. The hot clear solution allowed to cool; filtered to remove brucine. The cold mother liquor on standing gave more needle shaped crystals of brucine. The aqueous liquor extracted with chloroform.

Chloroform  
Extract  
(Brucine)

Yellow Aqueous Solution (B)

Evaporated to dryness.  
Yield = 0.416 g.  
No sharp melting point.  
Chromatographed in n-BuOH-HOAc- $\text{H}_2\text{O}$  (4:1:1).  
No spot

Figure 12

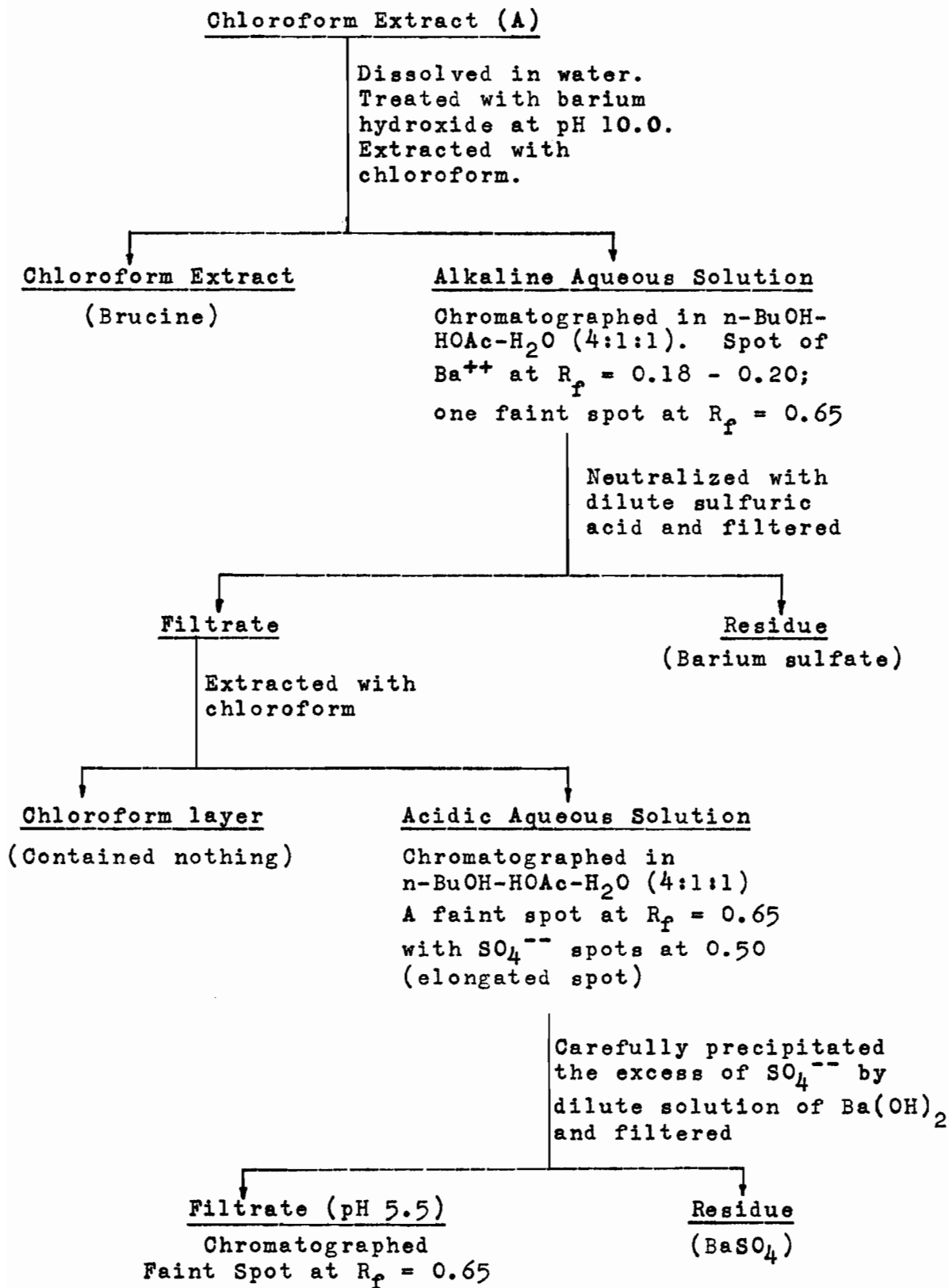


Figure 13

TREATMENT OF CHLOROFORM EXTRACT (A) (FIG. 12)

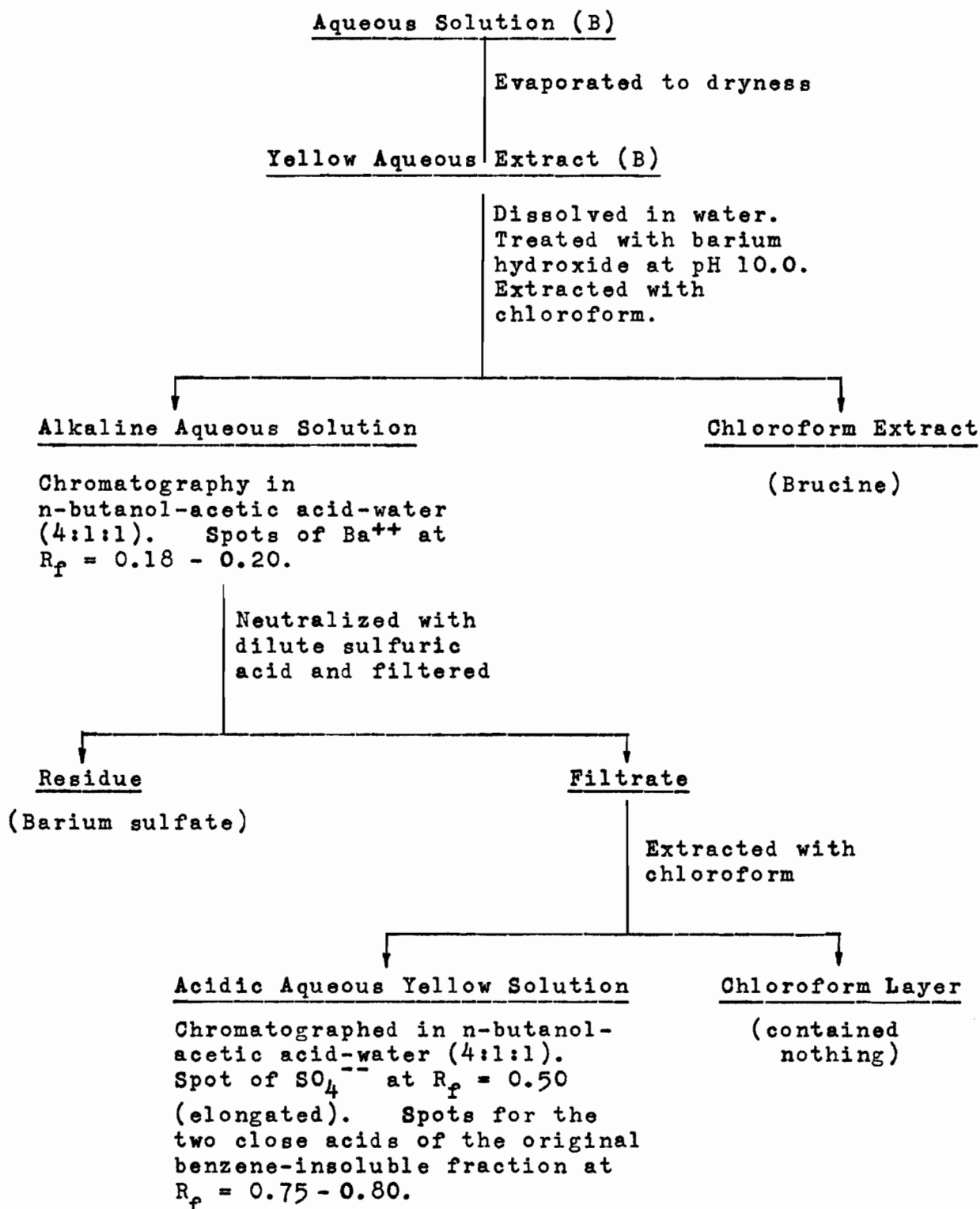


Figure 14

TREATMENT OF AQUEOUS SOLUTION (B) (FIG. 12)

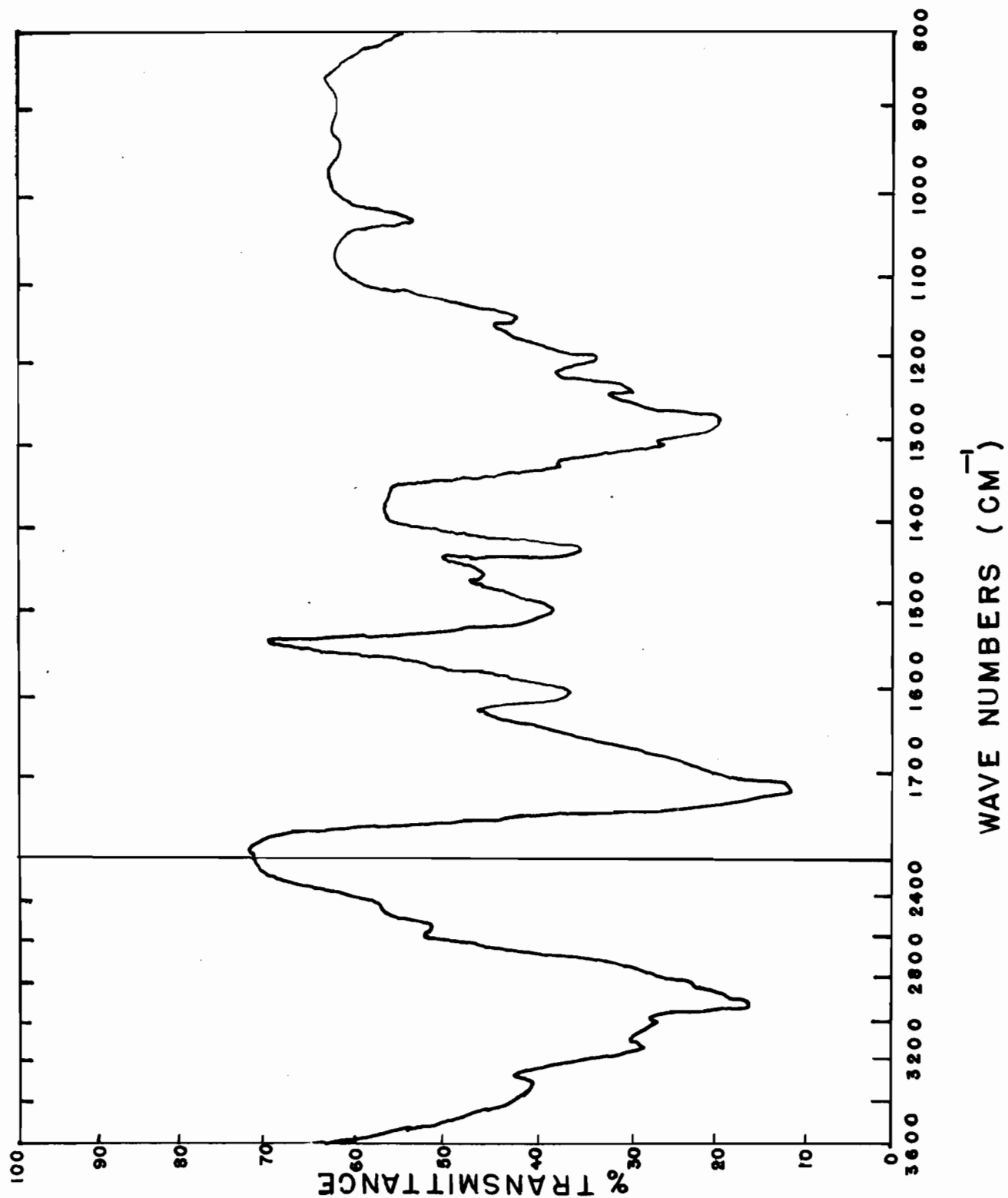


Figure 15

INFRA-RED SPECTRUM OF THE BENZENE INSOLUBLE  
FRACTION OF ETHER EXTRACT (B) (Fig. 11)

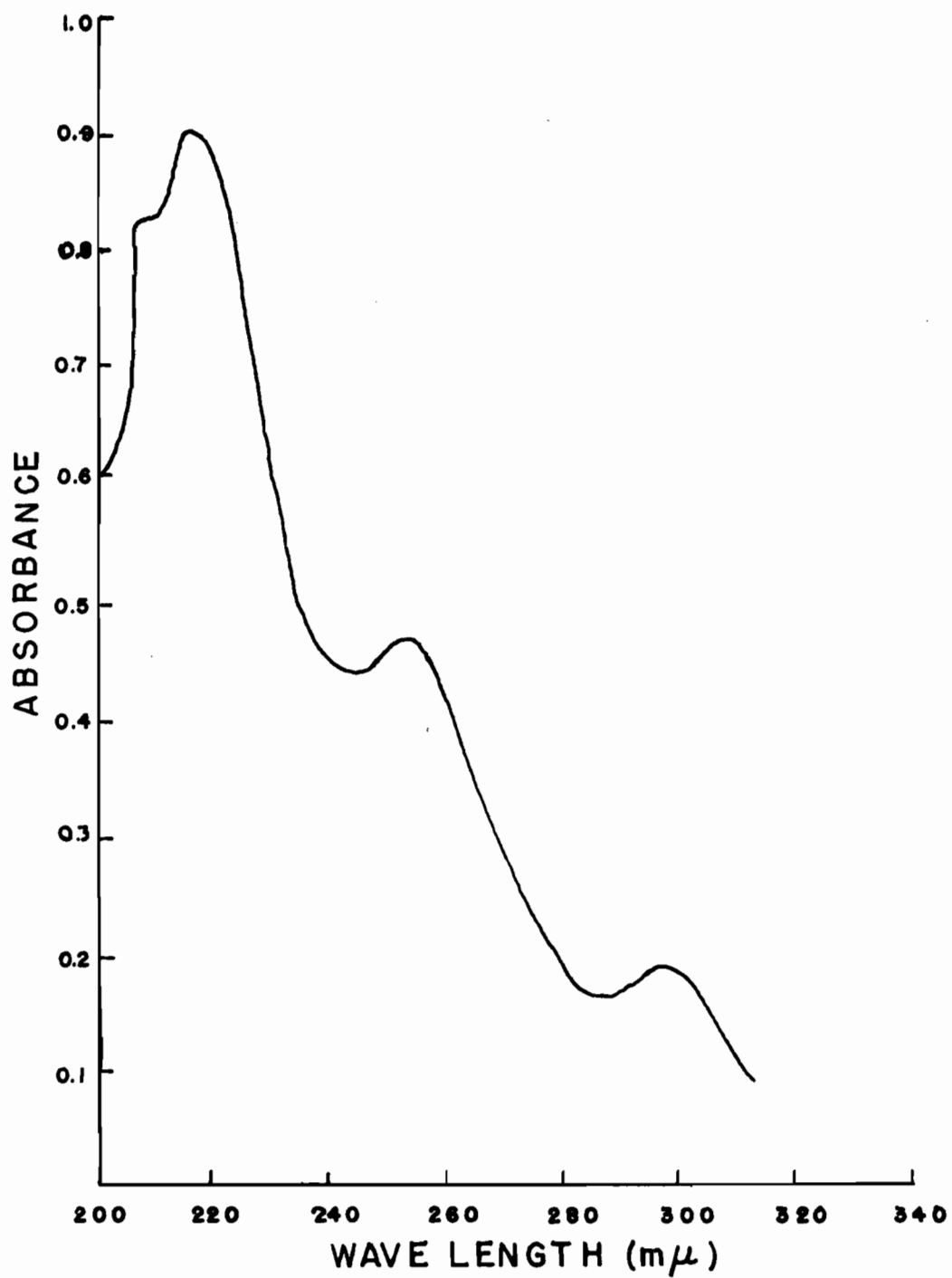
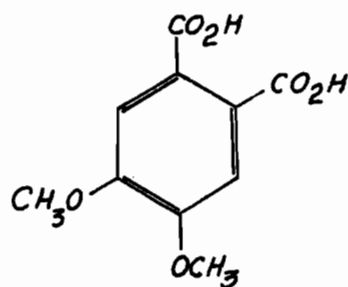


Figure 16

ULTRAVIOLET SPECTRUM OF THE BENZENE INSOLUBLE  
FRACTION OF ETHER EXTRACT (B) (Fig. 11)  
(in 95% ethanol)

substance was not useful as it failed to give rise to any sharp peaks. Elementary analyses, the methoxyl content, molecular weight and neutralisation equivalent agreed well with the molecular formula,  $C_{12}H_{12}O_2(OCH_3)_2(CO_2H)_2$ .

The degradation of the methylated formaldehyde periodate lignin with neutral permanganate was then undertaken, the lignin first being methylated with dimethylsulfate and sodium hydroxide to a constant methoxyl content of 13.1% from the original value of 9.35%. The methylated product in small amount was oxidized by neutral aqueous potassium permanganate and the liquors were processed in the same way as that just described for methylated periodate lignin. Paper chromatograms of the products were developed in methylisobutyl ketone - formic acid - water (10:1:1 v/v), but a bromophenol blue spray showed that all acidic material remained concentrated at  $R_f$  0.0. The failure to find veratric acid was consistent with the failure to recover vanillin when the original formaldehyde lignin was oxidized with nitrobenzene, and the failure to find metahemipinic acid (XXXVII) suggested that any veratric nuclei had not been substituted by a hydroxymethyl



XXXVII



group. The negative result was consistent with the possibility that the 'Vanillin' nuclei were substituted by methylene bridges in the formaldehyde lignin.

(D) Degradation in Alkali followed by  
Methylation and by Oxidation with  
Neutral Potassium Permanganate

The results of the foregoing oxidations suggested that the 'Vanillin' residues in periodate lignin might be substituted, or even cross-linked, by stable methylene units in the formaldehyde derivative. If so, the possibility remained that ether links might be cleaved to free phenolic groups by strong alkali. Identifiable products might be obtained by first degrading the formaldehyde lignin in this way, and then carrying out the previous methylation-oxidation sequence. The products might include members of the vanillic acid series, or compounds analogous to 4,5,4',5'-tetramethoxy diphenyl ketone-2,2'-dicarboxylic acid (XXII) obtained by Oliverio and Boumis<sup>(23)</sup> from the corresponding diphenylmethane. The work of these authors was described in the Historical Introduction.

Using the procedure of Freudenberg<sup>(51)</sup>, formaldehyde periodate lignin, and also the periodate lignin, for purposes of comparison, were degraded with an excess of concentrated aqueous potassium hydroxide at 160° for 90 minutes, and then methylated without isolation. The methylated products were oxidized with neutral aqueous potassium permanganate as

described in previous cases. Fig. 17 outlined the whole process.

Since there was a possibility of oxidizing the lignins in the treatment with strong alkali at a high temperature, the ether extracts (A') and (B') removed at pH 1 from both the periodate and the formaldehyde periodate lignin might contain phenolic acids, while the corresponding ether extract (C') should contain neutral substances. Paper chromatography was used to locate degraded fragment with the developer suggested by Freudenberg<sup>(51)</sup> for methylated acids, and by Eisenbraun<sup>(9)</sup> for benzene polycarboxylic acid. The results were summarized in Table XII. Although the ether extract (A') from the formaldehyde lignin showed a faint spot at  $R_f$  0.42, corresponding to veratric acid in butanol-morpholine-water, it failed to reproduce the same spot at  $R_f$  0.85 for standard veratric acid in benzene-acetic acid-water. Hence the spot at  $R_f$  0.42 in the former solvent system might be due to some other substance. Both the periodate lignin and formaldehyde lignin showed the presence of meta-hemipinic acid in the extract (B'). The extracts from both lignins behaved chromatographically in much the same way, showing a large number of very faint spots with nebulous streaking. In particular, the formaldehyde derivative yielded no spots peculiar to itself. Hence, the chromatographic separation and further investigation of the ether extracts were not carried out.

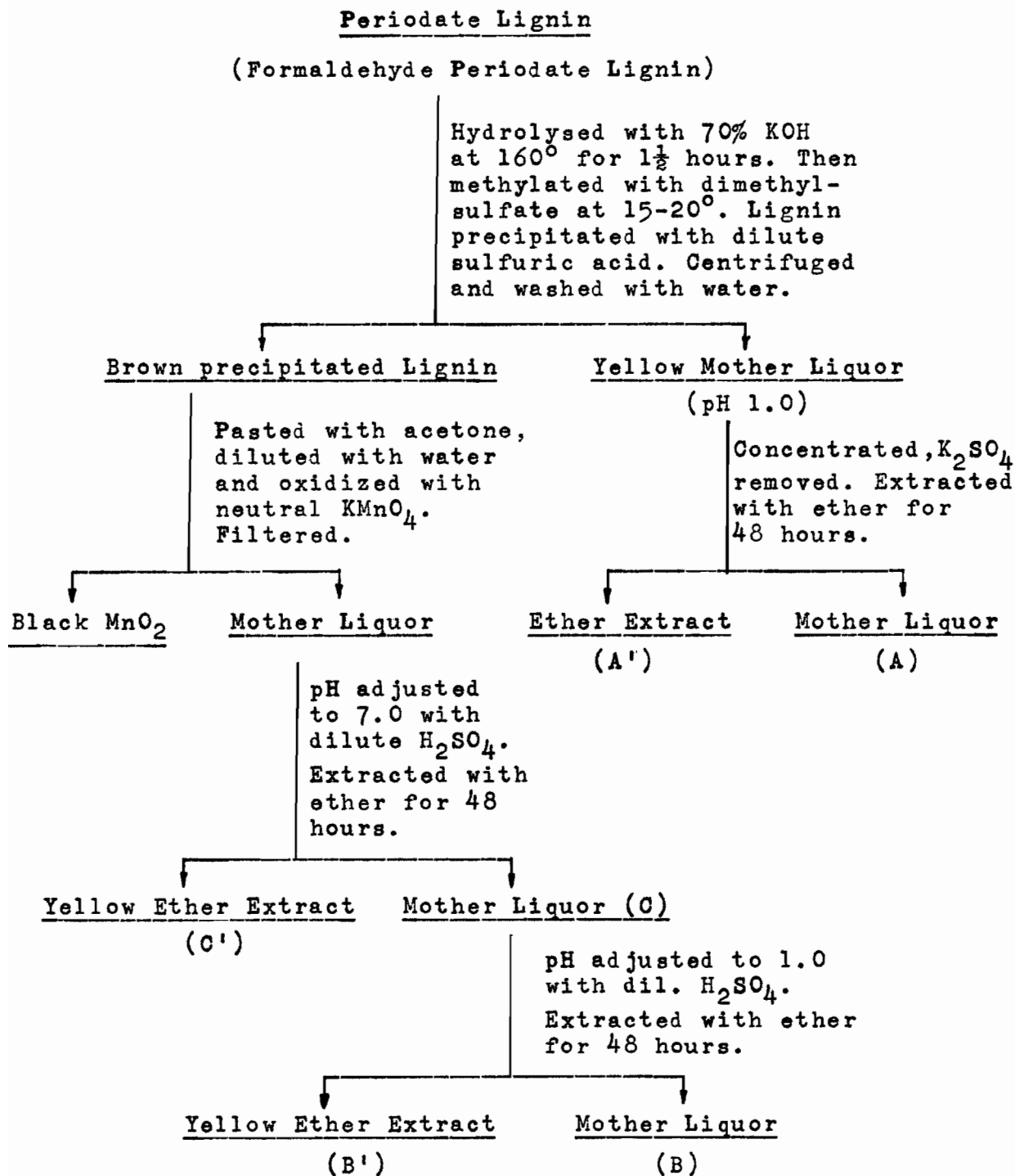


Figure 17

CAUSTIC FUSION OF LIGNINS FOLLOWED BY METHYLATION  
AND NEUTRAL PERMANGANATE OXIDATION

Table XII

Paper Chromatography of the Ether Extracts (Fig. 17)  
from both Periodate- and Formaldehyde Lignin

Type of Lignin	Butanol-morpholine -water (4:1:5 v/v) Rhodamine Spray and then followed by U.V.	Benzene-Acetic acid-water (8:11:4 v/v) Bromophenol Blue Spray	M.I.B.K. <sup>a)</sup> -formic acid-water (10:1:1 v/v) (a) Rhodamine and U.V. (b) Bromophenol Blue
<u>Periodate</u>			
<u>Lignin</u>			
A'	0.00, 0.30 <sup>+</sup> , 0.40 <sup>+</sup> , 0.50 <sup>++</sup> , 0.70 <sup>+</sup> , 0.83 <sup>+</sup>	0.00, 0.07 <sup>++</sup>	0.00 (streaking)
B'	0.00, 0.20 <sup>++</sup> , 0.40 <sup>+</sup> , 0.50 <sup>+</sup>	0.00, 0.07 <sup>++</sup> , 0.34 <sup>+</sup> , 0.51 <sup>+</sup> , 0.80 <sup>++</sup>	0.00 (streaking)
C'	streaking	streaking	streaking
<u>Formaldehyde</u>			
<u>Periodate</u>			
<u>Lignin</u>			
A'	0.00, 0.42 <sup>+</sup>	0.00, 0.07 <sup>++</sup>	0.00 (streaking)
B'	0.00, 0.20 <sup>++</sup>	0.00, 0.07 <sup>++</sup> , 0.34 <sup>+</sup> , 0.51 <sup>+</sup>	0.00 (streaking)
C'	streaking	streaking	streaking

<sup>a)</sup> Methylisobutyl Ketone

<sup>+</sup> Weak

<sup>++</sup> Strong

The mother liquors (A) and (B) (Fig. 17) from both the periodate and the formaldehyde lignin were almost completely freed from potassium sulfate by repeated precipitation from acetone, and the acetone solutions were developed in butanol saturated with 2% aqueous ammonia. Both the liquors (A) and (B) from the periodate lignin showed only long streaking. The mother liquor (A) from the formaldehyde lignin gave two good spots at  $R_f$ , 0.18 and 0.30, and the liquor (B) showed a clear spot also at  $R_f$ , 0.18. Two different sprays were used to detect the spots (Table XIII). Since these mobile substances were the first peculiar to formaldehyde lignin that had been encountered, the degradation with alkali, the methylation and the oxidation were repeated on a 20-g. scale. The substances of  $R_f$ , 0.18 and 0.30, were recovered from the formaldehyde lignin in yields of 1.26% and 4.1% respectively, after a quantitative paper chromatographic separation of the mother liquor (A), using n-butanol - 2% aqueous ammonia as eluent. The mother liquor (B) on similar treatment yielded only 0.16% of the substance of  $R_f$ , 0.18.

Both of the substances isolated from the mother liquor (A) were amorphous and hygroscopic. Both responded to the micro Nessler's test for the ammonium radical, and to tests for the carboxyl group. The presence of ash containing sulfate from the mother liquor (A) moved along with the mobile fractions, perhaps because too large samples had been spotted on the paper. A further purification of the fractions on

Table XIII

Paper Chromatography of the Mother Liquors (A) and (B)  
(Fig. 17)

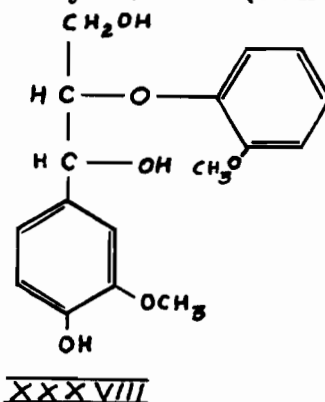
<hr/>	
<p style="text-align: center;">n-Butanol-2% aqueous ammonia. Whatman No. 1 or No. 3 paper.</p>	
Lignins	<hr/> <p>Spray: Neutral bromophenol blue or Rhodamine dye spray followed by ultraviolet light</p> <hr/>
<p><u>Formaldehyde</u> <u>Periodate Lignin</u></p>	
Mother Liquor (A)	$R_f = 0.00, 0.18, 0.30$ , elongated spot near the solvent front
Mother Liquor (B)	$R_f = 0.00, 0.18$ , elongated spot near the solvent front
<hr/> <p><u>Periodate Lignin</u></p>	
Mother Liquors (A) and (B)	Long streak with $R_f = 0.00$ and the elongated spot at solvent front
<hr/>	

paper resulted in the isolation of only 29 mg of the fraction with  $R_f$ , 0.18, and of 300 mg of the fraction,  $R_f$ , 0.30. Although now free from ash, both fractions were still hygroscopic, yellow powders, soluble in water. No reliable analyses could be made of the two substances due to the difficulty of handling them. The infra-red and the ultraviolet spectra of the purified substance of  $R_f$ , 0.30, were very difficult to interpret, since the former had a large number of irregular peaks, and the latter a flat spectrum with no indication of the aromatic ring and methoxyl group, found in all lignins. At this point, lack of material precluded

further examination of the substance, which seemed to be an ammonium salt.

Attempted Condensation of Guaiacyl glycerol -  
 $\beta$  - guaiacyl ether with Formaldehyde

It seemed desirable to investigate the reaction of a lignin model substance with formaldehyde under the conditions used for the preparation of the formaldehyde periodate lignin. Guaiacyl glycerol- $\beta$  - guaiacyl ether (XXXVIII), was chosen as

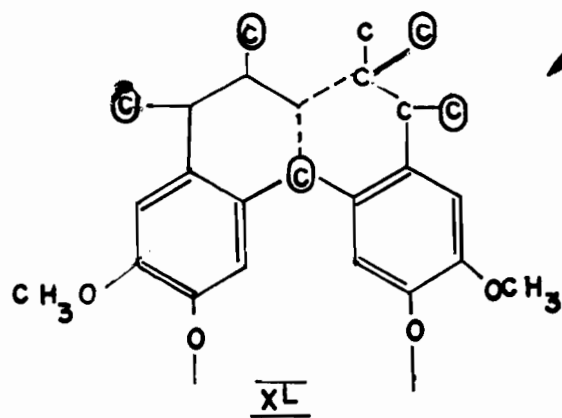
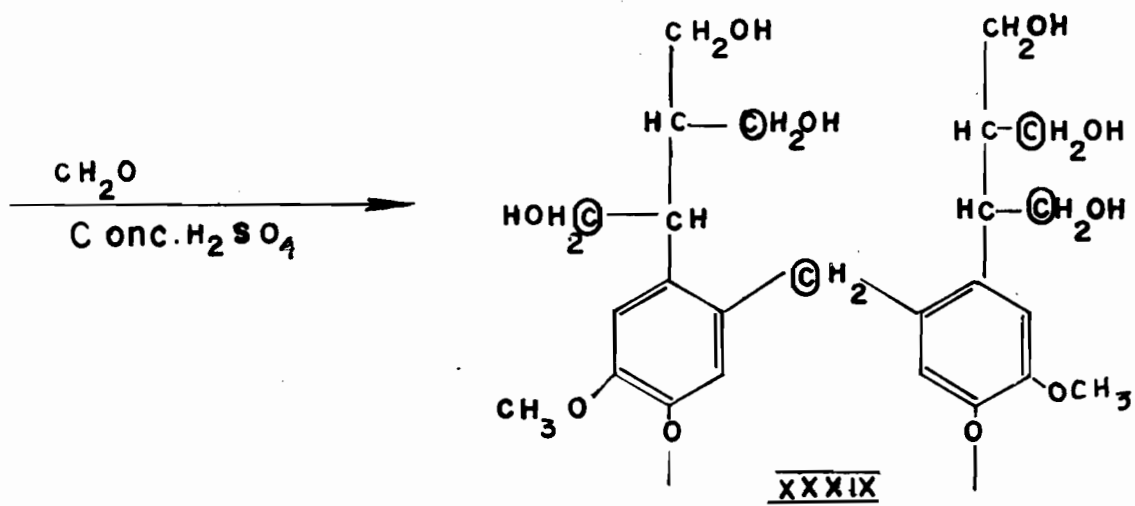
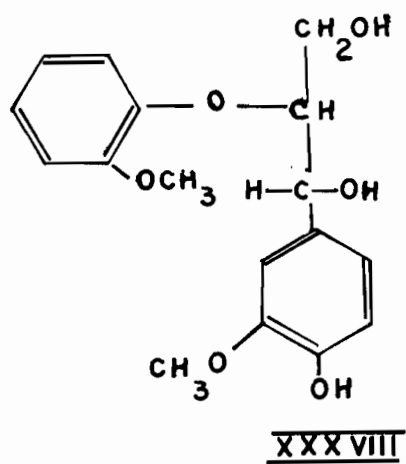


the model substance, and was synthesised from guaiacol by standard methods<sup>(56,57,58,59,60,61,62)</sup>. The guaiacyl ether, which was a yellow, viscous syrup, was subjected to a micro-oxidation with alkaline nitrobenzene, using the procedure of Eisenbraun<sup>(9)</sup>, and gave rise to vanillin and vanillic acid. An excess of formaldehyde with an excess of sulfuric acid was used in the condensation of the guaiacyl ether, the procedure being the same as that used for the condensation of the original periodate lignin. The product was a black tar with a methoxyl content of 12.2% in contrast to the 19.3% possessed by the original guaiacyl ether; this decrease, in conjunction

with the yield of 11.2% by weight, suggested that condensation with the formaldehyde was extensive. As expected, the condensed black tar did not yield any trace of vanillin or any identifiable substances when oxidized with alkaline nitrobenzene, but traces of benzene hexacarboxylic acid (mellitic acid) were formed on oxidation with potassium permanganate both in alkaline or neutral media. This observation might possibly be explained by the cyclisation of two guaiacyl glycerol ether units, substituted with hydroxymethyl groups, in presence of the strong sulfuric acid. Figs. XXXIX and XL illustrate this possibility (p. 79). Oxidation of the newly-formed carbocyclic rings in XL with potassium permanganate would be expected to yield some mellitic acid.

It is suggested that a detailed study of the condensation of formaldehyde with lignin model substances, such as guaiacyl glycerol- $\beta$ -guaiacyl ether, would be a worthy subject for future research. If the formation of mellitic acid by the drastic oxidation of the products is confirmed, then the isolation of this acid by the oxidation of formaldehyde periodate lignin might be attributed to the cyclisation of substituted phenylpropane units in the lignin. Eisenbraun's inference<sup>(9)</sup> that structures of the lignan type were the precursors of the mellitic acid might then prove to be unnecessary.





© Formaldehyde Carbon

## EXPERIMENTAL SECTION

### Analytical Methods

Unless stated otherwise, evaporations were carried out in a rotary evaporator at 30-35° under diminished pressure. All analyses were made at least in duplicate.

Methoxyl: The method of Vieboch and Schwappach<sup>(63)</sup> using 10-20 mg samples was employed, and the apparatus designed by Clark<sup>(64)</sup> was used. The liberated iodine was titrated with 0.03 N sodium thiosulfate.

Sulfur and sulfate: Sulfur was determined as described by Niederl and Niederl<sup>(65)</sup> by igniting about 20 mg of the sample with potassium nitrate, sugar and sodium peroxide in a steel bomb. All sulfur changed in the treatment to sulfate which was determined as barium sulfate.

Iodine: The method of Elek and Hill<sup>(66)</sup> was used. This method was based on the oxidation of the sample, 10-15 mg, with sodium peroxide in a modified Paar bomb. The sodium iodide was estimated as silver iodide.

Holocellulose: Ritter-Kurth method<sup>(67)</sup> was employed using chlorine gas followed by extraction with ethanol-monoethanolamine.

Klason Lignin: The standard method recommended by TAPPI<sup>(68)</sup> was employed using 72% sulfuric acid and 0.5-0.7 g. samples at 20° for 2 hours.

Ash: Samples, 10-20 mg, were ignited to a constant weight

in a micro-muffle furnace using a platinum boat<sup>(69)</sup>.

Neutralization Equivalent<sup>(66)</sup>: The sample, 10 mg, was dissolved in 5 ml of 50% neutral ethanol and titrated with 0.01 N sodium hydroxide standardized against 0.01 N potassium hydrogen phthalate, phenolphthalein being the indicator. The solution was heated to boiling as the end point was approached. If x ml of 0.01 N sodium hydroxide were consumed, then the N.E was calculated as

$$\text{Neutralization Equivalent} = \frac{\text{g. of substance} \times 1000}{x \text{ ml} \times (0.01 \text{ N NaOH})} \times 100$$

Paper Chromatography (Descending method): Sheets of Whatman No. 1 paper were used. A pencil line was ruled 7 cm from, and parallel to, one end. Then test solutions were applied from a micropipette to points 3 cm apart on this line and in such a way that the wetted spots would not exceed 1 cm in diameter. These spots were dried with a hot air current from an electric fan, and more solution was applied in a similar way until 0.5 - 5 mg of material had accumulated in each spot. The sheets were placed in chromatography tanks and allowed to equilibrate with the solvent vapour. Previously, the tightly covered tanks had been saturated with the vapour of the appropriate solvent mixture for 24 hours. Several hours later, the ends of the chromatograms were dipped into the solvent trays and the tank was closed tightly. When the solvent had run downward for about 35 cm, the paper sheets were taken out of the tank and air-dried. In the case of a developer containing volatile organic acids, the paper was

dried at  $100^{\circ}$  in an oven to remove the volatile acids in order to prevent interference. The mobile substances were detected with a suitable spray reagent.

In making up each solvent system, the components were thoroughly shaken and then allowed to stand overnight. The appropriate layer was then placed in the chromatography trays.

The following detecting reagents were used:

(a) Ferric chloride - potassium ferricyanide<sup>(44)</sup>: Water, 300 ml, 1% ferric chloride, 100 ml, and 1% potassium ferricyanide, 100 ml; prepared freshly as required. Phenolic zones turned blue but the colour was not permanent. If the paper was washed with very dilute hydrochloric acid immediately after spraying, fairly permanent spots were obtained.

Ammonium halides also responded to this spray.

(b) 2,4-Dinitrophenylhydrazine<sup>(70)</sup>: A 0.4% solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid.

Aldehydes and ketones turned brown, orange and pink, on a pale yellow background. The colour was fairly permanent.

(c) Bromophenol blue<sup>(71)</sup>: Bromophenol blue, 50 mg, was dissolved in 5 ml of distilled water and neutralized with sodium hydroxide to pH 7. The volume was then made up to 100 ml with ethanol. Acidic zones immediately turned yellow against a blue background, but the spots were not permanent. Prior to spraying a chromatogram run in acidic solvents, drying overnight at  $105^{\circ}$  was necessary to eliminate volatile acids from the solvent.

(d) Fast Red Salt GG (commercial name for the stabilized diazo salt of p-nitroaniline<sup>(72)</sup>): A 0.05% aqueous solution of the salt was sprayed on the paper immediately after exposure of the paper to ammonia vapour. The paper was allowed to dry in the air, and the colour of any spots was recorded. After 30 minutes the paper was sprayed with a saturated solution of sodium carbonate in water and allowed to dry. The colour of the spots was again recorded. Acidic, ketonic, and aldehydic compounds gave variously coloured spots.

(e) Rhodamine B dye<sup>(51)</sup>: Rhodamine B dye, 0.05 g., was dissolved in 100 ml of methanol. The dried paper after spraying was observed under ultraviolet light. Acidic zones showed as dark blue spots against a fluorescent violet background.

(f) Ammoniacal silver nitrate: The spray consisted of equal volumes of 0.1 N silver nitrate and 5 N ammonium hydroxide, and was used for the detection of phenols and carbonyl compounds, when brown or black spots were observed against a white background<sup>(73)</sup>.

### Preparations

#### Preparations of Periodate Lignin

A batch of periodate lignin was prepared from 800 g. of the dried spruce wood meal, 40 - 80 mesh in the following way. The meal was oxidized for 24 hours with 6 litres of 5% sodium paraperiodate buffered to pH 3.6 - 4.0 with acetic acid;

the suspension was filtered and hydrolysed with 0.1 N sodium hydroxide for 20 hours at room temperature, washed with water and re-suspended for 12 hours in water adjusted to pH 3.6 with acetic acid. After being recovered on a filter, the meal was subjected to four more oxidation-hydrolysis cycles; then the periodate lignin was washed with distilled water, recovered on a filter and air dried. The yield was 53% of the Klason lignin in the wood, the product containing 12.1% methoxyl, 0.8% ash and no holocellulose.

#### Preparation of the formaldehyde periodate lignin

The procedure followed was that given by Eisenbraun<sup>(9)</sup>. Periodate lignin, 40 g., dried over phosphorous pentoxide was immersed for 12 hours in a solution of 200 g. of commercial 36.8% formalin. Exactly 600 g. of 88.5% ice-cold sulfuric acid was then added very slowly with stirring, simultaneously with a little solid carbon dioxide so that the temperature did not go beyond 40-41°. Immediately after the addition of the sulfuric acid, the mixture was put into a water bath adjusted to 41-42°. The black gummy mass was then worked continuously with a glass rod for about one hour until no more solid particles could be detected, whereupon the viscous, black solution was poured very slowly with stirring into centrifuge tubes containing a five-fold volume of ice-cold water. The resulting precipitate was repeatedly washed on the centrifuge until there was no smell of formaldehyde in the wash liquor.

An emulsion eventually formed, and 5 ml of 88.5%

sulfuric acid was added in order to obtain a clear suspension. The precipitate was suspended in the minimum amount of water. Then N sodium hydroxide was added to the aqueous suspension to dissolve all the lignin, at which point the desired pH, 8-9, was reached. The dark brown solution was filtered through porous glass, and the formaldehyde lignin was reprecipitated by adding N sulfuric acid until pH 2 was reached. After being recovered on the centrifuge, the precipitate was extracted with the minimum quantity of acetone, the extract being evaporated on an evaporator. Finally, an excess of acetone was added to the extract and the inorganic material was removed on the centrifuge. The acetone solution was diluted with 200 ml of water, the acetone was evaporated and the remaining aqueous suspension neutralized to pH 5.7 and freeze-dried. Yield, 39.0 g. or 97.5%.

Found:  $\text{OCH}_3$  - 9.35, 9.36%; S - 1.2, 1.21%; Ash - 4.3%.

Synthesis of Guaiacyl Glycerol- $\beta$ -Guaiacyl  
Ether (XXXVIII)(56,57,58,59,60,61,62)

The synthesis was made starting from guaiacol (XLI) as shown in Fig. 18.

Acetovanillone (XLII): Guaiacol, 100 g., was dissolved in 88 g. of acetic anhydride mixed with 120 g. of powdered, anhydrous zinc chloride. The mixture stirred and was heated to boiling, whereby the zinc chloride went into solution, and a red colour developed. The solution was boiled for 3 minutes (temperature  $158^\circ$ ), then was chilled, and distilled with steam

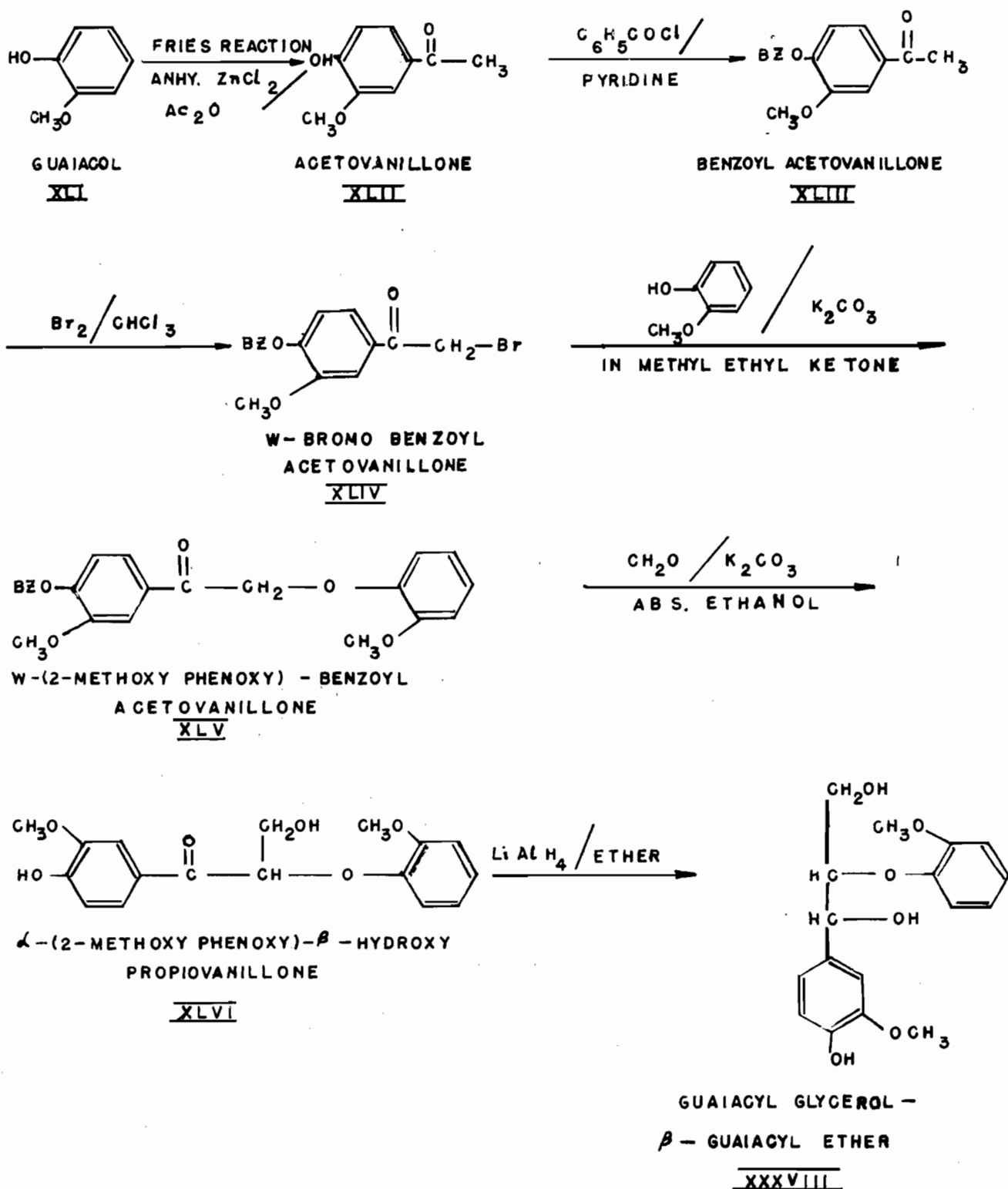


Figure 18

SYNTHESIS OF GUAIACYL GLYCEROL -  $\beta$  - GUAIACYL ETHER

(References: 56,57,58,59,60,61,62)



to remove the excess guaiacol. The residue after steam distillation was extracted with ether; the ethereal extract was dried over anhydrous sodium sulfate and evaporated under vacuum. Distillation of the residual oil at 0.5 mm pressure (bath temperature,  $200^{\circ}$ ) gave 59 g. boiling between  $125^{\circ}$  and  $150^{\circ}$ . This very thick oil was dissolved in 90 ml hot benzene, and on cooling, 30 g. of rose-coloured crystals were deposited. The melting point of  $112^{\circ}$  was that of acetovanillone (XLII).

Found:  $\text{OCH}_3$  - 18.6, 18.62%. Calc. for  $\text{C}_8\text{H}_7\text{O}_2$ .  $\text{OCH}_3$  - 18.7%.

Benzoyl-acetovanillone (XLIII): To 10 g. of the acetovanillone dissolved in 20 ml of anhydrous pyridine, 7 ml of benzoyl chloride was added in small portions. The temperature of the mixture increased, and finally a crystal slurry was formed. After about half-an-hour, 100 ml of water was added; a white, crystalline product separated, which was recovered, dried and dissolved in a small amount of benzene. Petroleum ether (b.p  $120-140^{\circ}$ ) was added until the solution just turned cloudy. Long, thin colourless needles of XLIII slowly separated, m.p  $107^{\circ}$ . (Correct m.p  $108-109^{\circ}$ ). Yield 12.1 g.

Found:  $\text{OCH}_3$  - 11.20, 11.19%. Calc. for  $\text{C}_{15}\text{H}_{11}\text{O}_3(\text{OCH}_3)$ :  
 $\text{OCH}_3$  - 11.50%.

w-bromobenzoyl acetovanillone (XLIV): Ten grams of XLIII was dissolved in 75 ml of chloroform. Bromine, 7.7 g., in 35 ml of chloroform was slowly added. The reaction was started by adding a drop of concentrated hydrochloric acid and by irradiation with ultraviolet light. After all the bromine

was added, the slightly red mixture was extracted with aqueous sodium bicarbonate, then with water, and the chloroform layer was dried over anhydrous sodium sulfate. The chloroform was evaporated under reduced pressure to leave an oil which crystallized on cooling. Recrystallization from ethanol yielded 10.5 g. of long colourless needles of XLIV melting at  $105^{\circ}$ . (Correct m.p  $105-106^{\circ}$ .)

Found:  $\text{OCH}_3$  - 10.12, 10.25%. Calc. for  $\text{C}_{15}\text{H}_{10}\text{O}_3\text{Br}(\text{OCH}_3)$ :  
 $\text{OCH}_3$  - 8.9%.

w-(2-methoxy phenoxy)-benzoyl acetovanillone (XLV): Anhydrous potassium carbonate, 10 g., was added to a solution of w-bromo-benzoyl acetovanillone (XLIV), 10 g., and guaiacol, 10 g., in dry methyl-ethyl ketone, 70 ml, and the mixture was heated on a water bath with vigorous stirring for 45 minutes, then filtered, diluted with water and extracted with chloroform. The chloroform extract was freed from excess guaiacol by extracting with sodium hydroxide solution, was washed with water, dried over anhydrous sodium sulfate and finally evaporated to a yellow viscous oil. Yield: 9.5 g.

Found:  $\text{OCH}_3$  - 15.62%. Calc. for  $\text{C}_{21}\text{H}_{14}\text{O}_4(\text{OCH}_3)_2$ :  
 $\text{OCH}_3$  - 15.81%.

$\alpha$ -(2-methoxy phenoxy)- $\beta$ -hydroxy propiovanillone (XLVI): Five millilitres of 40% formalin and 0.25 g. of potassium carbonate were added to a suspension of the XLV, 5 g., in 100 ml of absolute ethanol, and the mixture was heated at  $35^{\circ}$  with efficient stirring. After 20 minutes, benzoyl derivative

dissolved. Heating was continued for a further one hour and, when the mixture was cooled, an oil separated from the solution. The oil was recovered and extracted with chloroform, the extract then being dried over anhydrous sodium sulfate and evaporated. Yield: 3.0 g. of a yellow viscous oil (XLVI).

Found:  $\text{OCH}_3$  - 19.50%. Calc. for  $\text{C}_{15}\text{H}_{12}\text{O}_4(\text{OCH}_3)_2$ :

$\text{OCH}_3$  - 19.49%.

Guaiacyl glycerol- $\beta$ -guaiacyl ether (XXXVIII): Lithium aluminium hydride, 0.6 g., was suspended in 150 ml of dry ether contained in a three-necked round bottom flask (1 litre capacity) fitted with a mechanical stirrer, a reflux condenser and a separating funnel. The funnel contained 3 g. of XLVI in 250 ml of dry ether, and this solution was added drop by drop during 2 hours with vigorous stirring. Stirring for another 2 hours followed. The excess lithium aluminium hydride was decomposed with water. The ethereal layer, after drying over anhydrous sodium sulfate, was evaporated, giving a light yellow viscous syrup, XXXVIII; yield, 3.0 g. A comparison of the infra-red spectra of XLVI and XXXVIII showed complete reduction of the carbonyl group.

Found: C, 63.12; H, 6.01; O, 30.87;  $\text{OCH}_3$ , 19.31%.

Calc. for  $\text{C}_{15}\text{H}_{14}\text{O}_4(\text{OCH}_3)_2$ : C, 63.8; H, 6.25; O, 30.00;

$\text{OCH}_3$ , 19.34%.

The crystalline triacetate of XXXVIII melted at  $106^\circ$ .

(Correct m.p  $106 - 108^\circ$ ).

Preliminary Experiments on Degradation  
of the Formaldehyde Lignin

Extraction of Formaldehyde Lignin  
with 1% sodium bisulfite

A three-necked flask (250 ml) was fitted with a mechanical stirrer, reflux condenser and a thermometer. After 5 g. of dried lignin and 160 ml of 1% sodium bisulfite had been added to give a liquor to lignin ratio of 20:1, the assembly was heated on a steam bath. The temperature was 96-98°, and the acidity remained within the limits pH 4.9 - 5.1 during the experiment. After one hour, the black suspension was cooled to room temperature and dialysed through a cellophane membrane continuously for 120 hours. The non-dialysable fraction was concentrated to a suitable volume, freeze-dried and analysed. Yield, 4.2 g. 81%.

Found:  $\text{OCH}_3$  - 10.3; ash - 3.21, 3.24; sulfur - 0.8%.

Cleavage with Hydriodic Acid

The details of this experiment were taken from the work of Wieckowski<sup>(11)</sup>. The original formaldehyde lignin and the non-dialysable fraction from the above bisulfite treatment, 5 g. of each, were used. Each sample was placed in a three-necked flask equipped with a stirrer, a condenser, and a thermometer. Hydriodic acid (47%), 20 ml, was poured over the lignin, the flask was immersed in a thermostat at 50°, and the mixture was stirred for 8 hours. The resulting suspension was cooled to room temperature, diluted with about 20 ml of water and filtered through a sintered glass crucible. Any

free iodine was removed from the residue by repeated washing with 5% potassium iodide solution followed by water. The filtrate and washings were combined, and were dialysed for 96 hours. The non-dialysable fraction was recovered and analysed. The insoluble residue from the hydrogen iodide reduction was treated with sodium hydroxide, yielding an alkali-soluble and an alkali-insoluble fraction. The alkali-insoluble fraction was repeatedly washed with water, dried and analysed; the alkali-soluble fraction was dialysed for 72 hours, and the non-dialysable fraction was isolated and analysed. Table XIV summarized the composition of fractions from the original lignin and the non-dialysable portion (81%) after treatment with hydrogen iodide.

Concentration of the dialysable fraction from the alkali-soluble portion and subsequent neutralization to pH 7 with dilute sulfuric acid yielded a deep yellow solution which was treated with ethanol (30:70 v/v). Crystals of sodium sulfate were separated on a filter and the resulting yellow filtrate on freeze-drying gave a yellow gum, which was dried over phosphorous pentoxide to a constant weight. Yield, 12.5% and 13% respectively from formaldehyde lignin extracted with 1% bisulfite and from the original formaldehyde lignin.

The above yellow gum was examined by chromatography following the method of Jain<sup>(27)</sup>. About 100  $\mu$ g of the solution was spotted on Whatman No. 1 paper and the chromatogram was developed for 20 hours, in butanol-2% aqueous

Table XIV

Composition of Fractions from Original Lignin  
and the Non-dialysable Portion (81%)  
after Treatment with Hydrogen Iodide

	Non-dialysable NaHSO <sub>3</sub> Lignin	Original Lignin
	<u>%</u>	<u>%</u>
A. <u>Insoluble in HI</u>		
Yield	92	94
Ash	1.25	1.22
Sulfated ash	2.5	2.3
OCH <sub>3</sub>	9.2	9.01
I	traces	traces
B. <u>Sol. HI - non-dialysable</u>		
Yield	4	3.8
Ash	1.2	1.3
Sulfated ash	2.5	2.8
OCH <sub>3</sub>	3.3	3.4
I	traces	traces
C. <u>Alkali insoluble portion A</u>		
Yield	70	69.5
Ash	7.5	8.1
Sulfated ash	10.1	10.25
OCH <sub>3</sub>	9.45	9.45
I	traces	traces
D. <u>Non-dialysable alkali sol. portion of A</u>		
Yield	8	7.5
Ash	3.78	3.65
Sulfated ash	5.25	5.35
OCH <sub>3</sub>	8.6	8.75
I	traces	traces

ammonia, and sprayed with ferric chloride - potassium ferricyanide. A blue spot with  $R_f$ , 0.18, was observed. Jain<sup>(27)</sup> observed a similar spot with  $R_f$ , 0.26. The spot from the formaldehyde lignin directly degraded with hydriodic acid gave  $R_f$ , 0.16. In order to have enough of the degradation product, a large scale experiment was undertaken.

#### Larger-scale Degradation of Formaldehyde Lignin

Twenty-five grams of formaldehyde lignin were first treated with 1% sodium bisulfite, and the non-dialysable lignin fraction, 20 g., was extracted with 47% hydriodic acid in the same way as already described. Figs. 2, 3, and 4 on pages 27, 29 and 31 respectively showed the method of isolation of the yellow-brown, dialysed and alkali-soluble, crude gummy product, 3 g. (15% of the formaldehyde lignin fraction A). This material on exposure to air became resinous; it was soluble in water, giving a yellow-coloured solution of pH 6; soluble in ether and ethanol, and insoluble in benzene, chloroform and carbontetrachloride. A sample produced a deep violet colouration with 5% ferric chloride solution, thus showing a phenolic nature. When chromatographed with butanol - 2% aqueous ammonia, the material separated in three fractions - one which stayed on the starting line, another of  $R_f$ , 0.20 - 0.22, and a third streaking at  $R_f$ , 0.08 - 0.10. The infra-red spectrum shown in Fig. 5 (p. 32) was taken by the potassium-bromide technique, and the ultra-

violet spectrum (Fig. 6, p. 33) was determined in 95% ethanol with a concentration of 0.02 g. per litre.

Large-scale Separation of the Dialysable  
Mobile Fraction on a Cellulose Column

A chromatographic column was made of tubing 4.5 cm I.D and 60 cm long, fitted at the lower end with a perforated disc immediately above a stopcock. The wet method of packing the column was used. Water, 2.5 ml for each 10 g. of cellulose powder, and the mixture were intimately mixed until wetting was uniform and complete. The wet cellulose powder was then mulled using butanol - 2% aqueous ammonia (10 ml) and any lumps were broken before charging into the column by the wet method. Occasional pressing down of the cellulose during the addition was found helpful. Each 10 g. of the powders occupied about 7.5 cm in length.

The yellow gum, 2.0 g., was dissolved in 50 ml of the butanol - 2% aqueous ammonia eluent and was mixed with 10 g. of cellulose powder. This slurry was added to the top of the column. The solvent was allowed to pass through the column at a rate of approximately one drop per second. Fractions, 15 ml each, were collected in 300 tubes in an automatic fraction collector. Visible bands were developed, and the materials in them were collected in the various fractions of effluent until a sample no longer showed a blue spot when tested by paper chromatography and ferric chloride - ferricyanide spray.



The first 39 fractions did not show any spot on the paper. Fractions 40 - 170 gave a deep blue spot with  $R_f$ , 0.22, in butanol - 2% aqueous ammonia, and of  $R_f$ , 0.39 - 0.41 in butanol - pyridine - water (10:3:3 v/v). These effluents, when evaporated and dried, gave a yellow amorphous powder, amounting to 1.3 g. This product was isolated by evaporating as much as possible of the butanol under diminished pressure, displacing the remainder with water, and by drying the residue over phosphorous pentoxide. Fractions 171 - 300 gave a dark brown resinous mass staying on the starting lines of paper chromatograms, and amounting to 0.7 g. The main fraction, 1.3 g., of yellow amorphous powder, was examined under the microscope, which clearly showed the presence of crystalline material together with a yellow amorphous substance. A solution of the material in water was treated with decolourising carbon and filtered, the colourless filtrate being evaporated and dried. The original yellow colour returned. Attempts were then made to crystallize the yellow material by using suitable organic solvents. A concentrated solution in absolute ethanol when kept at 4° for 5 days began to crystallize. To the naked eye the crystals looked cubic, but when the mother liquor was removed the crystalline condition was lost and an amorphous powder resulted. The same phenomenon was observed with benzene-acetone and petroleum ether-ethanol mixtures.

The failure to obtain crystals led to the material,

now 800 mg in weight and still neutral, being ion exchanged with Amberlite IR-120 in a column 1 cm I.D and 13 cm long. The effluent, which had pH 2, was concentrated in a rotary vacuum evaporator under reduced pressure at 30° and the residue in the flask had a strong smell of iodine. When an attempt was made to remove the water completely at a slightly higher temperature of 38°, the whole of the material volatilized, as a violet vapour. The material from the condensing flask was extracted with chloroform, giving a violet solution. The free acid in this effluent was now thought to be hydriodic acid and the yellow amorphous material before the ion-exchange treatment was assumed to be ammonium iodide.

A survey of the literature<sup>(37)</sup> then showed that ammonium iodide, like the material under investigation, was very hygroscopic, formed colourless cubic crystals in ethanol, which disintegrated to an amorphous powder when dried; its solution in water turned yellow on standing. Ammonium iodide was then chromatographed in butanol - 2% aqueous ammonia and butanol - pyridine water (10:3:3 v/v) side by side with the material under study, and was found to give similar  $R_f$  values of 0.20 - 0.22 and 0.39 respectively. Therefore 9.7% of the 15% of dialysable material obtained after degradation of formaldehyde lignin with 47% hydriodic acid was ammonium iodide rather than any true degradation product.

The second fraction (0.7 g.), which stayed at the

top of the column, was recovered as oily drops on elution with water. After concentration and freeze-drying, the residue was a dark brown resinous mass, having an ash content of 17.5% and showing the presence of sulfate ions. A solution of the material in water had a pH of 8, and the solute could not be moved in paper chromatograms, using a number of solvent systems, and could not be crystallized by any means. The material was soluble in alcohol, leaving a white residue. By repeated treatment with alcohol, the ash was completely removed. The residue, 0.58 g., (pH of 8 in water) was ion exchanged with Amberlite IR-120. The effluent, pH 3, on evaporation formed a dark brown transparent film which was insoluble in all liquids except water and ethanol. To move the material in chromatograms, the solvent systems for aromatic acids, e.g. benzene - acetic acid - water (2:2:1), and n-butanol 1.5 N ammonia, were tried but without any success. In ethanol - ammonia - water (80:4:16), however, the substance gave three distinct yellow spots of  $R_f = 0.00$ , 0.30 and one faint spot of  $R_f = 0.38$ , with bromophenol blue in alcohol as the spray. When the spray was ferric chloride - ferricyanide solution, only one blue spot on the starting line was observed.

All of the ion-exchanged material (0.32 g.) was then submitted to quantitative paper chromatography. Samples, 30 mg, were spotted on each sheet (7" x 22") of Whatman No. 1 paper; twelve such sheets were used. The tank was first saturated with ethanol - ammonia - water (80:4:16) overnight and

the spotted papers were then equilibrated with the solvent for 12 hours before being developed for 16 hours. After drying the paper, half-inch widths were cut out from both sides of each strip to locate the position of the spot by spraying with bromophenol blue. Three fractions were noted; material having  $R_f = 0.00$ ,  $R_f = 0.30$ , and faint spots of  $R_f = 0.38$ . The paper-holding fraction was cut out and eluted with water, and the fractions were again ion-exchanged with Amberlite IR-120 separately. The third fraction ( $R_f$ , 0.38) after ion exchange did not show any residue and was therefore discarded. The first fraction ( $R_f$ , 0.00) gave a dark brown resinous mass, 66 mg, which failed to crystallize in any solvent. It had no ash, had a methoxyl content of 9.1, 9.15% and gave infra-red and U.V spectra characteristic of lignin.

The second fraction ( $R_f = 0.30$ ) was recovered after ion exchange as a yellow transparent film, 0.084, soluble in water and alcohol. A number of solvents were tried for crystallization but met with no result. Found:  $\text{OCH}_3$ , 8.94, 8.92%; ash, 6.8% (moisture and ash free basis). This fraction also showed an infra-red spectrum characteristic of lignin.

Even from 25 g. of formaldehyde lignin, the percentage yields of the degradation products were so small that the quantity available for identification purposes was inadequate. The results were so indecisive, that the study of degradation with 47% hydriodic acid was stopped at this stage.

Degradations of Periodate Lignin and  
Formaldehyde-Periodate Lignin with  
Sodium in Liquid Ammonia

Sodium-liquid ammonia reactions and the subsequent decompositions of by-product sodamide were carried out with extreme care. An eye mask and rubber gloves were worn, and manipulations were carried out in a fume hood.

The general procedure employed for these degradations was that of Freudenberg<sup>(40)</sup> as modified by Shorygina and co-workers<sup>(41,42,43)</sup>. Five-gram powdered samples of periodate lignin and of formaldehyde lignin were used. The sample was placed in a 1-litre Erlenmeyer flask and covered with 500 ml of anhydrous liquid ammonia previously cooled in an alcohol - solid carbon dioxide bath. Freshly cut sodium, 3.0 g., was added. Immediately afterwards, the flask was stoppered with a rubber bung pierced by a drying tube that contained sodium hydroxide pellets. The reaction vessel was then surrounded with vermiculite insulating material to maintain the temperature at approximately  $-33^{\circ}$ , the boiling point of ammonia. The mixture was shaken occasionally and, when necessary, more liquid ammonia was added to replace that lost by evaporation. If the blue solution became colourless, an additional 1.0 g. of sodium was added. After 66 hours, the flask was taken out of the insulating material so that the ammonia would evaporate rapidly. As soon as the contents of the flask had regained room temperature, they were completely covered with ether, and water was added dropwise. After the

reaction with sodamide and any sodium metal appeared to be complete, the mixture was allowed to stand for several hours. The product was then transferred to a liquid-liquid extraction, care being taken to keep the strongly alkaline mixture covered with ether to minimize oxidation by the air. Following an extraction with ether for 24 hours, the extract was dried over anhydrous sodium sulfate, filtered, and evaporated in a rotary evaporator at  $30^{\circ}$ . The yellow syrups (A, A') were dried over anhydrous calcium sulfate in a desiccator. Yield from periodate lignin (A) 1.02 g;  $\text{OCH}_3$ , 9.2, 9.2%. Yield from formaldehyde lignin (A') 0.14 g;  $\text{OCH}_3$ , 8.8, 8.9%. The treatment of the syrup (A, A') is described below in the section entitled 'Investigation of Solvent Extracts'.

The alkaline solution, after the extraction with ether, was acidified with dilute sulfuric acid to pH 1.8, when flocculent precipitate was formed. This precipitate was collected on the centrifuge, washed and dried. Yield from periodate lignin (D) 2.76 g;  $\text{OCH}_3$ , 14.1, 13.9%. Yield from formaldehyde lignin (D') 3.91 g;  $\text{OCH}_3$ , 9.4, 9.3%. The mother liquor (pH 1.8) was extracted with ether in a liquid-liquid extractor for 24 hours; the ethereal solution was dried over anhydrous sodium sulfate, filtered, and evaporated in a flash evaporator at  $30^{\circ}$ . The brown syrup (B, B') was dried over anhydrous calcium sulfate in a desiccator. Yield from periodate lignin (B) 0.21 g;  $\text{OCH}_3$ , 11.35, 11.38%. Yield from formaldehyde lignin (B') 0.419 g;  $\text{OCH}_3$ , 9.12, 9.13%.

The treatment of the syrup (B,B') is described below in the section entitled 'Investigation of Solvent Extracts'.

The acidic solution (pH 1.8) 300 ml, left after ether extraction was treated with a slight excess of dilute 10% barium chloride solution, was warmed on a hot plate, and the heavy white precipitate of barium sulfate was allowed to settle overnight, before being removed on a filter and washed with water. The filtrate and washings were concentrated in a rotary evaporator, then dried in vacuum over phosphorous pentoxide. Extraction of the dried residue with anhydrous acetone yielded a yellow extract which was evaporated and dried to water soluble yellow powders (C) and (C'). Yield from periodate lignin (C) 0.955 g;  $\text{OCH}_3$ , 5.32, 5.41%. Yield from formaldehyde lignin (C') 0.563 g;  $\text{OCH}_3$ , 8.98, 9.0%.

The acid-insoluble material (D) (Fig. 7, p. 38) from periodate lignin was submitted to two more cycles of sodium-liquid ammonia degradation. Each cycle was of 66 hours duration, and the amount of sodium and liquid ammonia used was in the same ratio as in the original degradation. The results, after processing as described above, are shown in Table V (p. 40).

#### Investigation of Solvent Extracts

Extract (A): The ethereal extract (A) at pH 12.0 from the degradation of periodate lignin was a brown oil. A first attempt to move the syrup by paper chromatography followed the procedure of Read<sup>(39)</sup>, and used two solvent systems,

hexane - dioxane - water (6:3:1 v/v) and hexane - pyridine - water (1:1:1 v/v). The oil moved giving a single distinct blue spot of  $R_f = 0.73$  and  $0.76$  respectively, with a ferric chloride-potassium ferricyanide spray. Hence the presence of a low molecular weight phenol was indicated.

The syrup was distilled in vacuo in a micro still that was designed to minimize loss from 'hang up'. From 1.02 g. of the syrup, 0.525 g. of distillate was obtained at 1 mm pressure and a bath temperature of  $230^{\circ}$ . Paper chromatography, using hexane - pyridine - water (1:1:1 v/v) gave again only one spot of  $R_f = 0.76$ .

The benzoate of this oil was made by a micro modification of standard methods<sup>(74)</sup>. Benzoyl chloride, 35 mg, together with 50 mg each of pyridine and the unknown distillate, were placed in a micro test tube made by sealing one end of a 6 x 10 mm piece of glass tubing. The mixture was heated with stirring at  $100^{\circ}$  for five minutes, and then allowed to cool. Excess water was added, and then the mixture was stirred with a micro stirring rod and allowed to settle. After the water had been removed with a capillary tube, 5% sodium bicarbonate was added, stirred, and removed as before. Washing with a few drops of distilled water followed, and then the solid was crystallized from ethanol, m.p  $73.5^{\circ}$ .

The reported  $R_f$  value of dihydroeugenol in hexane - pyridine - water (1:1:1 v/v) and the melting point for the benzoate of dihydroeugenol were respectively  $R_f = 0.76$ <sup>(39)</sup> and



74 - 75°(43,39).

Extract (B): The ethereal extract (B) at pH 1.8 of the degradation products from periodate lignin was a dark tar. When chromatographed in developers using the organic phase of the systems, hexane - pyridine - water (1:1:1 v/v) and hexane - dioxane - water (6:3:1 v/v), the tar showed mobile faint spots at  $R_f = 0.75$  and  $0.73$  respectively, with most of the material staying on the starting line. The sprays, bromophenol blue and ferric chloride - potassium ferricyanide, were both positive, indicating the presence of both phenolic hydroxyl and carboxylic acid groups. A paper chromatographic separation of the material from the tar was then made, using hexane - pyridine - water (1:1:1 v/v) as the eluent, and yielded 20 mg of a white, solid residue. This white solid was crystallized from very dilute ethanol to yield 13 mg of crystals with m.p  $207^\circ$ . The melting point reported for vanillic acid was  $210^\circ$ (75) and the  $R_f$  value reported for vanillic acid was 0.75 and 0.73 in hexane - pyridine - water (1:1:1 v/v) and hexane - dioxane - water (6:3:1 v/v) respectively(39), values which were identical with the above results. A mixed melting point with standard vanillic acid was  $208.5^\circ$ .

Extracts (A') and (B') (Table VII, p. 44): The ethereal extracts (A') and (B') of the degradation products from formaldehyde periodate lignin at pH 12.0 and 1.8, respectively, were examined in paper chromatography. A number of solvent

systems for the chromatography of phenols and phenolic acids<sup>(36,38)</sup> were tried, but without any success. The extract (B') moved almost up to the solvent front in hexane - pyridine - water (1:1:1 v/v), but the  $R_f$  value could not be measured as the spot showed too much tailing. This spot could be detected on the paper prior to spraying as a yellow streak which turned deep blue on treatment with ferric chloride - ferricyanide. A quantitative paper chromatographic separation of 0.4 g. of the brown syrup, using 40 Whatman No. 1 paper of size (6" x 22") and hexane - pyridine - water (1:1:1 v/v) as eluent, yielded 0.12 g. of a yellow gum. This gum behaved rather strangely on rechromatography, showing a long streak from  $R_f = 0.50$  to  $R_f = 0.95$ . All attempts to crystallize the gum were unsuccessful, and it was not further investigated.

Water-soluble material (C) and (C') and Acid-insoluble material (D) and (D') (Tables V and VII): The water-soluble and acid insoluble degradation products from both periodate lignin and formaldehyde lignin were yellow amorphous powders, apparently of high molecular weight and phenolic in nature, and with infra-red and ultraviolet spectra identical to those of the parent lignins. All four were subjected to alkaline nitrobenzene and alkaline potassium permanganate oxidations on the micro-scale, using the procedures of Eisenbraun<sup>(9)</sup>. The results of the paper chromatography of the oxidation products are shown in Table XV. Both fractions (C) and (D)

Table XV

Paper Chromatography of the Water-soluble and  
the Acid-insoluble Fractions after Oxidation

Fractions <sup>a)b)</sup>	Alkaline Nitrobenzene Oxidation <sup>c)</sup>	Alkaline Potassium Permanganate Oxidation <sup>d)</sup>
	Solvent: Butanol-2% aq. ammonia	Solvent: Methyl Isobutyl ketone-formic acid-water (10:1:1 v/v)
	R <sub>F</sub> value	R <sub>F</sub> value
Water-soluble Fraction (C)	0.44	0.12, 0.30
Water-soluble Fraction (C')	-	0.00, 0.12
Acid-insoluble Fraction (D)	0.44	0.12, 0.30
Acid-insoluble Fraction (D')	-	0.00, 0.12

a)b) Described in Table V and VII.

c) Spray: Ferric chloride - ferricyanide

d) Spray: Neutral bromophenol blue

on alkaline nitrobenzene oxidation yielded vanillin with  
R<sub>F</sub> = 0.44 in n-butanol - 2% aqueous ammonia; the fractions  
(C') and (D') on similar oxidation failed to show anything  
identifiable by paper chromatography.

The oxidation products of fractions (C) and (D) from  
alkaline potassium permanganate were chromatographed in

methylisobutyl ketone - formic acid - water (10:1:1 v/v) and were sprayed with neutral bromophenol blue.  $R_f$  values of 0.12 and 0.30, characteristic of benzene penta-carboxylic acid and 1,2,4,5-tetra-carboxylic acid respectively, were observed. On the other hand,  $R_f$  values of 0.00 and 0.12, characteristic of benzene hexa- and penta-carboxylic acids, were given by the fractions (C') and (D') from formaldehyde lignin.

### Degradations by Oxidation

#### Oxidation of Formaldehyde Lignin with Nitrobenzene

The method was based on those of Freudenberg<sup>(76)</sup> and Stone and Blundell<sup>(47)</sup>, and the procedures both of Jain<sup>(27)</sup>, who used 4 N sodium hydroxide, and of Eisenbraun<sup>(9)</sup>, who tried the 2 N alkali, were followed. Samples, 250 mg of the lignin and 5 ml of freshly distilled nitrobenzene, were sealed with 5 ml of 4 N or 2 N sodium hydroxide in a bomb made of 98% nickel, and the assembly was heated at 175° for three hours. The bomb was then rapidly cooled, and the contents were filtered and washed with distilled water. Filtrate and washings were concentrated, neutralized with dilute sulfuric acid, and then extracted with ether. The ether extract was spotted on equilibrated paper and developed for 15 to 16 hours, using n-butanol saturated with 2% aqueous ammonia and n-butanol - pyridine - water (10:3:3 v/v) solvent systems. After being dried, the paper showed a yellow spot of  $R_f = 0.88$  (n-butanol - 2% aqueous ammonia) and  $R_f = 0.82$  (n-butanol -

pyridine - water - 10:3:3 v/v) before spraying.

This yellow spot became intense on spraying with 2,4-dinitrophenyl hydrazine. Even when freshly distilled and redistilled nitrobenzene samples were used, all types of lignin - periodate spruce, formaldehyde-periodate spruce, periodate birch, and even wood meal and substances like vanillyl alcohol, gave the same yellow spot; all except formaldehyde lignin also giving a spot for vanillin ( $R_f = 0.44$ , in n-butanol - 2% aqueous ammonia, and  $R_f = 0.87$ , in n-butanol - pyridine - water - 10:3:3 v/v). Benzyl alcohol and glucose also showed the same yellow spot. When conducted in a stainless steel bomb, the oxidations failed to yield the yellow spot. Starting with 250 mg of lignin, 15 mg of the yellow material was recovered by paper chromatography as yellow needles which melted at  $112^\circ$  and responded to the micro-tests for nitro and phenolic hydroxyl groups. The melting point of p-nitrophenol was  $114^\circ$ . The substance was identified as p-nitrophenol, since the mixed melting point was  $113^\circ$ .

#### Oxidations with Cupric Hydroxide<sup>(77)</sup>

(a) Micro scale: Two separate experiments were performed - one with periodate spruce lignin and the other with formaldehyde periodate lignin. The lignin, 50 mg, together with 300 mg of cupric hydroxide and 1 ml of 4 N sodium hydroxide, was sealed in a 2 ml capacity bomb and kept in an air oven at  $175^\circ$  for three hours. During the heating period, the bomb

was twice shaken vigorously. After the reaction, the contents of the bomb were directly spotted on the chromatographic paper. The paper with the dried spots was held for a minute over a beaker containing glacial acetic acid to neutralize the free alkali, and was then developed using the solvent systems - n-butanol saturated with 2% aqueous ammonia and also n-butanol - pyridine - water (10:3:3 v/v). After the paper had been equilibrated overnight, the chromatogram was run for 15 hours, and was then sprayed with diazotised p-nitroaniline. No spots were observed with formaldehyde - periodate lignin, except a long streak, but periodate lignin showed one violet spot at  $R_f$ , 0.44 (butanol - 2% aqueous ammonia) and  $R_f$ , 0.86 (butanol - pyridine - water - 10:3:3 v/v), which was the same as for authentic vanillin. In another oxidation of this lignin with alkaline cupric hydroxide, the contents of the bomb, instead of being directly spotting on the paper, after filtration were neutralized with dilute sulfuric acid and then were extracted with ether. The ethereal extract gave distinct streaking with bulging at some points when chromatographed in the solvent systems mentioned above. Another quantitative oxidation was next made on the semi-micro scale with 250 mg of formaldehyde lignin and, after proper processing, the dark brown ethereal extract, 25 mg, was spotted in high concentration. When developed in butanol - 2% aqueous ammonia and sprayed with diazotised p-nitroaniline, five distinct spots of  $R_f$ , 0.015, 0.037, 0.07,

0.12 and 0.82 appeared, among which the spots at  $R_f$ , 0.07 and 0.82 were the most interesting. The chromatogram when sprayed with four different reagents for phenols and phenolic acids showed spots of different colour and intensity.

(b) Larger scale: In a standard experiment, the mixture of formaldehyde periodate lignin (6 g.), 6 ml of 4 N sodium hydroxide and 36 g. of cupric hydroxide was sealed in a 130 ml capacity stainless steel bomb. Heating was in a glycol oil bath, equipped with an electrically driven rotor and with a relay system for maintaining the desired temperature of  $175^{\circ}$ . After three hours with constant stirring, the contents were chilled to room temperature, were removed, filtered and the cuprous oxide sludge washed thoroughly with water. The filtrate and the washings were acidified with dilute sulfuric acid, then centrifuged and the residue washed with water. The clear brown, centrifuged solution was concentrated, and a continuous extraction with ether removed 0.80 g. of dark brown oil. When chromatographed on paper, using butanol - 2% aqueous ammonia, this oil showed the same mobile spots observed in the micro-scale experiment discussed above. All of the above oil, 800 mg, was placed on a column of cellulose powder packed by the wet method, and an attempt was made to separate the components using n-butanol - 2% aqueous ammonia as the eluent and an automatic fraction collector. No separation could be achieved, and similar failure met attempts using two other columns of different dimensions. After recovery, the

remaining oil, 600 mg, was finally separated by quantitative paper chromatography, and three fractions were collected. The first fraction,  $R_f$ , 0.00 consisted of 200 mg of a brown oil; the second fraction, with the initial  $R_f$ , 0.07 in the mixture, showed a  $R_f$  value of 0.15 after chromatographic separation, and amounted to 30 mg of a brown resinous mass. The third fraction also showed a new  $R_f$  value, increasing from 0.82 to 0.86, and yielded 18 mg of a yellow powder. It seemed necessary to repeat the work on a still larger scale.

(c) Thirty grams of formaldehyde lignin, 30 ml of 4 N sodium hydroxide and 180 g. of cupric hydroxide were placed in a one-litre capacity stainless steel bomb and heated for three hours at 180°. The processing of the oxidation products was shown in the flow chart (Fig. 8, p. 49), and the chromatographic behaviour of the extracts was discussed in Table VIII (p. 50). After failing to separate extract A (Fig. 8) on a cellulose column, all of the oil, 4.0 g., was separated on paper using butanol - 2% aqueous ammonia as the eluent. The procedure for this large-scale separation, obtained from Dr. Timell, gave an effective separation of the three components present in the extract (A), as shown below.

(1) First fraction, 2.50 g., eluted from the paper by cold absolute ethanol. A dark brown oil,  $R_f$ , 0.00, both in n-butanol - 2% aqueous ammonia and n-butanol - pyridine - water (10:3:3 v/v). The oil gave an infra-red spectrum of a typical lignin nature.



(2) Second fraction (0.40 g.) eluted from the paper by cold absolute ethanol. A dark brown resinous substance,  $R_f$ , 0.15 and 0.81, in butanol - 2% aqueous ammonia and butanol - pyridine - water (10:3:3 v/v) respectively. The resin failed to crystallize in a number of solvents tried. Found by the Schwarzkoff Microanalytical Laboratory, New York. Found: C, 49.0; H, 6.24; O, 44.75;  $\text{OCH}_3$ , 11.0%. Calcd. for  $\text{C}_{11}\text{H}_{15}\text{O}_7(\text{OCH}_3)$ , mol. wt. 290: C, 49.7; H, 6.2; O, 44.1;  $\text{OCH}_3$ , 10.7%. The ultraviolet spectrum of the material showed the peak at 210  $m\mu$  and 280  $m\mu$ , typical of lignin and its degradation products (Fig. 9, p. 52). The infra-red spectrum was also similar to a typical lignin, except that it showed a very strong carbonyl absorption at 1675  $\text{cm}^{-1}$  (Fig. 10, p. 53). Nevertheless, the substance formed neither a bisulfite derivative nor a 2,4-dinitrophenyl hydrazone. Although tests for the phenolic group were positive, attempts to prepare an acetate were indecisive. Fruitless attempts at crystallization from many solvents exhausted the fraction.

(3) The third fraction (0.375 g.) was eluted with hot absolute ethanol, as a yellow shining powder,  $R_f$ , 0.86 and 0.84 in butanol - 2% aqueous ammonia and butanol - pyridine - water (10:3:3) respectively. The chromatographic data, infra-red and ultraviolet spectra were in good accord with those for bivanillyl found in the literature. Found by the Schwarzkoff Microanalytical Laboratory, New York: C, 70.03; H, 6.59;  $\text{OCH}_3$ , 22.59%. Calcd. for bivanillyl,  $\text{C}_{14}\text{H}_{12}\text{O}_2(\text{OCH}_3)_2$ :

C, 70.05; H, 6.57;  $\text{OCH}_3$ , 22.63%. The compound, melted at  $159^\circ$ , and the acetate prepared from it at  $136^\circ$ . An authentic sample of bivanillyl, secured through the courtesy of Dr. I.A. Pearl, The Institute of Paper Chemistry, Appleton, U.S.A., melted at  $161-162^\circ$  and its diacetate at  $139^\circ$ . Mixed melting points with these authentic samples were undepressed.

The infra-red spectrum of the first fraction (1) (2.50 g.) separated from extract (A) was the same as those of extracts (B) and (C) (Fig. 8, p. 49). So the fraction (1) and the extracts (B) and (C) were combined, and the total amount of oil was 5.48 g. An attempt was made to distil 1 g. of the oil at 2 mm pressure, but decomposition started above  $140^\circ$ , without any distillate. On adding petroleum ether (b.p  $40 - 60^\circ$ ) to the rest of the 4 g. of oil, a brown flocculent precipitate separated which was washed with petroleum ether and dried. The infra-red and ultraviolet spectra of the brown powder again showed the same characteristics as those of lignin. Analysis showed an ash content of 2.3% and methoxyl of 9.12, 9.2%, the same as the original formaldehyde lignin. The material when subjected to alkaline nitrobenzene oxidation did not give any vanillin, but when oxidized with alkaline potassium permanganate gave benzene hexa- and penta-carboxylic acid, identified by paper chromatography.

#### Oxidation with Alkaline Potassium Permanganate

The oxidation, and the chromatographic separation of

the oxidation products were carried out exactly according to the procedure followed by Jain<sup>(27)</sup> and hence were not mentioned here.

Oxidation of the Formaldehyde Periodate Lignin  
with Neutral Potassium Permanganate

This oxidation followed a procedure of Richtzenhain<sup>(49)</sup>. Four grams of the formaldehyde lignin were suspended in 115 ml of water in a 500 ml three-necked flask fitted with a thermometer and a mechanical stirrer, and heated to 90° with constant stirring. Finely pulverized potassium permanganate, in 1-g. portion, was gradually added, and, as the colour of the permanganate disappeared, the pH of the solution was maintained at 7 by the gradual addition of dilute sulfuric acid. A total of 23 g. of permanganate was added in ten hours.

After the oxidation, the manganese dioxide was removed on a filter, and was washed with hot water. The filtrate and washings were concentrated to 200 ml, acidified with dilute sulfuric acid to pH 1.8, and extracted with ether in a separating funnel. The ethereal solution was dried over anhydrous sodium sulfate, evaporated, and the residue was dried over phosphorous pentoxide in a vacuum desiccator, to give a yellow amorphous material. Yield: 0.015 g. or 0.37%,  $R_f$  = streaking in methylisobutyl ketone - formic acid - water (10:1:1 v/v).

The yellow aqueous solution (200 ml) was then treated

with 10% barium chloride solution; the precipitate of barium sulfate was recovered on the filter, and the filtrate was again made acidic with dilute hydrochloric acid. To remove the excess barium, a few drops of dilute sulfuric acid in equivalent amount was added, and the barium sulfate was removed on the centrifuge. The yellow aqueous filtrate was concentrated to semi-dryness, and then extracted with acetone. The yellow acetone extract containing traces of potassium chloride was chromatographed in methylisobutyl ketone - formic acid - water (10:1:1 v/v), the spray being bromophenol blue. Nebulous yellow streaking near the solvent front was found along with a distinct round spot at the starting line. A paper quantitative separation of the acetone extract was made using the above eluent, the spot at  $R_f = 0.00$  was cut out and eluted with cold water. The aqueous solution, on evaporating and drying over phosphorous pentoxide, gave a white crystalline powder, melting at  $285^{\circ}$ . Yield: 0.01 g. or 0.25%. A mixed melting point with an authentic sample of mellitic acid was  $286^{\circ}$ .

Oxidation of Periodate Lignin with  
Neutral Potassium Permanganate

The procedures for the oxidation and the isolation of the products were the same as those described for the formaldehyde lignin. Four grams of periodate lignin consumed 25 g. of permanganate during the process. Using the same analytical procedures discussed above, no identifiable materials were formed from such an oxidation.

Methylation of Periodate Lignin with  
Dimethyl sulfate and Sodium hydroxide<sup>(50)</sup>

Periodate lignin, 10 g., was placed in a three-neck one-litre flask with 295 ml of water. The flask was fitted with a mercury-sealed stirrer and two separatory funnels, containing 190 ml of dimethyl sulfate and 310 ml (472 g.) of aqueous sodium hydroxide, respectively. The entire reaction was carried out in a nitrogen atmosphere, and the flask was cooled with running water so that the temperature did not exceed 30°. The reaction was started by running the sodium hydroxide and dimethyl sulfate into the flask. After about ten minutes, all but 100 ml of the sodium hydroxide solution had been added. The remaining sodium hydroxide and the dimethyl sulfate were then added over a period of 4.5 hours. After the reaction was complete, the residue was washed with water under a nitrogen atmosphere until neutral.

The residue was freeze-dried and finally dried over phosphorous pentoxide under vacuum for two days to constant weight. Two more methylations were performed to give a constant methoxyl content. Yield: 8.5 g. Found:  $\text{OCH}_3$ , 17.35, 17.32%.

Oxidation of Methylated Periodate Lignin  
with Neutral Potassium Permanganate

The procedure used was that of Freudenberg<sup>(51)</sup>. Finely powdered methylated periodate lignin, 8.5 g., was suspended in 300 ml of water and boiled. After that, the

mixture was stirred and the temperature maintained at 55 - 60°, while potassium permanganate was added at intervals in 4 - g. portions. After each addition, when the colour was discharged, the pH was adjusted to 6 - 7 by the dropwise addition of dilute sulfuric acid. A total of 54 g. permanganate was added before the colour remained pink for 20 minutes. The suspension was heated to 90 - 95° and another 2 g. of permanganate was added and kept for ten minutes, after which the colour was discharged. Thus, a total of 56 g. of permanganate was consumed over a period of about  $3\frac{1}{2}$  hours. The suspension was filtered and washed with hot water five times. The bright yellow filtrate was concentrated to 100 ml and then extracted with ether for 48 hours. The ethereal solution was dried over anhydrous sodium sulfate, evaporated and finally dried over phosphorous pentoxide in a vacuum desiccator to a yellow friable material. Yield: 0.023 g.

The aqueous yellow solution, 100 ml, was made to pH 1.8 - 2.0 with dilute sulfuric acid and extracted with ether for 48 hours. The ethereal extract on drying to a constant weight was a yellow oil. Yield: 1.3 g. or 15.3%; Ash, 0.00%. As shown in Table XI (p. 62), the material moved only in one solvent system, butanol - acetic acid - water (4:1:1 v/v), giving two close spots at  $R_f$ , 0.75 - 0.80, identified as acids by the bromophenol blue spray. A paper chromatographic separation of the two spots, using the above solvent system, was not successful.

The electrophoretic behaviour of the oil was then observed. A Whatman No. 1 paper (3" x 18") was soaked with the 3 N ammonium hydroxide used as the electrolyte, the paper being dipped in the solution placed in the two cells; then at the mid-point of the paper, 5 mg of the sample in ammoniacal solution was spotted. Immediately 600 volts was applied to the ends of the paper for 90 minutes. The paper after drying was then sprayed with bromophenol blue. Most of the material stayed on the central starting line, but a faint yellow spot with streaking appeared at a distance of 10 cm on the anode side, showing no effective separation.

The remaining 1.23 g. of the oil was found to be only partly soluble in benzene, giving an insoluble fraction of 0.60 g., which was yellow powder. Both fractions were chromatographed in butanol - acetic acid - water (4:1:1 v/v). The benzene soluble fraction stayed on the starting line, while the insoluble fraction showed the same two close spots at  $R_f$ , 0.75 - 0.80, and the separation appeared clean cut.

At this stage, another 23.5 g. of the methylated periodate lignin was oxidized under similar conditions, yielding 1.65 g. of the same benzene insoluble fraction. An attempt was next made to purify the insoluble fraction by the formation of brucine salts. The procedure of Bell and Morgan<sup>(52)</sup>, used for the resolution of 1:1' dinaphthyl - 5:5' dicarboxylic acid was adopted. Figs. 12, 13 and 14 (pp. 65, 66 and 67 respectively) described the method and the analytical

details. The acidic yellow solution in Fig. 14, on chromatography in butanol - acetic acid - water, showed the two close spots at  $R_f$ , 0.75 - 0.80 of the original benzene insoluble fraction. Hence there was no effective separation of the two components.

The infra-red spectrum (Fig. 15, p. 68) of the powder was taken using potassium bromide pellet and showed sharp peaks, suggesting the material was homogeneous. The ultraviolet spectrum of the substance (0.02 g./litre of 95% ethanol) showed three new peaks at 218, 253 and 295  $m\mu$  (Fig. 16, p. 69). Schwaroff Laboratory, New York, provided the following analysis - Found: C, 56.5; H, 6.39; O, 37.11;  $OCH_3$ , 17.68; ash, 0.0%; mol. wt. 329. Calcd. for  $C_{12}H_{12}O_2(OCH_3)_2(COOH)_2$ ; mol. wt. 340: C, 56.47; H, 5.9; O, 37.77;  $OCH_3$ , 18.2%. The neutralization equivalent of the substance was found to be 158, 160. Calcd. neut. eq. 170. The nuclear magnetic resonance spectrum of the substance in dimethyl sulfoxide and carbon tetrachloride (1:1 v/v) failed to show any sharp peaks.

#### Oxidation of Methylated Formaldehyde Lignin with Neutral Potassium Permanganate

Five grams of formaldehyde lignin ( $OCH_3$  9.35%) was methylated with dimethyl sulfate and alkali to a constant methoxyl content of 13.1% in three methylations, using the procedure described before. Yield: 5 g.

Three grams of the methylated lignin was oxidized



with 22 g. of potassium permanganate near pH 7, the procedure adopted was also described previously. Paper chromatography of the oxidation products in the aqueous liquor after the extraction with ether revealed a product with  $R_f$  value = 0.00 in methylisobutyl ketone - formic acid - water (10:1:1 v/v), when sprayed with bromophenol blue. This  $R_f$  value might correspond to that of benzene hexacarboxylic acid.

Degradation in Alkali, followed by  
Methylation and Oxidation with  
Neutral Potassium Permanganate

The procedure adopted was that of Freudenberg<sup>(51)</sup>. Two preliminary experiments, with 5 g. each of periodate lignin and formaldehyde periodate lignin, were performed. The lignins were carefully mixed with a solution of 90 g. of potassium hydroxide in 45 ml of water contained in a 500 ml three-necked flask fitted with a reflux condenser and a mechanical stirrer. The solution was heated under reflux for 1.5 hours at 160°. After being cooled to room temperature, with dilution with 200 ml of water, the flask and contents were kept at 15° while dimethyl sulfate, 140 ml, was added drop by drop over a period of 1.5 hours. Stirring was continued for another 8 hours. After decomposing the excess of dimethyl sulfate by adding 5 g. of potassium hydroxide, the solution was stirred for 4 hours and acidified by dilute sulfuric acid to pH 1.0. The precipitated lignin was recovered on the centrifuge and was washed with water. The yellow acidic mother liquor was stored. The methylated

lignins were oxidized with potassium permanganate under neutral conditions, as described before. The degraded methylated, periodate lignin and formaldehyde lignin consumed 15 g. and 18 g. of permanganate, respectively. After working up the products of the oxidations as described previously, paper chromatography of the ether extracts gave the results described in Table XII (p. 74).

The acidic mother liquors (A) and (B) (Fig. 17, p. 73) from both periodate- and formaldehyde lignin were evaporated to semi-dryness, and acetone was added. Most of the potassium sulfate was removed from the yellow acetone solutions in six crystallizations. The acetone solutions were then chromatographed in a number of solvent systems, the most successful of which was butanol saturated with 2% aqueous ammoniumhydroxide. As shown in Table XIII (p. 76), the mother liquors (A) and (B) from periodate lignin gave long streaking with an elongated spot at the solvent front, but liquor (A) from formaldehyde lignin showed two distinct spots at  $R_f = 0.18$  and  $R_f = 0.30$ , and liquor (B) gave one clear spot at  $R_f = 0.18$ . Bromophenol blue, in ethanol neutralized to pH 7.0 was the spray. Another spray, a methanolic solution of Rhodamin B dye, followed by observation in an ultraviolet light, confirmed the above results. The last spray was used by Freudenberg<sup>(51)</sup> for the detection of methylated aromatic carboxylic acids.

Larger Scale Degradation of Formaldehyde  
Periodate Lignin

A larger-scale experiment with 20 g. of lignin was conducted, the flow chart being shown in Fig. 17. The mother liquors (A) and (B) showed the same chromatographic behaviour as encountered with the above smaller-scale experiment. A quantitative paper chromatographic separation of the two fractions present in liquor (A) and of the one in liquor (B), using butanol saturated with 2% aqueous ammonia was finally tried. A total of 160 Whatman No. 3 papers of size 8" x 22" were used for the separation A and 85 of the same size for liquor (B). Water was used for eluting the developed papers. The yields were 0.252 g. of  $R_f$ , 0.18 and 0.823 g. of  $R_f$ , 0.30 from the mother liquor A, and 0.031 g. of  $R_f$ , 0.18 from mother liquor B.

Although rechromatography indicated that the yellow, resinous, very hygroscopic products were homogeneous, they contained varying amounts of ash. All the fractions showed the presence of the carboxylic acid group and responded to the micro Nessler's test for the ammonium radical, and the ash contained sulfate. The fractions were again submitted to quantitative paper chromatography for further purification. The fraction with  $R_f$ , 0.18 amounted to only 0.029 g., which was not worked up further. The fraction with  $R_f$ , 0.30, which amounted to 0.300 g., was recovered free of ash, but was still a very hygroscopic, yellow powder soluble in water. The

infra-red and the ultraviolet spectra of the substance failed to reveal any indication of the structure. Found: C, 16.90; H, 6.17; O, 69.24; N, 7.69%.

Reactions of guaiacyl glycerol  
 $\beta$ -guaiacyl ether (XXXVIII)

(a) An oxidation of the substance was made in the micro-scale (50 mg) with alkaline nitrobenzene, following the procedure of Eisenbraun<sup>(9)</sup>. The paper chromatogram had spots of  $R_f$ , 0.44 and 0.10, in butanol saturated with 2% aqueous ammonia, and of  $R_f$ , 0.87 and 0.41, in butanol - pyridine - water (10:3:3 v/v) solvent systems. Standard samples of vanillin and vanillic acid were found to give similar  $R_f$  values when developed in these two solvent systems.

(b) A 2.5 g. sample of the ether was first soaked overnight in 5 g. of formalin (36.8%). Ten grams of 88.5% ice-cold sulfuric acid were then added very slowly with stirring, simultaneously with a little solid carbon dioxide so that the temperature did not go beyond 40 - 41°. The rest of the procedure was the same as that for the preparation of the formaldehyde lignin. The final acetone liquor was diluted with water, and evaporation of the acetone left an aqueous emulsion of pH 7.0. This emulsion was evaporated first on a flash evaporator at 35°, and was finally dried over phosphorous pentoxide in a desiccator under vacuum to a black tar. Yield, 2.8 g. Found: ash, 0.00;  $OCH_3$ , 12.1, 12.21%.

(c) A sample, 250 mg, of the above tar was oxidized with alkaline nitrobenzene, using the standard procedure. The paper chromatogram of the products failed to reveal any spots on the chromatograms, which were developed in butanol - 2% aqueous ammonium hydroxide or in butanol - pyridine - water (10:3:3 v/v) solvent system. Ferric chloride - ferricyanide and diazotised p-nitroaniline were the sprays.

(d) The procedures used for the oxidation with potassium permanganate both in alkaline and in neutral media were described previously in this thesis. One-gram samples were used and 10 - 11 g. of the permanganate were consumed in each case. The standard chromatographic procedure for the identification of benzene polycarboxylic acids as described by Eisenbraun<sup>(9)</sup>, using a methylisobutyl ketone - formic acid - water (10:1:1 v/v) system and a neutral bromophenol blue spray, was followed. The chromatograms showed only the presence of mellitic acid ( $R_f = 0.00$ ) in both cases.

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. Mild sulfonation with 1% aqueous sodium bisulfite near pH 5 at 98° for an hour separated the formaldehyde periodate lignin into an insoluble, non-dialysable fraction (80%) with a sulfur content of 0.8% and a soluble, dialysable fraction (20%) with a sulfur content of 5.2%. This confirmed the work of Jain, thus showing that the formaldehyde lignin was not homogeneous in nature. The failure of the major, insoluble fraction to become extensively sulfonated suggested that the sulfonatable positions in the original lignin had been substituted by formaldehyde residues.

2. An attempted degradation of the major fraction described in (1) by constant-boiling hydriodic acid at 50° for 8 hours had little effect. Since these conditions were adequate for the cleavage of dialkyl- and Benzyl aryl ethers, but not of aryl alkyl ethers, the first two types of ether link were probably of minor importance in the lignin structure. These results confirmed previous observations.

3. Of the 15% of dialysable material isolated from the treatment described in (2), about 9.7% travelled as a unit in paper chromatography with a  $R_F$ , 0.22 in butanol - 2% aqueous ammonia, and was identified as ammonium iodide. A previous opinion that this fraction was a complex phenol, although wrong, was understandable, because the extensive similarity in

behaviour between ammonium iodide and phenols in colour tests and chromatographic behaviour was not realized prior to the present research.

4. A reductive cleavage of any ether linkages, by sodium in liquid ammonia, was attempted by other workers on lignin sulfonates and on various lignins. When periodate lignin was submitted to this reaction, cleavage of ether occurred, as shown by extensive demethylation. About 20% of dihydro-eugenol and 0.26% of vanillic acid were formed. Failure to isolate any identifiable material from the formaldehyde lignin suggested that its structure contained additional carbon-carbon bonds, perhaps methylene linkages derived from the formaldehyde.

5. Failure to obtain vanillin or 6-carboxy vanillin from the oxidation of the formaldehyde lignin with nitrobenzene in alkali substantiated earlier work. An oxidation with cupric hydroxide in alkali, however, resulted in the isolation of a phenolic compound,  $C_{11}H_{15}O_7(OCH_3)$ , in about 1.3% yield. Although the dark resinous mobile substance could not be identified, its infra-red spectrum showed a strong carbonyl group frequency at  $1675\text{ cm}^{-1}$  and its ultra-violet spectrum the maxima at 210 and  $280\text{ m}\mu$  found in all lignins. This material was not obtained from the original periodate lignin.

6. In addition to (5), oxidation of the formaldehyde

lignin by alkaline cupric hydroxide also resulted in the isolation of 1.25% of bivanillyl, the formation of which could not be explained, since vanillin was absent.

7. The isolation of 0.9% and 2.5% of benzene hexa- and benzene penta-carboxylic acid from the formaldehyde lignin, and of 2.36% and 0.66% of benzene penta- and 1,2,4,5-tetra-carboxylic acid from the periodate lignin, by oxidation with potassium permanganate in alkali, substantiated previous work. It appeared probable that the precursor of the tetra-carboxylic acid in periodate lignin condensed with formaldehyde in such a way that it became the precursor of the hexa acid in the formaldehyde lignin.

8. A neutral potassium permanganate oxidation of the formaldehyde lignin resulted in the isolation of 0.25% of benzene hexa-carboxylic acid, suggesting that a new aromatic ring, fully substituted by carbon atoms, had been formed in the presence of the strong sulfuric acid. Periodate lignin under similar treatment failed to produce any benzene carboxylic acids.

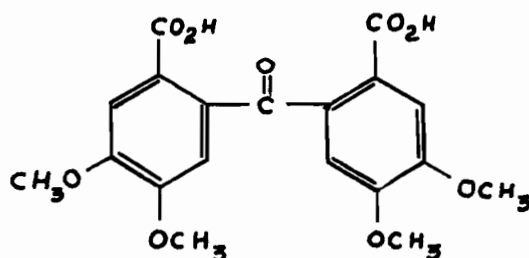
9. Failure to isolate veratric acid by the neutral permanganate oxidation of the completely methylated periodate lignin pointed to the absence of any free phenolic hydroxyl groups in the vanillin precursors it was known to contain. A new compound, however, with the molecular formula,  $C_{12}H_{12}O_2(OCH_3)_2(CO_2H)_2$ , was isolated in about 7% yield. This



yellow amorphous powder showed two close spots at  $R_f$ , 0.75 - 0.80 in butanol - acetic acid - water (4:1:1 v/v) and was at first thought to be a mixture of two acids. Extensive attempts at separation failed, and a sharp infra-red spectrum suggested that the product was homogeneous. The substance showed three peaks at 218, 253 and 295  $m\mu$  in its ultraviolet spectrum, unlike those of any lignin derivatives.

10. As expected, a neutral potassium permanganate oxidation of the completely methylated formaldehyde lignin showed the formation of benzene hexa-carboxylic acid, but the absence of metahemipinic acid suggested the absence of any free or etherified hydroxymethyl group at the 6-position of the benzene nuclei.

11. A drastic treatment of the formaldehyde lignin with caustic potash, followed by methylation and oxidation with neutral potassium permanganate, failed to produce any substances detectable by chromatography. This suggested that methylene linkages might unite more than two aromatic nuclei, since a compound like



could have been identified.

12. Guaiacyl glycerol- $\beta$ -guaiacyl ether was synthesised by standard methods and condensed with formaldehyde in the same way as that used for periodate lignin. The resulting black tar failed to produce any vanillin on oxidation, either with nitrobenzene or cupric hydroxide in alkali. A neutral potassium permanganate oxidation formed benzene hexacarboxylic acid, as revealed chromatographically. This result was important because it showed that a single phenylpropane unit of the lignin type could be cyclized in strong acid to yield the precursor of the mellitic acid.

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