ACTION OF REDUCING AGENTS ON A LIMIT HYPOCHLORITE OXYLIGNIN

by

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GENERAL INTRODUCTION

The present work should be considered as a part of a long term research program, initiated at the Pulp and Paper Research Institute of Canada in 1946 by the preparation of a suitable starting material, periodate lignin. The subsequent work consisted of the determination of the chemical characteristics of this material, improvements in its preparation and its oxidation with the technical bleaching agents, chlorine dioxide and sodium hypochlorite.

The stable end-product obtained in about 30% yield by the latter method, and termed oxylignin, has been subjected over the period of the last 8 years to an extensive study. In brief, the oxylignin has been shown to contain chlorine, and methoxyl, carboxyl, aliphatic hydroxyl, but no free phenolic groups, and perhaps a few quinone groups. Attempts to degrade the oxylignin by oxidative methods, and thereby throw light on its structure, gave discouraging results.

The present thesis describes the experiments on the lability of the chlorine substituent in the oxylignin, and on the probable presence of quinone groups. Attempts were made with many reducing agents to degrade the oxylignin to compounds of lower molecular weight, care being taken to use the mildest possible conditions and thereby avoid the autocondensation of the oxylignin.

The most promising agents found were 47% hydriodic acid at 50° and sodium in liquid ammonia. The work was supplemented by studying the action of alkaline hypochlorite and of 47% hydriodic acid on a small number of model compounds.

HISTORICAL INTRODUCTION

Action of Alkaline Hypochlorite on Lignins and Lignin Model Compounds

Aqueous solutions of chlorine are used extensively as bleaching agents for wood pulps, but the nature of the changes and the mechanism of the reactions remain largely unknown. The reactions of chlorine in aqueous solution, however, have been extensively discussed by McCarthy (1) and reviewed by Green (2). Only relevance to the present research justifies the inclusion of the following brief summary in this introduction.

When chlorine is dissolved in water, a reversible hydrolysis occurs:

$$Cl_2 + H_2O \rightleftharpoons H^+ + Cl^- + HOCl$$

The hypochlorous acid then ionises according to the following equation

$HOCl \rightleftharpoons H^+ - OCl^-$, the constant for this process being

$$K = \frac{(H^+)\ (OCl^-)}{(HOCl)} = 5.6 \times 10^{-8} \quad (3)$$

Hence, an aqueous chlorine solution contains three oxidants, namely free chlorine, hypochlorous acid and the hypochlorite ion, the relative amounts of which depend on the pH of the solution. McCarthy (1) and Hisey and Koon (4) calculated the above ratios and found that molecular chlorine was predominant below pH 2, hypochlorous acid between pH 2 and 7, and the hypochlorite ion above pH 7; thus substitution as well as oxidation might occur with aqueous chlorine, especially at low pH values.

Cross and Bevan (5) were the first to isolate a lignin containing about 27% chlorine by the action of chlorine gas on moist jute. Half of the chlorine used up was converted into hydrogen chloride, thus indicating that only substitution took place. Heuser and Sieber (6) concluded from their study of the chlorination of spruce wood pre-extracted with benzene-alcohol that oxidation was the main process, while Jonas (7), working with hydrochloric acid spruce lignin, claimed that both substitution and oxidation occured. Hagglund (8), on subjecting the same material to a similar treatment, obtained a mixture of highly chlorinated lignins (Cl about 40%); Rassow and Zickman's product (9) from hydrochloric acid spruce lignin contained, after extensive chlorination, about 38% of chlorine. Hibbert and Taylor (10) studied the action of hypochlorous acid on glycol lignin and on methylated glycol lignin under both acidic and basic conditions. They found that in all cases where the lignin was dissolved in alkali prior to the addition of hypochlorous acid, there was a sharp initial rise in the rate at which chlorine was consumed, followed by a much less rapid change. They concluded that the first fast reaction was due to chlorination of the phenolic material, while the almost horizontal section of the rate plot corresponded to a much slower oxidation process. Later, Hibbert and coworkers (11), by studies of the reaction of lignin with hypochlorite at different pH values, concluded that the initial rapid reaction consisted of chlorination and demethoxylation of the lignin, followed by a relatively slow oxidation.

The systematic study of the action of sodium hypochlorite solution on periodate lignin was initiated by Whittall (12), whose experiments, carried out over a range of pH values and with varying times of reaction, showed the presence of an initial fast reaction, similar to that observed by Hibbert (10), at all pH values. A slow reaction followed if the pH was kept above 11. Most of the periodate lignin dissolved readily in the first fast stage of the reaction and the product, termed oxylignin, could be precipitated from the solution by acidification to pH 2. Whittall found it was essential to destroy the excess of hypochlorite by the addition of acetone if a swift and uncontrollable chlorination was to be avoided. A comparison of yields and composition of the products obtained at different pH values showed that at pH 12 the yield of oxylignin was highest and its chlorine content lowest. Detailed examination of this material by methylations both with dimethyl sulphate and with diazomethane suggested that this oxylignin contained four methoxyl, two aliphatic hydroxyl, two carboxylic acid groups, two chlorine atoms, but no free phenolic groups. The determination of molecular weight and neutralisation equivalents indicated a molecular weight of about 1000.

The nature of the oxylignin was investigated in more detail by Brounstein (13) who confirmed Whittall's (12) tentative indication of the absence of free phenolic groups in this material, which had been exposed to the action of alkaline hypochlorite for 16 - 20 hours. Attempts to degrade the oxylignin molecule to fragments of lower molecular weight, using a variety of reagents, failed. The methylated oxylignin, however, was not stable to the further action of alkaline hypochlorite solution, which appeared to break down the original molecule into components of lower molecular weight, but the products were much more heavily chlorinated.

The oxidation liquors left over after the separation of the oxylignin were also studied by Brounstein (13), but in spite of considerable work, no identifiable compounds were isolated. The ether-soluble portion of the oxidation liquor was methylated twice with diazomethane and then distilled under high vacuum, but only 4.5% of the material passed over although the temperature reached 265°. The butanol-soluble portion of the oxidation liquor was examined by chromatography on a Magnesol column and three distinct fractions were collected.

The oxidation of periodate lignin with alkaline hypochlorite was studied next by Sacks (14), who used a somewhat different approach to this problem. He examined the UV spectrum of the oxylignin obtained by Brounstein (13) and noticed that the maximum at 280 $m\mu$, characteristic for untreated lignin, had almost disappeared, while the spectrum of the products recovered from the oxidation liquors was completely different from that given by the oxylignin.

Systematic study of the constituents of the aqueous liquors showed that they consisted mainly of aliphatic acids and some water soluble, highly coloured, acidic material which was not clearly defined. This material exhibited some properties characteristic of quinones. The oxidation of periodate lignin with sodium hypochlorite at pH 12 was then continued for 145 hours, and a new oxylignin, resistant to the further action of sodium hypochlorite, was obtained in about 33% yield. Both the new oxylignin and the residual liquor were examined, the former only to a limited extent. The new oxylignin was found to be a mixture of acids of molecular weight between 3000 and 450, fairly resistant to mild oxidants, while drastic oxidation degraded it completely to short chain aliphatic acids. The original oxidation liquors yielded products amounting to 60% of the original material, from which oxalic acid, maleic acid and a small quantity of glycollic acid was isolated. A chlorinated fatty acid, most probably α,α-dichloro-propionic acid, was also isolated. The rest of the material was separated as an amorphous brown solid of low methoxyl content, which failed to yield any identifiable products.

The interpretation of the results of oxidation of lignin by alkaline hypochlorite was facilitated by the investigation of the action of this oxidant on known organic compounds, particularly on those related structurally to lignin. The present review of the work done in this field will be limited to the experiments carried out on aromatic compounds and in alkaline solutions, because different products were formed in acidic media, as might be expected from the brief discussion of the chemistry of aqueous chlorine already given. The oxidation of the side chains of aromatic compounds by alkaline hypochlorite has been recognised for many years, and Vanarendonk and Cupery (15) obtained carboxylic acids, free of chlorine, in over 90% yield when acetophenone and its derivatives listed in Table I were oxidised.

Table I

Action of Hypochlorite on Acetophenone and its Derivatives

Compound	Yield (%) of the corresponding benzoic acid derivative
Acetophenone	85
4-Methoxy acetophenone	90
4-Chloro acetophenone	93
4-Methyl acetophenone	96
3-Methyl-4-methoxy acetophenone	92
2-Methyl-4-methoxy acetophenone	90

However, 3-Nitro acetophenone and p-hydroxy acetophenone did not yield the corresponding carboxylic acids, and no mention of products was given. It was very likely that chlorine entered the above compounds during the reaction. Smith (16) obtained a mixture of all three isomers of monochloro benzoic acid, when sodium benzoate was subjected to the action of alkaline hypochlorite.

Soper and Smith (17) found that simple monohydric phenols reacted with hypochlorous acid at a rate directly proportional to the hydroxyl ion concentration in the region of pH 7, but the rate became inversely proportional near pH 12. More recently, Hopkins and Chisholm (18) obtained nuclear chlorinated products by the action of sodium hypochlorite on various substituted benzoic acids, aryl acetic acids, aryl oxyacetic acids and aryl aldehydes; the positions of the entry of the chlorine atom and the yields are given in Table II.

Table II

Action of Sodium Hypochlorite on Aromatic Acids and Aldehydes

Starting material	Substitution product	Yield, %
Anisic acid	3-chloro	80
Vanillic acid	5-chloro	85
p-hydroxy benzoic acid	3,5-dichloro	50
m-methoxy benzoic acid	6-chloro	60
2-Hydroxy-4-acetoxy benzoic acid	5-chloro	40
Anisyl acetic acid	3-chloro	50
Vanillin	5-chloro	90
Piperonal	6-chloro	poor
2,4-Dihydroxy benzaldehyde	5-chloro	80
Phenoxyacetic acid	2,4-dichloro	75
2-Chlorophenoxy acetic acid	2,4-dichloro	80
2-Methylphenoxy acetic acid	4-chloro	85

Thorn and Purves (21) investigated the rate of reaction of sodium hypochlorite with vanillin and pyrogallol in the range pH 8 to pH 12 at 25°, and found that the rate was governed by the acidity of the solution, being slowest at pH 12. In all cases, the plots of moles of hypochlorite per mole of vanillin (or pyrogallol) against time showed a very rapid initial reaction, which was shown, by the isolation of 5-chloro vanillin and trichloro pyrogallol, to be mainly chlorination. The rapid reaction was followed by a considerably slower consumption of hypochlorite.

Richtzenhain and Alfredsson (19) (20) extended the scope of the investigation by studying the effect of alkaline hypochlorite on a number of phenols and phenol ethers believed to be structurally related to lignin, as well as on various lignin preparations. An increase of temperature from 20° to 95° increased the rate of the reaction by a factor of about 3, but the initial fast reaction, referred to previously, was present in all cases investigated. The methylation of lignins with diazomethane greatly reduced the rate of reaction which was further reduced if the lignin was fully methylated by diazomethane followed by dimethyl sulphate. This observation led the authors to the conclusion that free phenolic groups in the lignins investigated (sulphate lignin, hydrochloric acid lignin and lignosulphonic acid) were responsible for the initial rapid reaction with hypochlorite. To illustrate the effect of methylation, their figure 1 has been reproduced.

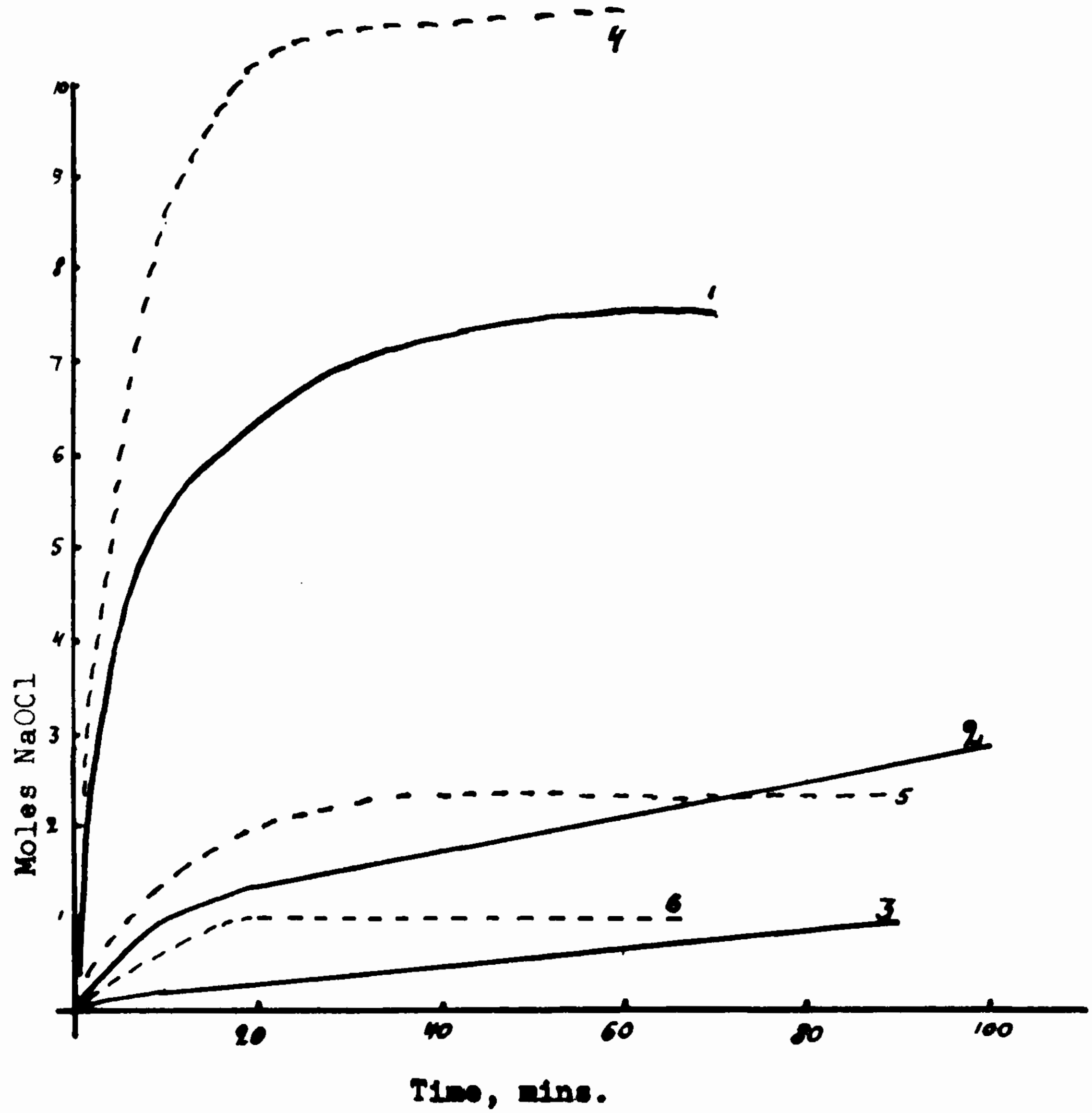


Figure 1

Rate of consumption of moles of sodium hypochlorite per molar equivalent of methoxy group in various lignins

Hydrochloric acid lignin, full lines ————
Plot 1 - not methylated. Plot 2 - methylated with diazomethane (OCH_3, 21.0%). Plot 3 - methylated with diazomethane and dimethyl sulphate (OCH_3, 29.0%).
Sulphate lignin, broken lines - - - - - - - - -
Plot 4 - not methylated. Plot 5 - methylated with diazomethane (OCH_3, 24.6%). Plot 6 - methylated with diazomethane and dimethyl sulphate (OCH_3, 26.4%).

A comparison of the behaviour of phenols and of phenol ethers, when subjected to the action of alkaline hypochlorite is illustrated in figure 2, also reproduced from Richtzenhain and Alfredsson (19).

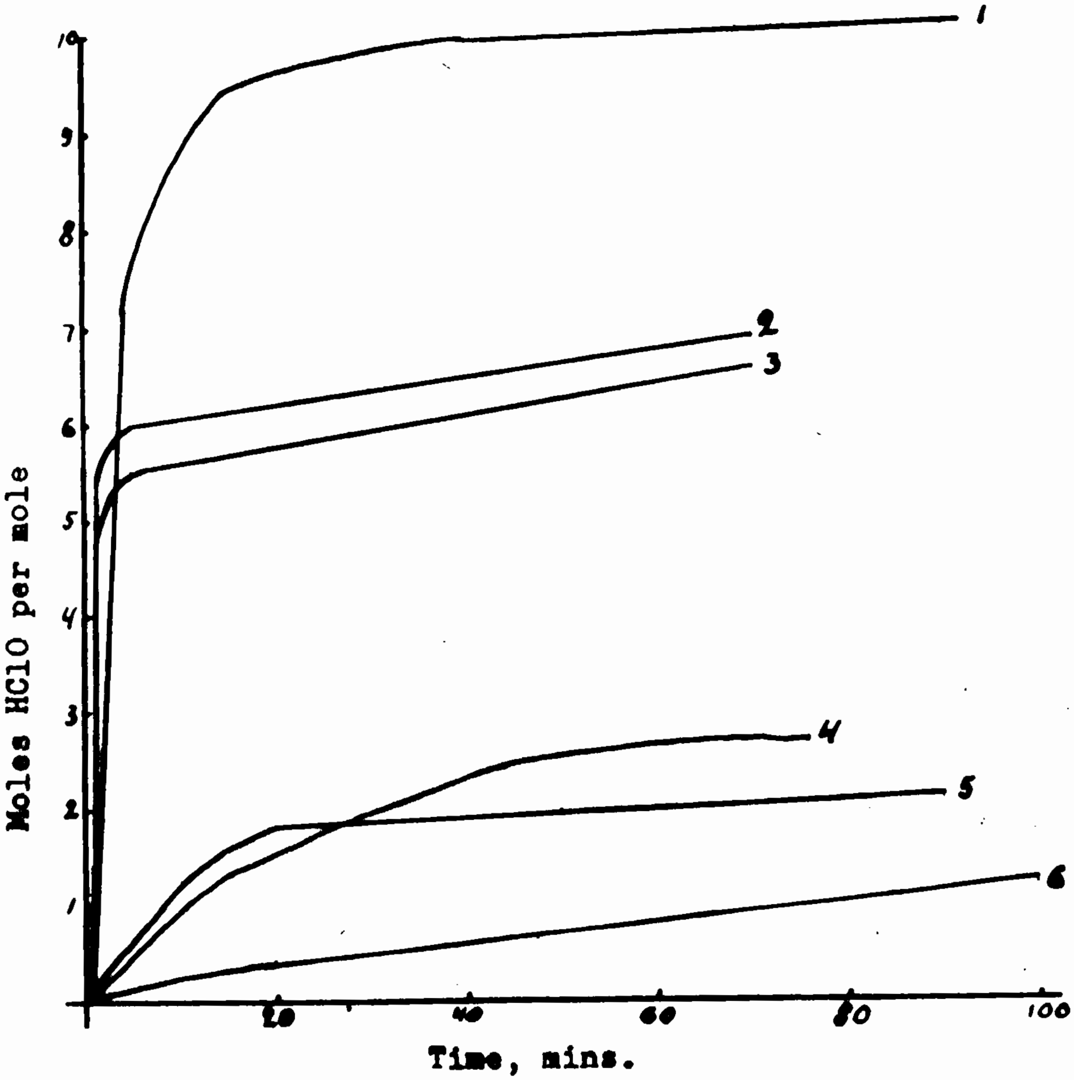


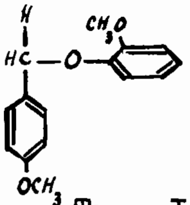
Figure 2

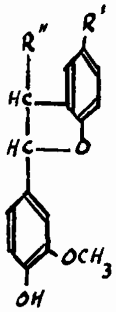
Consumption of hypochlorite by phenols and phenol ethers

Plot 1 - isoeugenol. Plot 2 - vanillic acid. Plot 3 - guaiacol. Plot 4 - isoeugenol methyl ether. Plot 5 - veratryl alcohol. Plot 6 - eugenol methyl ether.

The phenols (plots 1, 2 and 3) rapidly reacted with hypochlorite, while phenol ethers were much more resistant to this reagent. About 5 - 7 moles of hypochlorite was required for the cleavage of the aromatic phenolic nucleus (more if the phenol contained an oxidisable side chain, eg. isoeugenol) while phenol ethers consumed hypochlorite only in the amount required for the oxidation of the side chain. In contrast with phenols, phenol ethers were not chlorinated and no aromatic cleavage was observed. The similarity of the initial consumption of hypochlorite by lignins to that observed in the case of phenols is surprising, because the phenolic content of the former is much smaller than that of the latter. This anomaly could be explained either by the presence in lignin of some unknown groups, which were rapidly degraded by the hypochlorite, or by the attack of hypochlorite on phenolic ethers with the resulting cleavage of the ether bonds and subsequent formation of free phenols. The first possibility, in view of our limited knowledge of the constitution of lignin, is not amenable to a systematic study. To test the second possibility, Richtzenhain and Alfredsson (20) subjected a number of synthetic phenol ethers to the action of alkaline hypochlorite. The compounds investigated can be divided into three groups, according to the position of the ether linkage.

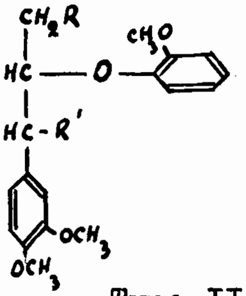
The first group, having the ether linkage attached to the first carbon atom of the side chain, was further subdivided into general type Ia and Ib.

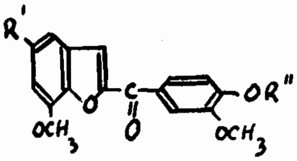

Type Ia


Type Ib

Compounds of type Ia were stable to hypochlorite even at 95°, while compounds of type Ib rapidly consumed hypochlorite with simultaneous introduction of chlorine into the phenolic portion. If R^1 was CH = CH-CH$_2$OH and R^{11} was CH$_2$OH, then both phenolic and phenol ether rings were degraded, thus supporting Freudenberg's (22) theory, that lignin consisted to an extent of 40 - 50% of such dehydrodiconiferyl alcohol units.

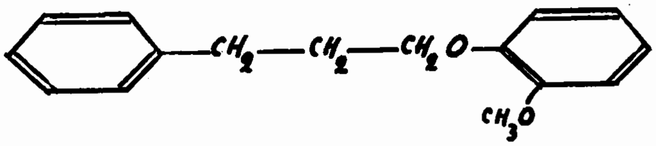
The second group of compounds, having the ether group joined to the β carbon atom of the side chain as shown below, were fairly rapidly attacked by the hypochlorite in spite of the absence of free phenolic groups and yielded veratric acid as the degradation product.


Type IIa


Type IIb

Thus it is possible that lignin contains some groupings of this type in combination with the dehydrodiconiferyl alcohol groups mentioned above. Freudenberg (23) suggested that about 15% of the lignin could be linked through β carbon atom. Adler and Lindgren (24) attached great importance to structure IIa where both R and R^1 were hydroxyl groups, as this formulation explained the sulphonation and alcoholysis of lignin. Compounds of type IIb were stable to hypochlorite, provided that R^{11} was not hydrogen.

The third group (III), linked through an ether bond attached to the γ-carbon atom of the side chain, was found to be stable to hypochlorite, thus throwing considerable doubt on the mechanism of the action of hypochlorite on lignin suggested by Hibbert and coworkers (11).



III

Just recently, Sutherland (25) studied the action of alkaline hypochlorite on phenolic substances, which are given in Table III together with the products.

Table III

Action of Alkaline Hypochlorite on Phenolic Substances at pH 11-12 and 25°

Compound	Products	Yield, %
p-hydroxy benzaldehyde	3-chloro and	27
	3,5-dichloro derivatives	67
Anisaldehyde	3-chloro anisic acid	4
3-Hydroxy-1-(3,4 dimethoxy phenyl)-2-propanone	Not identified Consumption of hypochlorite observed	
p-hydroxy propiophenone	Mpt. 116-117° Not identified	
Propioveratrone	Veratric acid	19

Action of Alkaline Earth Metals in Anhydrous Ammonia on Lignin and Related Compounds

Solutions of the alkali metals in liquid ammonia were first investigated by Weyl (26) who, according to Johnson and Meyer (27), observed that sodium and potassium were soluble in this medium and produced highly coloured solutions. He considered, in line with the theories prevailing at the time of his work, that the metals were joined to the nitrogen of ammonia, but no quantitative data were presented to support this statement. Seely (28) (29) (30) contended that the metals dissolved in ammonia as such and did not form any compounds with it.

The behaviour of alkali metals dissolved in liquid ammonia was studied systematically and in great detail by Kraus (31) (32) (33) (34) (35) (36), who showed that an ionic equilibrium existed between positively charged metallic ions and the negatively charged carrier, which was identical for all metals and which exhibited abnormal conductive power in more concentrated solution. From studies of conductivity, Kraus was able to conclude that a solution of an alkali metal in liquid ammonia formed the connecting link between electrolytic and metallic conductors, and that the process of conduction was effected by a negatively charged carrier of sub-atomic dimensions, which he identified with an electron. Thus, in concentrated solution, one might picture the metal atoms dissociating in accordance with the equation

$$M \rightleftharpoons M^+ + \epsilon^-$$

The electrons had much the same mobility which they had in the solid, so that the solution possessed a very high conductance. When the solution was diluted, the above dissociation was accompanied by a second reaction, namely the association of ammonia molecules with the electrons

$$\epsilon^- + xNH_3 \rightleftharpoons \epsilon^-(NH_3)x$$

and the mobility of the ammonated electron was much smaller than that of free electrons. As this process became more dominant upon dilution, more of the electrons became associated with ammonia and the conduction acquired properties characteristic of electrolytes. With the above picture of the nature of metal solutions in mind, one may logically expect that the chemical reactions exhibited by these solutions will be of two types: first, ionic double decompositions and second, reductions; many examples of both types are known. Excellent reviews of the action of sodium in liquid ammonia on a wide variety of both organic and inorganic compounds are available (37) (38) (39) and the present account will be restricted to organic reductions of interest in the lignin field. Numerous workers (40) in the lignin field postulated the guaiacylpropane unit as a building stone of the lignin molecule. The exact mode of linkage between the monomers is the crucial, unsolved problem of lignin chemistry which, in spite of years of extensive studies, still awaits its solution. Overwhelming evidence points, however, to the presence of some kind of an ether bond on one of the carbon atoms of the side chain, as a major link between the monomers. Thus the cleavage of such an ether bond and its replacement by a hydroxyl group under conditions which would retain the 3-carbon side chain, and leave the other parts of the molecule intact, would reveal the exact position of the ether link and greatly facilitate the further elucidation of the structure.

The action of sodium in liquid ammonia on ethers was first investigated by Kraus (41) who claimed that none occured. This statement was found by Sowa (42) to be true only for aliphatic ethers, as a series of diphenyl ether derivatives of the type ROR^1 were cleaved yielding phenols ROH and R^1OH. The same author found that the linkage between the oxygen and the substituted phenyl group R or R^1 was strengthened against cleavage by the following substituents, listed according to their increasing effectiveness: o-CH_3, p-CH_3, p-OCH_3, o-NH_2, p-NH_2. The ether bond was weakened by the following groups, similarly listed: o-CH_3, o-COONa, p-COONa. Similar series were derived for meta substituents; in this case m-CH_3 and m-NH_2 strengthened the linkage between oxygen and substituted phenyl group (43) against cleavage, while m-OCH_3 and m-COONa had the opposite effect. The same author (44) also examined series of $4,4^1$-disubstituted diphenyl ethers and found that the ether linkage is strengthened against cleavage by p-CH_3, p-$C(CH_3)_3$ and p-NH_2, listed according to increasing effectiveness.

A considerable amount of work on the action of sodium in liquid ammonia was carried out by Birch (45) (46) (47) (48) (49) (50) using alcohol as the solvent. It was very soon realised (45) however, that sodium in liquid ammonia, in presence or absence of available hydrogen from alcohols, showed very different reducing properties. The former combination was capable of reducing sodium naphthoxides, methoxy-alkyl benzenes and alkyl benzenes to the α,δ dihydro compounds. Since, as discussed previously, sodium in liquid ammonia behaved as a solution of metal cations and ammonated electrons, it might be expected to reduce by electron addition, but the presence of such a ready proton source as alcohol rendered reduction by nascent hydrogen a possibility. Since in alcohol solution the products of reduction were different, it was almost certain that the mechanism was not identical with that operating in liquid ammonia alone; consequently the presence of alcohol in these reductions involved not only its action as a solvent but also its active participation in the reduction process. For this reason, subsequent discussion will be limited to the reactions carried out in a truly inert solvent (e.g. aliphatic ethers) or directly in liquid ammonia.

Table IV illustates the results of Birch (48), concerning the action of sodium in liquid ammonia on a variety of ethers.

Table IV

Action of Sodium in Liquid Ammonia on Ethers.

Compound	Product	Yield, %
Anisole	Phenol	27
o-tolyl methyl ether	o-cresol	17
m-tolyl methyl ether	m-cresol	9
p-tolyl methyl ether	p-cresol	4
Veratrole	Guaiacol	89
Resorcinol dimethyl ether	Resorcinol monomethyl ether	71
4-Methyl veratrole	3-hydroxy-4-methoxy toluene	78
Guaiacol n-propyl ether	Catechol n-propyl ether	42
Guaiacol iso-propyl ether	Catechol iso-propyl ether	Not given
Guaiacol benzyl ether	Guaiacol) Toluene)	25 5
2-Methoxy phenoxy acetic acid	Guaiacol	40

The action of alkali metals dissolved in liquid ammonia on lignin was examined for the first time by Freudenberg and coworkers (51); cupraammonium (cuoxam) lignin was shaken for 24 hours with potassium in liquid ammonia, using a specially reinforced steel bomb maintained at 20°. The products were completely soluble in alkali, from which they could be precipitated by carbon dioxide. Some methoxyl groups were lost after the first treatment, but subsequent treatments failed to lower the methoxyl content further. This partial demethylation was accompanied by a cleavage of other ether bonds, as the resulting lignin could be methylated to a higher degree than the untreated material. Sodium in liquid ammonia was apparently less reactive than potassium, although similar in action toward lignin.

Further experiments, using a solution of potassium in liquid ammonia, under pressure at room temperature, were carried out by Freudenberg (52), who examined the effect of this reagent on spruce wood, beech wood, cuoxam lignin, as well as on some phenol ethers.

A single treatment of wood meal with potassium in liquid ammonia resulted in two main fractions, which were separated into methanol-soluble and methanol-insoluble portions; the former yielded 16 - 18% of material originating exclusively from lignin and containing 12.6% of hydroxyl groups, which was considerably more than normally encountered in lignin. The methanol-insoluble fraction, amounting to 77.4% of the original material, contained cellulose including 4.8% of pentosans.

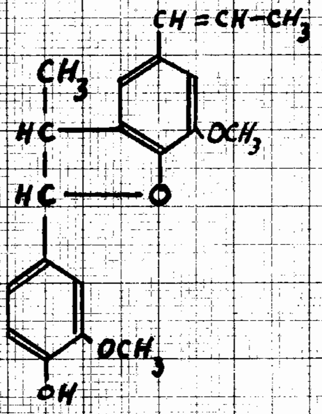
When cuoxam lignin was subjected to the same treatment, all the lignin component of the cuoxam lignin (which apparently contained some polysaccharides) was rendered soluble in ammonia and methanol. The methoxyl content of the products was unchanged, but the phenolic hydroxyl content, after two potassium-liquid ammonia treatments, reached 15.8%, thus indicating that many ether linkages other than methoxyl had been split. Methylation of the products followed by oxidation with permanganate yielded 5% of veratric acid, 3 - 4% of isohemipinic acid and 4% of dehydrodiveratric acid.

The action of potassium in liquid ammonia on phenols and phenol ethers revealed the high degree of specificity of this reagent. The compounds investigated and the products of the reaction are summarised in Table V.

Table V

Action of Potassium in Liquid Ammonia on Phenols, Ethers and Phenol Ethers

Compound	Product
Anisole	Phenol
Veratrole	Guaiacol and catechol
Propylveratrole	4-methoxy-3-hydroxy propylbenzene
Dihydroeugenol	Recovered unchanged
Vanillin	Vanillic alcohol
Vanillic acid	Recovered unchanged
Dehydrodiisoeugenol (Compound IV (page 21)	Compound V (page 21)
Dehydrodiisoeugenol Methyl ether	Compound V (page 21)
Egonol Compound VI (page 21)	Compound VII (page 21)



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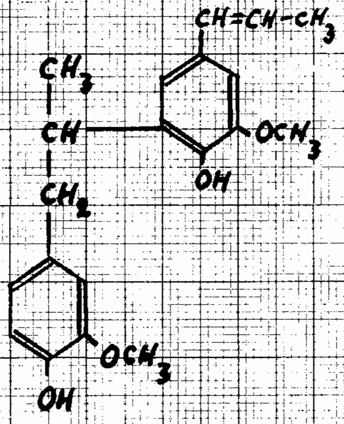

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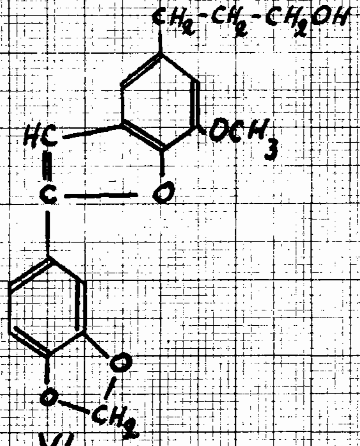

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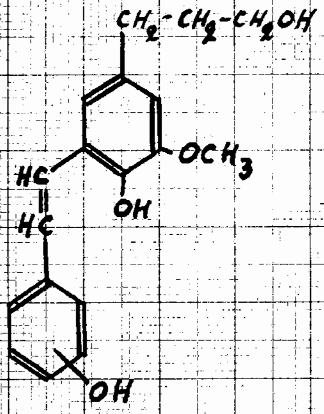

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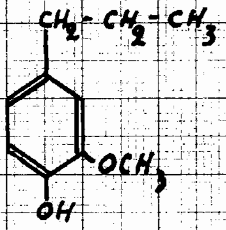

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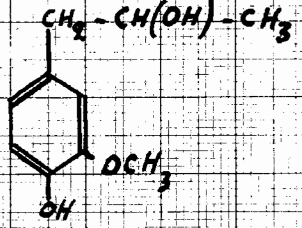

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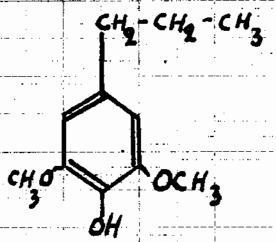

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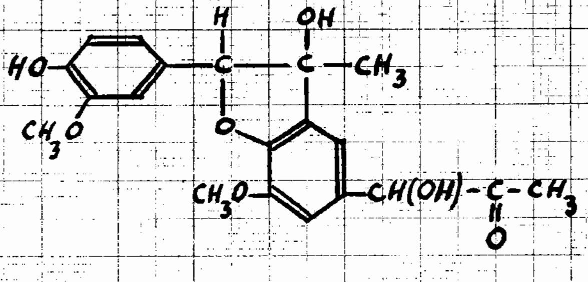
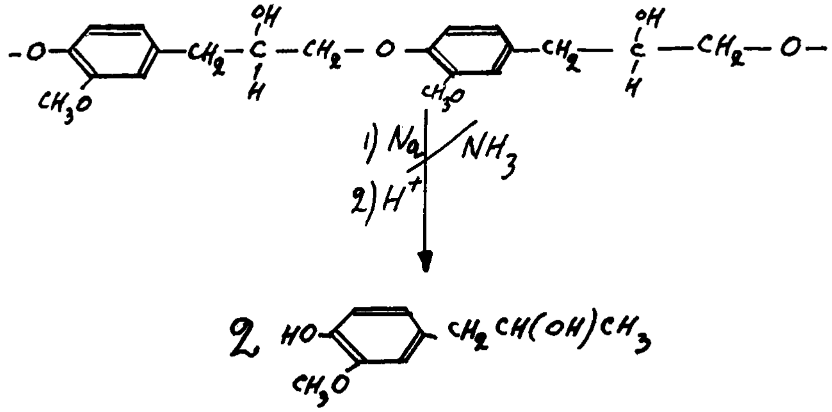


XI

Lignin was found by Shorygina and coworkers (53) to undergo decomposition by repeated treatment with sodium in liquid ammonia at atmospheric pressure and -33°. The products were found to be a mixture of ether-soluble compounds (8%), acids and phenols (19%), high molecular weight compounds resulting from incomplete reaction (11%) and water-soluble material (50%). No change in the elementary composition of cuoxam lignin was observed (54), when it was analysed for carbon and hydrogen after each of nine sodium in liquid ammonia cycles while the methoxyl and hydroxyl content reached a constant value after the first cycle. The average molecular weight of the products decreased gradually, thus indicating a degradation from the original D.P. of 25 to 17. (Molecular weight of the elementary unit was taken as 180.) The alkaline solution of the products (55) was extracted with ether, yielding a mixture (8.4% by weight of the original material) of two compounds, one of which was identified as dihydroeugenol (VIII) while the other was a hydrocarbon, probably $C_{18}H_{34}$. The above solution, when acidified and extracted with ether, yielded 3.6% of 1-(4-hydroxy-3-methoxy phenyl) propanol-2 (IX) (56). Similar treatment (57) of aspen cuoxam lignin yielded about 2% of dihydroeugenol (VIII) and about 2.3% of an oil, identified as 1-(4-hydroxy-3,5-dimethoxy phenyl) propane (X), while fir wood (58) gave 1.23% of dihydroeugenol (VIII) and 6.3% of an oil which was not identified.

Although the yields of the products were very small, the authors (59) drew rather definite conclusions regarding the mechanism of the reaction and the location of the ether bond in lignin. The formation of the phenolic hydroxyl group, observed in all the products, was attributed to the cleavage of an ether bond by sodium in liquid ammonia, while the aliphatic hydroxyl in (IX) was, according to the authors, present in the original lignin. The formation of (VIII) and (X) was explained by the hydrogenation of (IX) by sodium in liquid ammonia; to verify the above statement, coniferyl alcohol was subjected to the action of sodium in liquid ammonia and yielded 86% of dihydroeugenol (VIII) (55). Hence this reagent was capable of saturating the double bond and reducing the primary alcohol group. Unfortunately, no evidence was given for the alleged replacement of secondary hydroxyl groups as in (IX) by hydrogen, using sodium in liquid ammonia as the reducing agent.

On the basis of the above interpretation, Shorygina and coworkers (59) postulated the following mechanism for the scission of lignin by sodium in liquid ammonia, thus supporting Hibbert's theory (60) that β-hydroxy coniferyl alcohol was the basic structural link in lignin.



The formation of the monomeric products discussed above was, according to Shorygina, contrary to Freudenberg's suggestion, that lignin was based on a benzofuran structure (XI), because this carbon skeleton would have been unattacked.

Action of Hydriodic Acid on Lignin and Oxidised Lignin

The first recorded use of hydriodic acid under controlled conditions of temperature and concentration was that of Irvine and Hynd (61), who obtained D-glucose from tetramethyl-D-glucose by heating the latter under reflux with 45% hydriodic acid at 95° for 10 hours. In the field of lignin, hardly any experiments with hydriodic acid were recorded in the literature. Willstätter and Kalb (62) studied the action of constant-boiling hydriodic acid (sp.gr. 1.96) and red phosphorus on hydrochloric acid lignin. The mixture was heated under reflux for 10 hours (b.p. 127°), yielding a soft, brown resin which, after extraction with alcohol, gave a product containing 14.8%

of iodine, half of which could be split off by boiling with zinc in acetic acid. The partially dehalogenated material was not uniform and seemed to consist principally of two products exhibiting slightly acidic properties. The same authors repeated the reaction in sealed tubes maintained at 250° for 4 - 5 hours. The products were washed with water and then extracted with ether, the aqueous residue (23% by weight) not being further investigated. The ethereal extract was treated with sodium to split off the combined iodine, and at the same time to convert the acidic compounds into sodium salts. After being separated, the sodium crusts were combined with the remainder of the acid compounds, which were extracted from the ether solution by dilute sodium hydroxide. The combined alkaline extracts were acidified and extracted with ether, giving a resin which was not examined in detail. The mixture remaining in the original ether extract was isolated as a viscous oil, which was separated into an acetone-soluble, liquid hydrocarbon fraction, and a solid fraction, only slightly soluble in acetone. The liquid hydrocarbons were fractionally distilled under reduced pressure and yielded seven fractions, differing in elementary composition and molecular weight (range 166 - 350). The solid fraction, referred to above, had a molecular weight of 720 - 840 and appeared stable to the repeated action of hydriodic acid and red phosphorus.

At the time of the work just described, a close structural relationship between lignin and carbohydrates was believed to exist. To confirm this hypothesis, the above authors subjected mannitol, glucose, xylose, cellulose and a humus-like substance, prepared from glucose, to the same reaction, and found that these compounds gave a hydrocarbon mixture very similar to that obtained from lignin, although in lower yield. On this basis they concluded that the hydrocarbons were polycyclic hydrogenated ring compounds originating from furane derivatives formed as intermediates, which condensed to form polycyclic material.

MacDougall and DeLong (63) studied the effect of constant-boiling hydriodic acid on the amount and nature of lignin isolable from young plants containing considerable amounts of protein. This reagent was found to give a much larger residue of "lignin" when compared to Klason method using 72% sulphuric acid (64).

The action of 45% hydriodic acid on oxylignin was studied by Brounstein (13), who allowed the reaction to proceed in a carbon dioxide atmosphere at room temperature for 18 hours and then increased the temperature to 50°. After 4.5 hours at this temperature, when a total of 5.4% of methoxyl group had been evolved as methyl iodide, the reaction was stopped. The resulting mixture, after dilution with water, neutralisation with sodium bicarbonate and filtration, gave an 85% yield of an insoluble precipitate, while the filtrate, when extracted with ether, yielded 7.6% of crystalline needles, melting point 128 - 129°, which were not further investigated.

The action of several other reducing agents on the oxylignin was investigated during the research now to be described. Since these agents were well-known, and the results they gave with oxylignin were indecisive, a detailed description of their general chemical behaviour seems unnecessary.

DISCUSSION OF RESULTS

Behaviour of Wood During Oxidation with Aqueous Periodate

The periodate lignin was prepared by the process of Ritchie and Purves (65) as modified by Sacks (14) who used cold, dilute sodium hydroxide to decompose the oxycellulose instead of boiling water. Several cycles of oxidation-hydrolysis were necessary. The product was believed to have undergone only an insignificant amount of self-condensation, as no elevated temperatures or strong acids or bases were employed in its preparation. Furthermore, the time required for the preparation was shortened: less than three months of intermittent work being sufficient to prepare about 400g of periodate lignin free of any appreciable amount of holocellulose. The loss of methoxyl groups of lignin, encountered during its isolation by the above method, was undoubtedly a disturbing factor. As seen from figure 3 the decrease of methoxyl content was rapid during the first two cycles of oxidation and hydrolysis, but later only a slow and gradual change occurred. A possible explanation of this behaviour could be based on the experiments of Adler and Hernestam (66) who subjected a number of compounds containing one or more guaiacyl nuclei to the action of periodic acid and found that under these conditions the aromatic nucleus was split with the formation of unsaturated acids and methanol; the latter was liberated in a yield of 0.9 mole per mole of guaiacol.

Recently, Japanese workers (67) subjected a variety of lignins to the action of periodic acid and observed a considerable consumption of the oxidant; methylation of the lignins with methanol-hydrogen chloride gas, or with diazomethane, reduced the consumption of periodic acid by about one-half, while if the lignins were fully methylated with dimethyl sulphate, no reaction occurred. The same authors (68) also studied the behaviour of lignin model compounds and of lignin derivatives toward the same reagent. They observed that guaiacyl compounds containing free hydroxyl groups were readily oxidised with a simultaneous partial loss of methoxyl groups. From the results with lignin derivatives, they postulated the presence of α-β glycols and hydroxy ketone groups in the side chain of the samples investigated. Unfortunately, the present author was unable to obtain a translation of the original papers and for that reason no detailed discussion is possible.

On the basis of the work described above, it would be logical to suggest that the lignin originally present in wood possessed a few free phenolic groups, the oxidation of which caused the breakdown of a few methoxyl groups to methanol in the initial stages of the preparation.

Lange's work (69) on UV spectra of lignin revealed

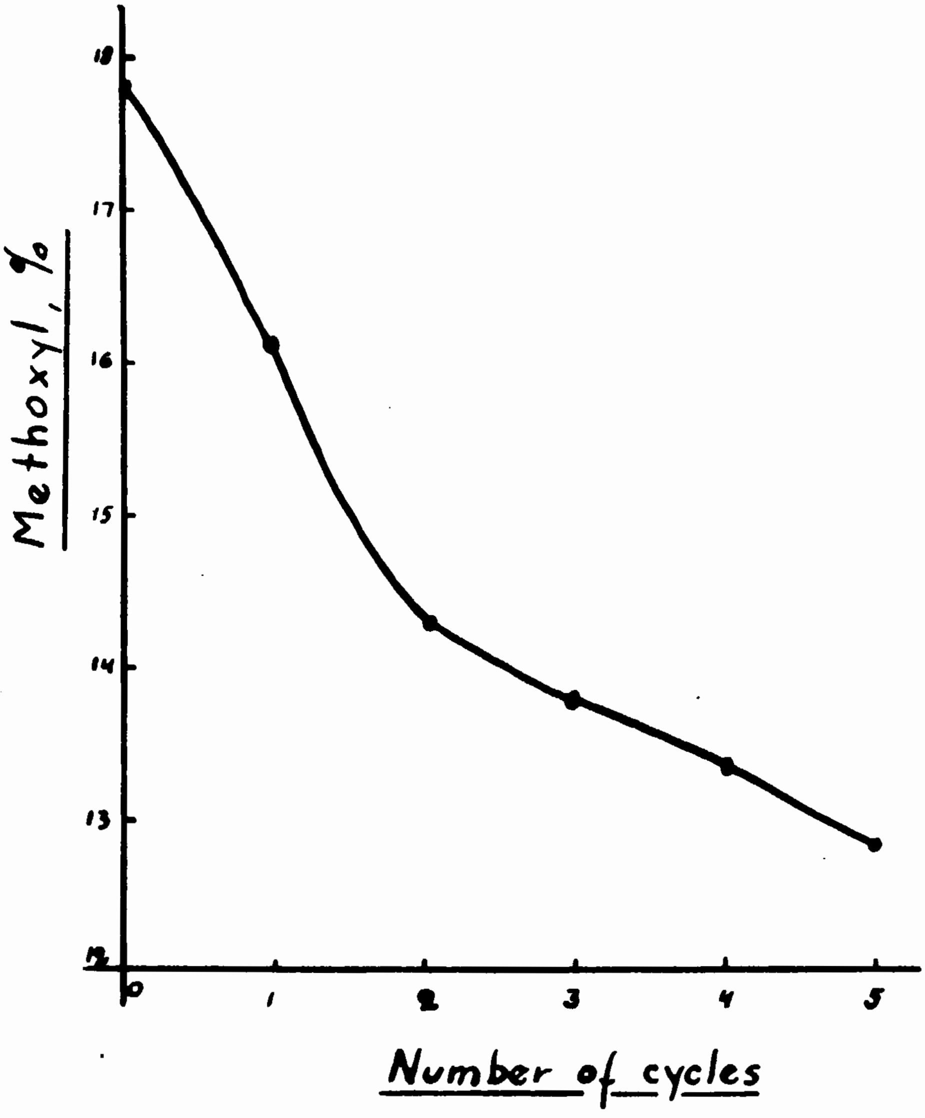


Fig. 3- Loss of methoxyl groups on periodate oxidation.

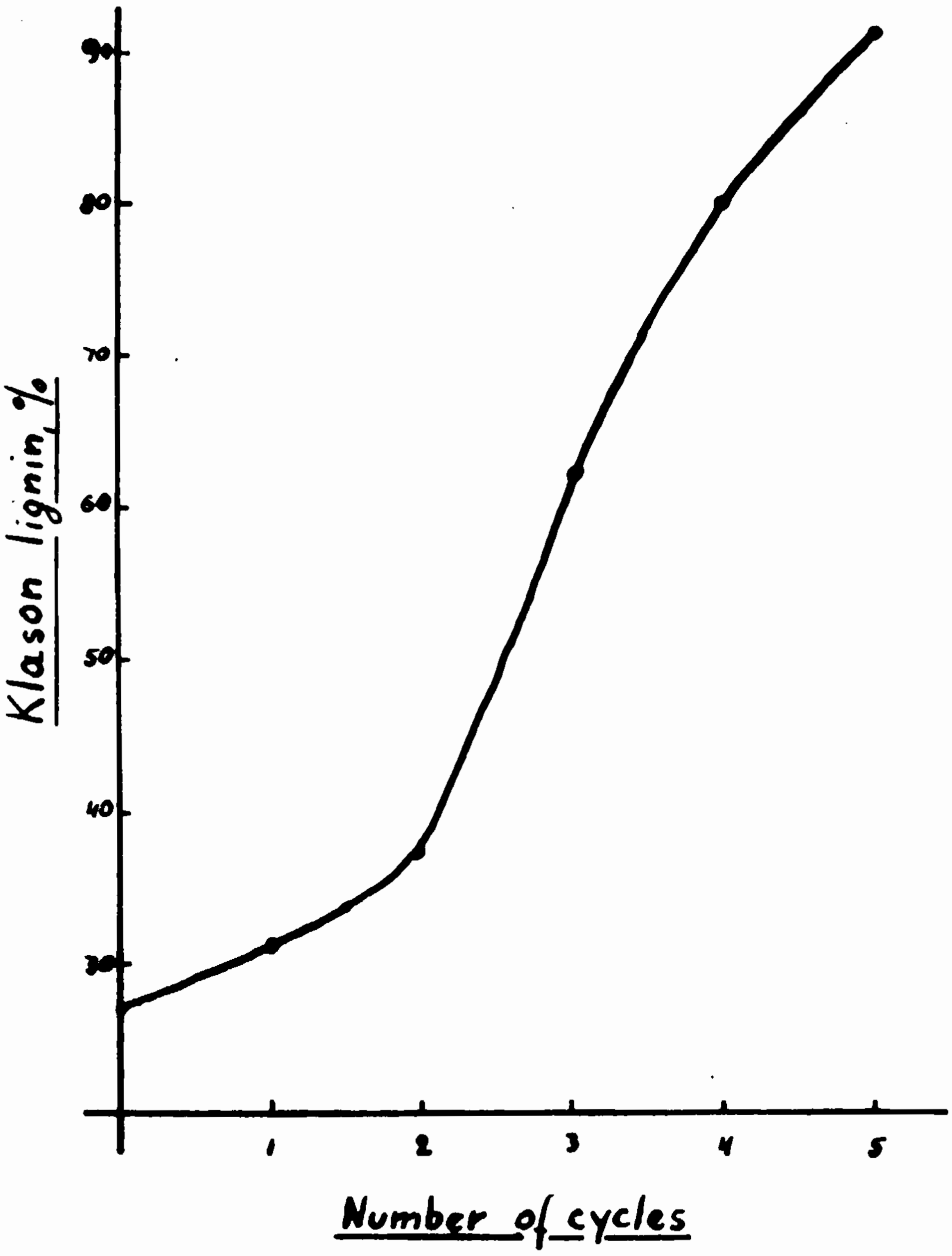


Fig. 4 - Increase in the Klason lignin content during preparation of the periodate lignin.

few, if any, free phenolic groups. Alternatively, a few phenolic groups might be combined with carbohydrate or other units and be liberated when such units were oxidised with periodate.

The oxidation by periodate of the bulk of the carbohydrate material was, however, rather slow as shown by the relatively small increase of lignin content after two cycles (figure 4). The analytical results at the end of the third cycle showed a rapid increase in the lignin content and a small decrease in the methoxyl value, thus indicating that at this stage the oxidation of phenolic material was largely completed and the predominant reaction was one of oxidation of the carbohydrate portion of the wood. Also, if lignin were further degraded by the periodate solution, then the slope in the final cycles (figure 3) should be much steeper because at these later stages the lignin was most exposed to the action of the periodate solution. The absence of any appreciable amount of free phenolic groups in periodate lignin was confirmed by the methylation studies of Cabott (70) and the author. Brickman (71) observed only a slight increase in the methoxyl content when the periodate lignin was exposed to the action of diazomethane for 24 hours, but repeated treatments with this reagent increased the methoxyl content by about 4%. Sacks (14) found a small increase in the methoxyl value of periodate lignin when it was subjected to the identical treatment; he also subjected the methylated product to the action of sodium hypochlorite solution under conditions that were found to dissolve all of the original periodate lignin. Only 74% of the methylated sample dissolved. The insoluble residue perhaps was a nitrogen-free polymer always formed (72) on the decomposition of diazomethane and for that reason the methoxyl values obtained by exposing lignin to the action of diazomethane over an extended period of time should be treated with extreme caution.

Spencer (73) found that lignins methylated with diazomethane contained small amount of nitrogen; this was interpreted as evidence that lignins contained a coumarin type of lactone linkage. Thus a small change in methoxyl value resulting from the reaction of lignin with diazomethane does not always represent the true extent of methylation, as the formation of diazo polymer and the possible reaction of diazomethane with other groups in lignin, referred to above, could cause an appreciable error.

The Lability of the Chlorine in the Oxylignin

The oxidation of the periodate lignin with sodium hypochlorite at pH 12 was reported by Brounstein (13) and by Sacks (14) to be accompanied by the introduction of chlorine. This process was studied in greater detail during the present research. The crude oxylignins were reprecipitated from acetone solution by the addition of a large quantity of water until their ash content was zero or almost zero. Table VI shows that the chlorination was completed in a very small fraction of the total time of reaction. Since the overall reaction was chlorination and oxidation, the first fast chlorination reaction was accompanied by a much slower oxidation process.

Table VI

Introduction of Chlorine during Hypochlorite Oxidation of Periodate Lignin

Time, hours	Chlorine %	Time, hours	Chlorine %
0	0	48	5.15, 5.20
3	5.10, 4.95	72	5.25, 5.20
12	5.33, 5.69	96	5.18, 5.27
24	5.20, 5.35	120	5.15
36	5.25, 5.37	144	5.30

To determine the lability of the chlorine, advantage was taken of the fact that aliphatic chloride could be replaced by a cyanide group under conditions in which aromatic chloride was inert. Therefore the chlorine remaining in the oxylignin after the treatment with potassium cyanide must have been aromatic in its stability. This nitrogen containing product was termed cyanolignin. Hydrolysis of the product with alkali, followed by analysis for chlorine, served as a check of the "aromatic" chlorine content and hence the approximate distribution of chlorine could be calculated. It was found that the original oxylignin, with Cl, 5.25%, yielded a cyanolignin, N, 1.16%, and that saponification of the latter gave a product with Cl, 2.42%. Although the changes in the base molecular weight of the three lignin derivatives were unknown, they were probably too small to invalidate the following estimates. The nitrogen content, 1.16%, corresponded to $(1.16x35.5)/_{14}$ or 2.9% of chlorine. Hence 55% of the original chlorine was replaceable by cyanide and was "aliphatic", while 2.4%, or 46%, was stable to cyanide and also to alkali, and was therefore "aromatic". A similar investigation by N. S. Thompson (74) concerned the 17% of chlorine introduced into periodate lignin by chlorine dioxide. He used silver nitrate as the precipitating agent, and found the ratio of "aliphatic" to "aromatic" chloride to be 10:7.

An attempt was made to determine the position of chlorine on the aromatic nucleus of the oxylignin molecule. The most promising approach appeared to be the oxidation of the oxylignin with alkaline nitrobenzene. This treatment, when applied to lignin (75) or lignosulphonic acids (76), yielded vanillin, while the halogenated lignins gave rise to halogen substituted vanillins. Thus 6-chlorovanillin was isolated by Jayne (77) from lignin oxidised with sodium chlorite solution, and 5-iodovanillin was separated by Lautsch and Piazolo (76) from cuoxam spruce iodolignin using the nitrobenzene oxidation. To obtain the aromatic nucleus in optimum yield, the oxylignin and nitrobenzene were heated in sodium hydroxide solution under pressure at 175° (78) for 2 hours; these drastic conditions certainly hydrolysed all of the aliphatic chlorides and even the aromatic chlorides could possibly be affected. Purves and Brickman (79) proved that the conditions were drastic enough to split an alkyl-aryl ether bond, as they obtained vanillin from veratraldehyde in about 30% yield. The oxylignin, when subjected to the alkaline nitrobenzene oxidation, yielded only vanillin and vanillic acid; chlorine containing compounds were present only in very small amounts and could not be isolated. It was of interest to note that an alkaline nitrobenzene oxidation of periodate lignin previously oxidised by chlorine dioxide did not yield any vanillin or other aldehyde (74). Thus alkaline hypochlorite and chlorine dioxide must operate through different mechanisms. The industry, indeed, acts as if these two bleaching agents supplemented each other, for in the production of high purity dissolving pulps the bleaching process often consists of alkaline hypochlorite followed by a chlorine dioxide stage, the combination being found more satisfactory than either oxidant by itself.

Tests for Functional Groups and Fractionation of the Oxylignin

The UV spectra of an 0.1% solution of the oxylignin in methanol were observed in order to check the chemical similarity of the oxylignin prepared by W. Sacks (14) with

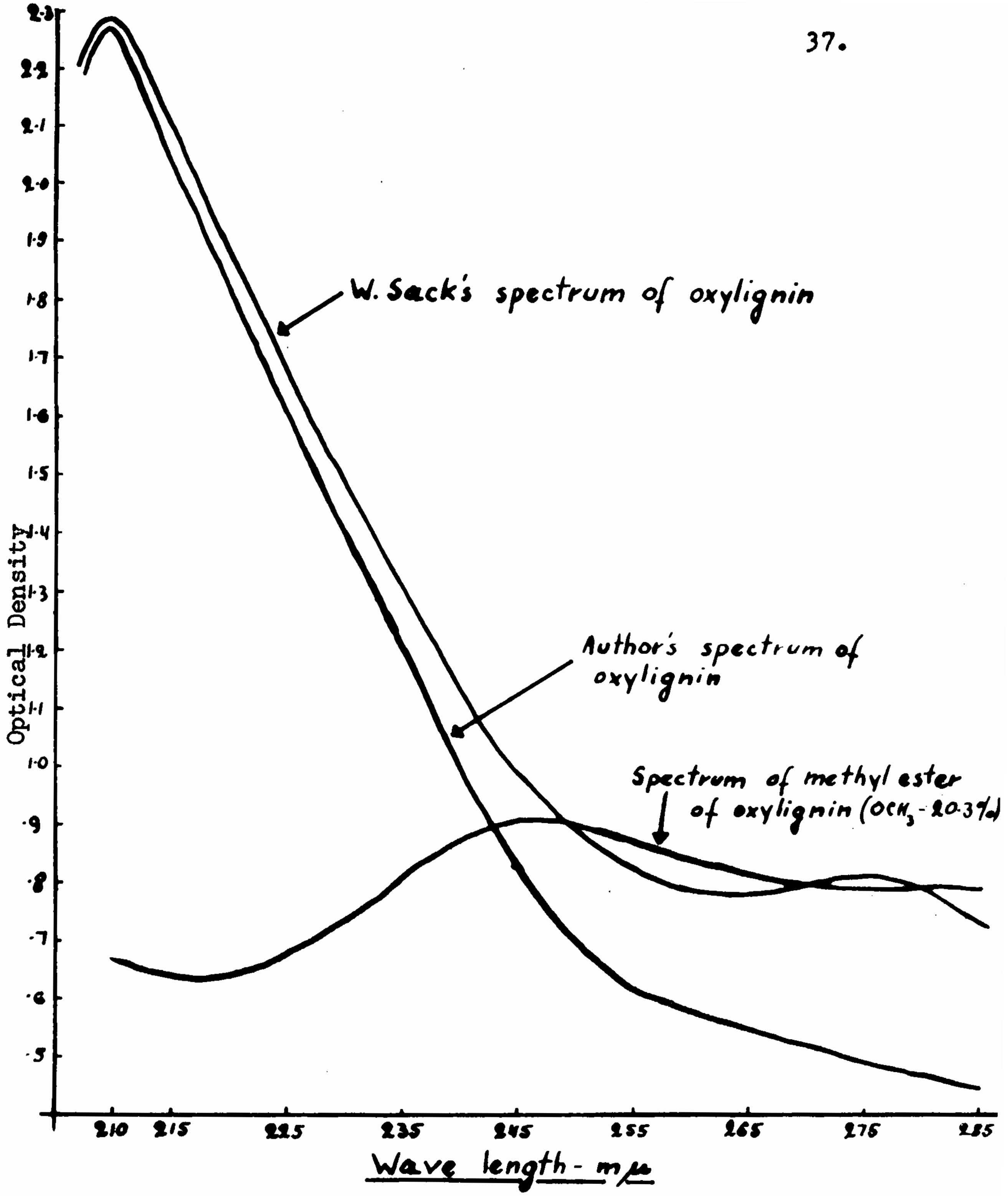


Fig. 5 - Comparison of the U.V. spectra of oxylignins

that prepared by the author. The two plots (figure 5) were almost identical up to 245 mμ, but at longer wave-lengths unexplained differences were apparent. A spectrum of the methylated oxylignin, prepared by the author, was very similar at longer wave-lengths to that given by oxylignin obtained by Sacks. It was therefore possible that the higher methoxyl content of Sacks' oxylignin (about 9.4% compared with 8.8% for author's product) caused the difference in the longer wave-length region of the spectrum.

The "quinone" properties of the oxylignin, first observed by Sacks (14) and noted in the present work by means of tests with sodium hydrosulphite and with acidified potassium iodide solution, were lacking supporting evidence. In order to secure more convincing indications of the presence of quinone groups, a series of reactions typical of quinones was carried out on oxylignin. Table VII illustrates the methods used. (Page 41)

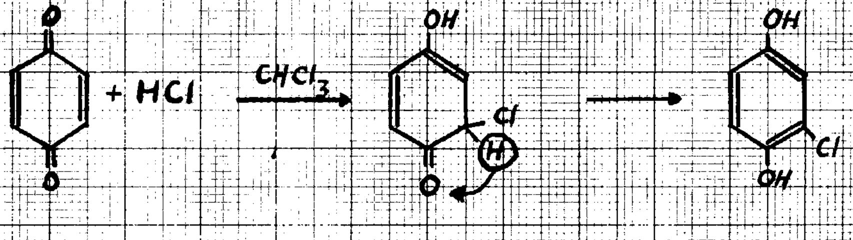
The reaction with dry hydrogen chloride gas (80) is specific for quinones and can be represented as 1:4 addition followed by enolisation, although the exact mechanism is unknown (XII to XIV). (Page 40) The chlorine content should therefore show an increase. When the oxylignin was subjected to the action of dry hydrogen chloride gas in chloroform, the chlorine content increased from 5.25 to 5.9%, thus indicating, but not proving, that a reaction of the type shown above might have occurred. If so, then about 0.02 atoms of chlorine per 100 grams could be attributed to quinone units.

Another specific reaction was the addition of methanol to a quinone (81) (XV to XVI), when the two were heated under reflux in the presence of zinc chloride as catalyst.

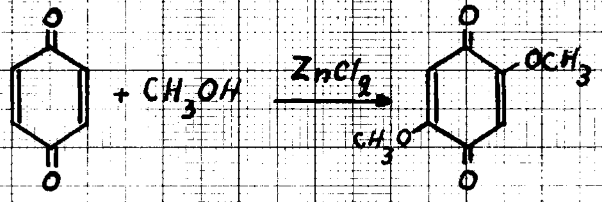
Since the oxylignin contained carboxylic acid groups which would be esterified during the reaction, it was decided to employ a large excess of methanol to ensure complete esterification, and then to reduce the methyl ester to the alcohol using lithium aluminium hydride. Thus, any net increase in the methoxyl content from the original 8.8% could not be attributed to methyl ester groups, but only to the introduction of the new methoxyl groups by the type of reaction shown above. In the actual experiment with oxylignin, a small but definite increase (from 8.8 to 10.0%) of methoxyl content was found, corresponding to about 0.04 groups per 100 grams.

Acetylation studies were undertaken to determine the extent to which the oxylignin could be acetylated, and in particular to establish whether or not the Thiele procedure (82) for the acetylation of quinones gave a higher acetyl value than did acetylations in neutral or alkaline media. The Thiele procedure could be represented by the scheme illustrated by formulae XVII to XIX, page 40. Thus, if a quinonoidstructure were present in the oxylignin, the

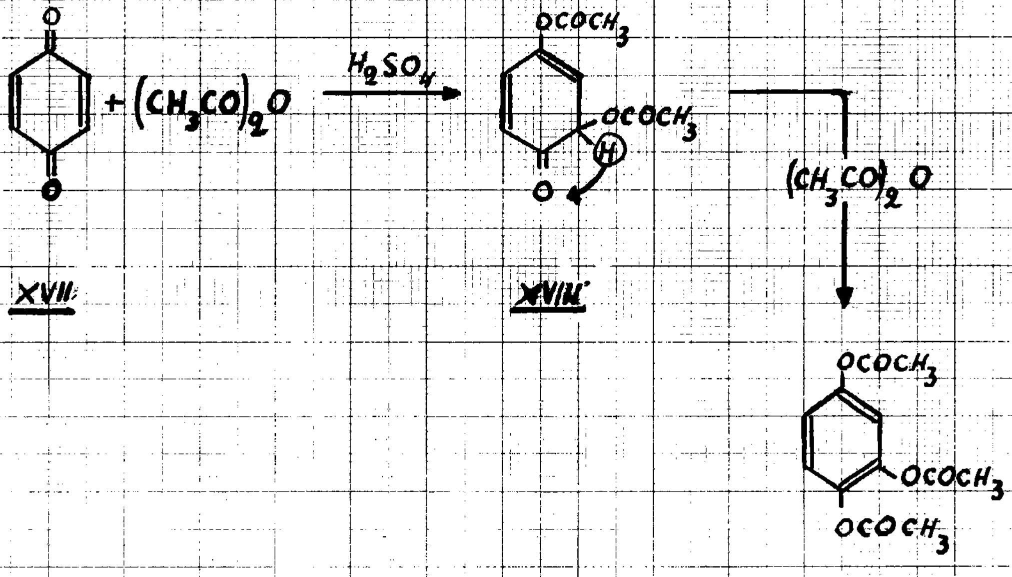
Typical reactions of quinones



XII XIII XIV



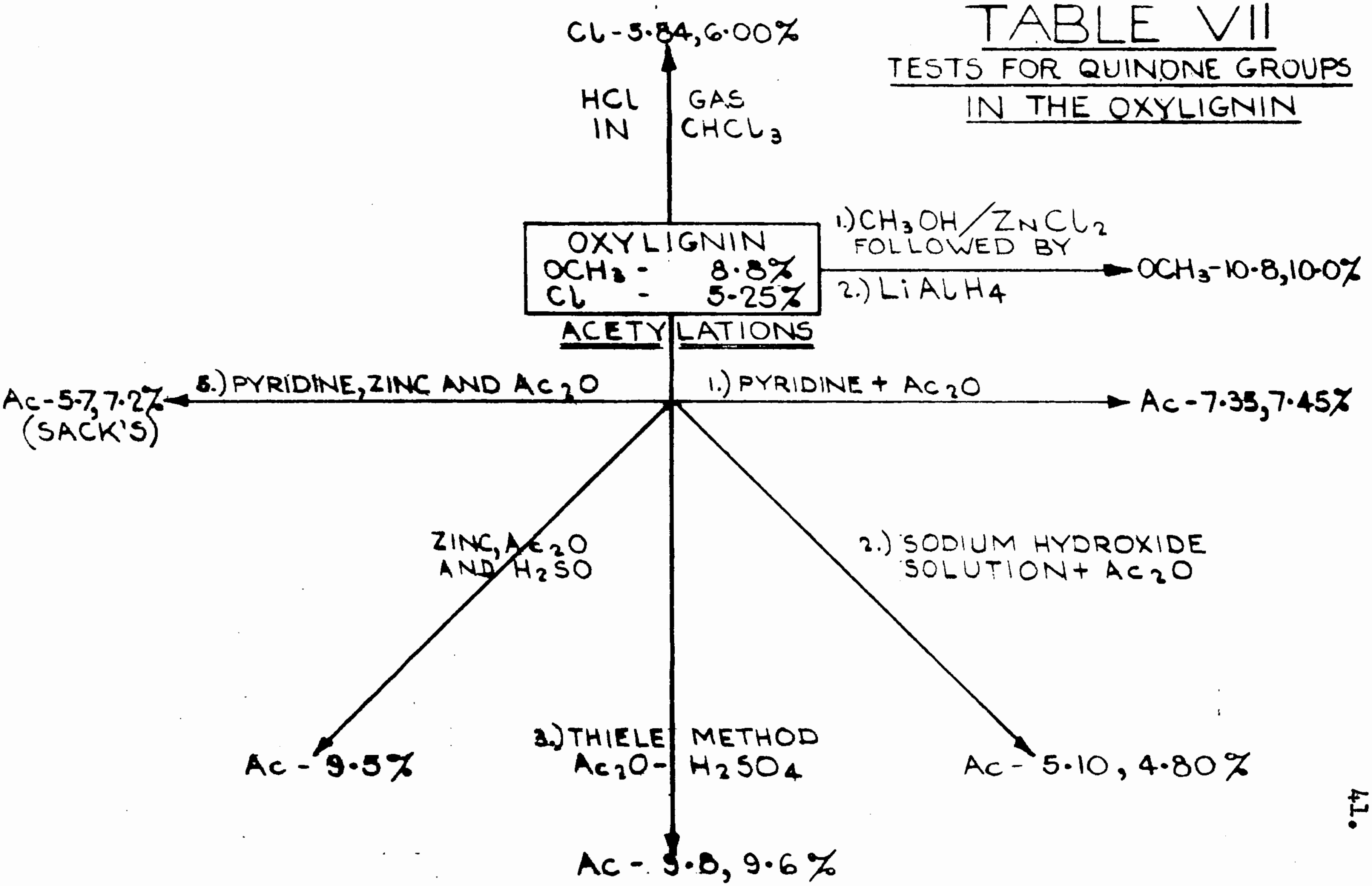
XV XVI



XVII XVIII XIX

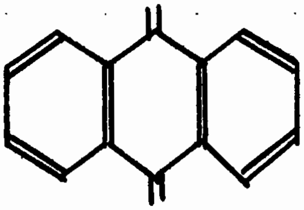
TABLE VII

TESTS FOR QUINONE GROUPS IN THE OXYLIGNIN



Thiele acetylation should yield the acetate of highest acetyl content. The analytical data, summarised in Table VII, showed that the Thiele methods 3 and 4 were more effective and that about (9.7-7.2) or 2.5% of acetyl groups might have been those of an acetylated quinone. This amount corresponded to about 0.06 moles of acetyl group per 100 grams.

The three experiments described above all suggested that about 0.02 base moles of quinone per 100 g, or about 2%, were present in the oxylignin. In the opinion of the author, however, the observed changes in chlorine, methoxyl and acetyl contents were too small to be fully convincing, and each of them could originate from other types of reaction. Furthermore, if the hydrogen atoms of the quinone ring were substituted by stable groups or aromatic rings as in structure XX,



XX

then none of the above reactions could have taken place. The evidence could therefore only be considered as an indication, and not as a proof, that the quinonoid structure might be a part of the oxylignin polymer.

The fractionation of the oxylignin (Table VIII), carried out by the addition of petroleum ether (boiling range (30-60°) to an acetone solution, gave results which were similar to those obtained by Sacks (14). The oxylignin was a mixture of acidic, chlorinated compounds of apparent molecular weight 350 - 3000, the average number molecular weight being about 2300. The first fraction (about 70% of the oxylignin) had a smaller chlorine content and a higher molecular weight and neutralisation equivalent than did the subsequent fractions, which exhibited a gradual increase in chlorine content and a gradual decrease in molecular weights and neutralisation equivalents.

The methoxyl values of various fractions did not show any definite trend.

In an attempt to obtain a chlorine-free oxylignin, the fraction of lowest chlorine content was refractionated using the same system as before, and five fractions whose chlorine content varied from 3.2 to 5.3% were isolated, as shown in Table IX. It was therefore probable that all members of the heterogeneous mixture constituting the oxylignin polymer were chlorinated to a certain degree.

Table VIII

Fractionation of the Oxylignin(a)

Fraction	Pet.ether added-cc	Ppt.in grams	% by weight	% Cl	% OCH_3	N.E.	M.Wt.of methyl ester	of methoxyl content
1	75	10.5	70.00	3.80	9.05	282,314	2800(b)	14.5
2	150	1.3	8.65	4.25	7.22	225,231	2020	18.9
3	225	0.8	5.35	4.85	8.00	208,218	1390	14.7
4	300	0.5	3.33	5.25	6.85	211,216	1050	17.4
5	450	0.7	4.65	5.75	8.42	185,187	825	19.0
6	Evap.	0.8	5.35	6.70	5.90	- -	350	17.1

(a) The oxylignin, 15.0 g, was dissolved in 300 cc. of acetone. All analytical figures were averages of concordant duplicate or triplicate estimations.

(b) Not fully soluble; result doubtful.

Table IX

Subfractionation of the Head Fraction of Oxylignin(a)

Subfraction	Volume of pet.ether added-cc	Weight of the precipitate in g	% by weight of fraction 1	% by weight of oxylignin	% Cl	% OCH_3
1a	5	1.25	36.2	25.0	3.3 3.2	8.8 9.0
1b	5	1.08	31.2	21.6	3.5 3.4	
1c	5	0.44	12.8	8.8	3.6 3.7	
1d	15	0.41	11.9	8.2	4.0 4.0	
Residue	Excess	0.14	4.1	2.8	5.3 5.4	9.4 9.2

(a) A solution of 3.45 g. in 70 cc. of acetone.

Action of Reducing Agents on Periodate Lignin and Oxylignin

Introduction. One of the most powerful tools for the elucidation of the structure of an organic compound is step-by-step degradation by oxidising agents. This method, when applied to lignin field, yielded short-chain aliphatic acids and carbon dioxide (83) (84) as well as polycarboxylic benzene acids (85) (86) (87) and mixtures of metahemipinic, isohemipinic and veratric acid, all in yields too low to tell much about structure. Oxidation of lignins with alkaline nitrobenzene (88) (89) gave better yields of vanillin and similar aromatic compounds having a carbon side chain.

As already mentioned, attempts to oxidise hypochlorite oxylignin by Sacks (14) and Brounstein (13), using potassium permanganate, chlorine dioxide, hydrogen peroxide, aqueous chlorine, silver oxide and chromium trioxide, failed to yield any identifiable products. The failure might be attributed, at least in part, to the inaccessibility of their starting material, to the low yields of ether-soluble or crystalline products, and to the fact that these products almost invariably contained closely related compounds difficult to separate.

The zinc-hydrochloric acid procedure originally used by Clemmensen (90) (91) (92) was satisfactory for the reduction of compounds appreciably soluble in the acidic medium. On account of the extremely low solubility of the oxylignin in acid, a modification (93) of the Clemmensen method was employed and dioxane was chosen as the solvent for the oxylignin. Another modification which, according to Windaus and Rahlen (94), inhibited the formation of insoluble reduction products on the surface of the zinc, employed a hydrocarbon solvent. In this case, the reduction occurring in the aqueous acid layer took place at such a high dilution that polymolecular condensations were kept at a minimum. Even those precautions failed to prevent extensive resin formation, and most subsequent reductions were carried out in neutral or alkaline solution.

A customary method of reduction in an alkaline medium, due to Kishner (95) and Wolff (96), employs hydrazine hydrate. The method generally succeeded with compounds of high molecular weight, but in all cases heating at about 180° for 6 - 8 hours was recommended. Since such a high temperature might cause extensive condensation of the lignin, as well as partial cleavage, it was decided to test the reaction at a lower temperature. A pilot experiment failed to reduce acetophenone to the expected ethyl benzene when pyridine (boiling point 117°) was used as the solvent. Hence the use of a high boiling solvent (triethylene glycol) could not be avoided when the reduction of the periodate lignin and of the hypochlorite oxylignin was attempted.

The ether extract from the reduction of oxylignin exhibited phenolic properties but was too small in amount to be examined by classical methods. Consequently, chromatographic analysis was employed. Among many (97) (98) (99) (100) methods of identifying phenols by paper chromatography, the one due to Hossfeld (100) appeared to be the most convenient. The method consisted of coupling phenols with diazotised sulphanilic acid (101) and applying the dye so formed to a strip of Whatman No. 1 paper, which had been previously sprayed with 4% sodium carbonate solution and dried. A total of 15 known phenols were chromatographed in the manner described, and the method appeared to be quite general. When this technique was applied to the ether-soluble products obtained from the oxylignin reduced by the Wolff-Kishner method, three spots corresponding to guaiacol, phenol and eugenol were observed. All of these phenols had been previously obtained from lignin (102) (103) by thermal decomposition, and hence it was quite likely that in this case also they were formed as high temperature artifacts.

A similar Wolff-Kishner reduction of the original periodate lignin gave, in the ether soluble portion, a small quantity of m-ethyl phenol, which had never before been isolated from lignin. Unfortunately, the overall yield was only about 1%, and therefore no definite conclusions as to the origin of this material could be made. The <u>para</u> isomer was obtained by Freudenberg and Adam (104) in 1.1% yield by destructive distillation of hydrochloric acid spruce lignin in a current of hydrogen.

The action of milder reducing agents, which are normally employed at more moderate temperatures, was then studied. The amorphous materials obtained by the attempted reduction of oxylignin with lithium aluminium hydride (105) were characterised by a considerable decrease in their chlorine content and by wide variations in their methoxyl values.

A recent modification (106) in sodium borohydride reductions, consisting of the addition of a solution of anhydrous aluminium chloride in diethylene glycol dimethyl ether, made it possible to reduce esters, carboxylic acids and nitriles. When applied to the oxylignin and its methyl ester, this modification failed to yield any identifiable products. Aluminium chloride was found to demethoxylate the oxylignin drastically, and the products still contained free carboxylic acid groups. The action of sodium borohydride on a solution of oxylignin in dilute sodium hydroxide apparently failed to produce any changes. Over 60% of the starting material was recovered; the analysis and UV spectrum of this fraction were very similar to those given by the original oxylignin.

Reductions of the oxylignin with metallic zinc, both in glacial acetic acid and in alkaline solutions, failed to yield any ether-soluble products. The amorphous materials obtained in both cases had lost most of the original chlorine, but no other changes seemed to have occurred. Since the above reducing agents seemed to be too mild to split the oxylignin molecule, more powerful reducing agents, known to be capable of reductive cleavage, were subsequently employed.

Reductive Scission of Periodate Lignin and of Oxylignin with Hydriodic Acid

In a preliminary experiment, the rate of loss of methoxyl groups from periodate lignin was studied under conditions of temperature and hydriodic acid concentration identical to those recommended (107) for methoxyl determinations. However, the 1-g sample used was far greater than that normally employed in the methoxyl determination, and this circumstance probably explained the incomplete demethoxylation after heating under reflux for 6.5 hours. (Table X)

Table X

Cleavage of Methoxyl Groups from Periodate Lignin by Hydriodic Acid(a)

Minutes	OCH_3 lost %	Minutes	OCH_3 lost %
30	1.11	210	5.42
60	1.74	270	6.13
90	3.08	330	8.70
120	3.93	390	10.83
150	4.78		

(a) A 1-g sample in 21 cc of 47% hydriodic acid.

The residual, largely demethoxylated periodate lignin, was fairly heavily iodinated (I,-10.6%) and was considerably darker than the starting material. The physical characteristics were unfavourable for further work, as the material was no longer finely divided and had an almost tar-like appearance. In order to reduce the extent of iodination and of condensation, which invariably occurred when lignin was subjected to elevated temperature, the reduction was carried out at a lower temperature both in the presence and in the absence of red phosphorus.

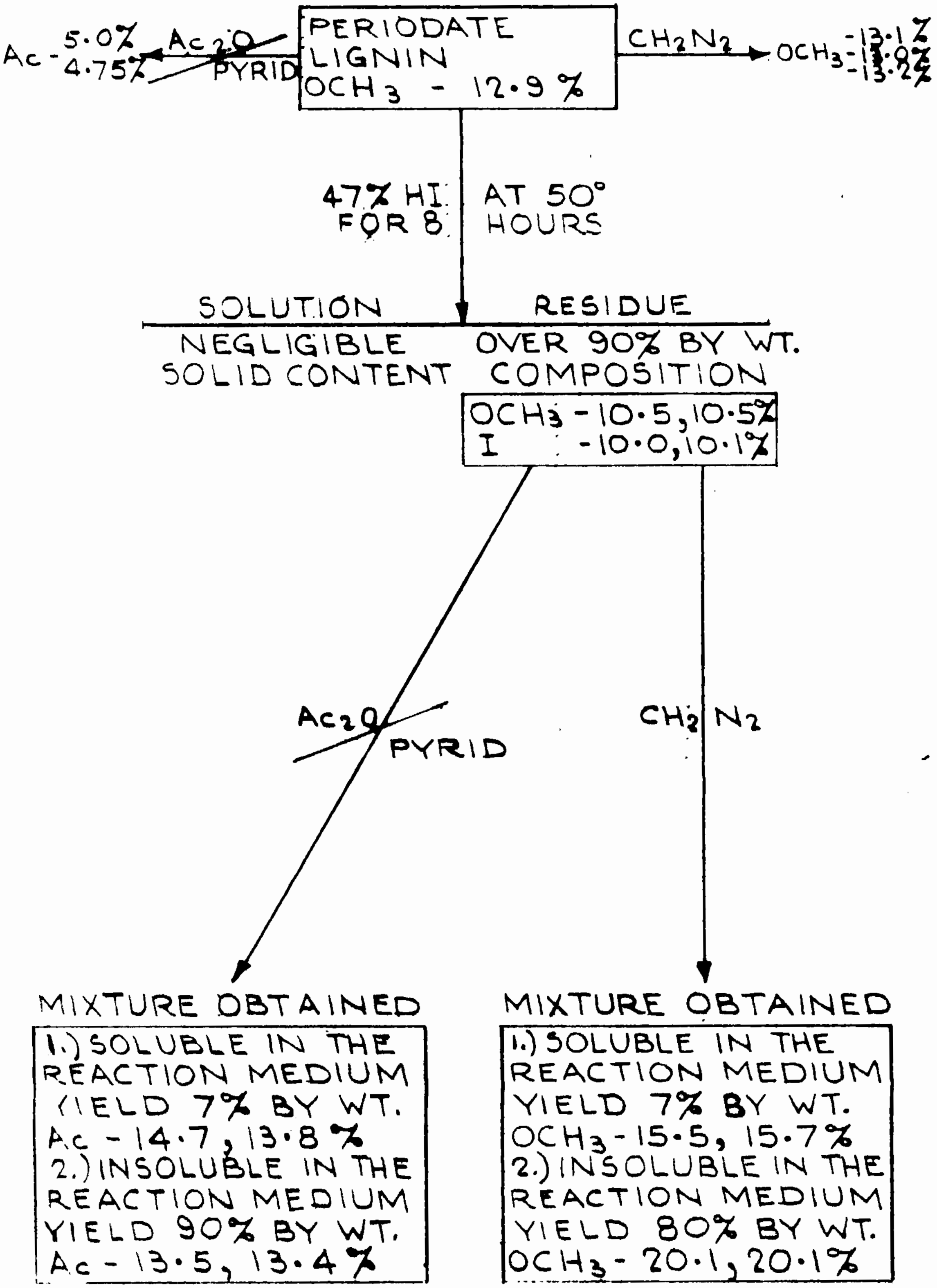
In the reductions in the presence of red phosphorus the hydriodic acid was liberated by the action of phosphoric acid on potassium iodide; this method (108) lowered the boiling point of the reaction medium and most likely diminished any condensation of the lignin. Attempts to separate the products from the excess phosphorus depended upon their solubility in sodium hydroxide solution, but were only partially successful, since only 60% by weight of the periodate lignin was recovered. Unfortunately, this product could only be isolated as a thick tar although it was free of iodine and largely demethoxylated. These unfavourable results led to the use of a still lower temperature and the omission of the phosphorus.

The action of 47% hydriodic acid on the periodate lignin for 8 hours at 50° yielded over 90% by weight of a product which was resistant to the further action of hydriodic acid under the conditions of the experiment. This product retained over 80% of the original methoxyl groups and had an iodine content of 10%. No carboxylic acid groups were detected. In view of the high degree of methoxyl retention and the practically unchanged physical characteristics, it was decided to examine this product in greater detail and to compare its properties with those of untreated periodate lignin. The examination consisted of estimating the number of unsubstituted phenolic plus aliphatic hydroxyl groups by acetylation with acetic anhydride-pyridine, and the unsubstituted phenolic groups alone by methylation with diazomethane in anhydrous conditions. Although carboxylic acid groups would have responded to the methylation, there was no evidence of their presence in periodate lignin.

The data in Table XI showed that the periodate lignin had very few, if any, hydroxyl groups capable of being methylated with diazomethane and must have been almost devoid of phenolic hydroxyl groups. Consequently, the acetyl groups introduced by acetylation could only be formed from aliphatic hydroxyl units. Methylation of the periodate lignin treated with hydriodic acid yielded a product containing 20.1% of methoxyl group, (a small, 7% yield of product of OCH_3 content 15.6% was disregarded) thus showing that new phenolic hydroxyl groups to the equivalent of OCH_3 (20.1-13.1) or 7% must have been formed. The corresponding increase in acetyl was (13.5-4.9) about 8.6%. Since both the methylation and the acetylation increased the substitution by 0.20 to 0.23 units per 100 g, it was probable that all the new

TABLE XI

ACTION OF 47% HYDRIODIC ACID ON PERIODATE LIGNIN



hydroxyl groups were phenolic in nature.

In order to establish whether or not the iodine present had an "aliphatic" or "aromatic" character, the product from the periodate lignin (iodine content 10%) was treated with 5% sodium hydroxide solution at room temperature. (Table XII) Partial solution occurred and the insoluble residue, amounting to 77% by weight, contained 3.0% of iodine. The soluble portion (12% by weight), recovered by adding hydrochloric acid and collecting the precipitate, had 18.3% of iodine. Thus the average iodine content was

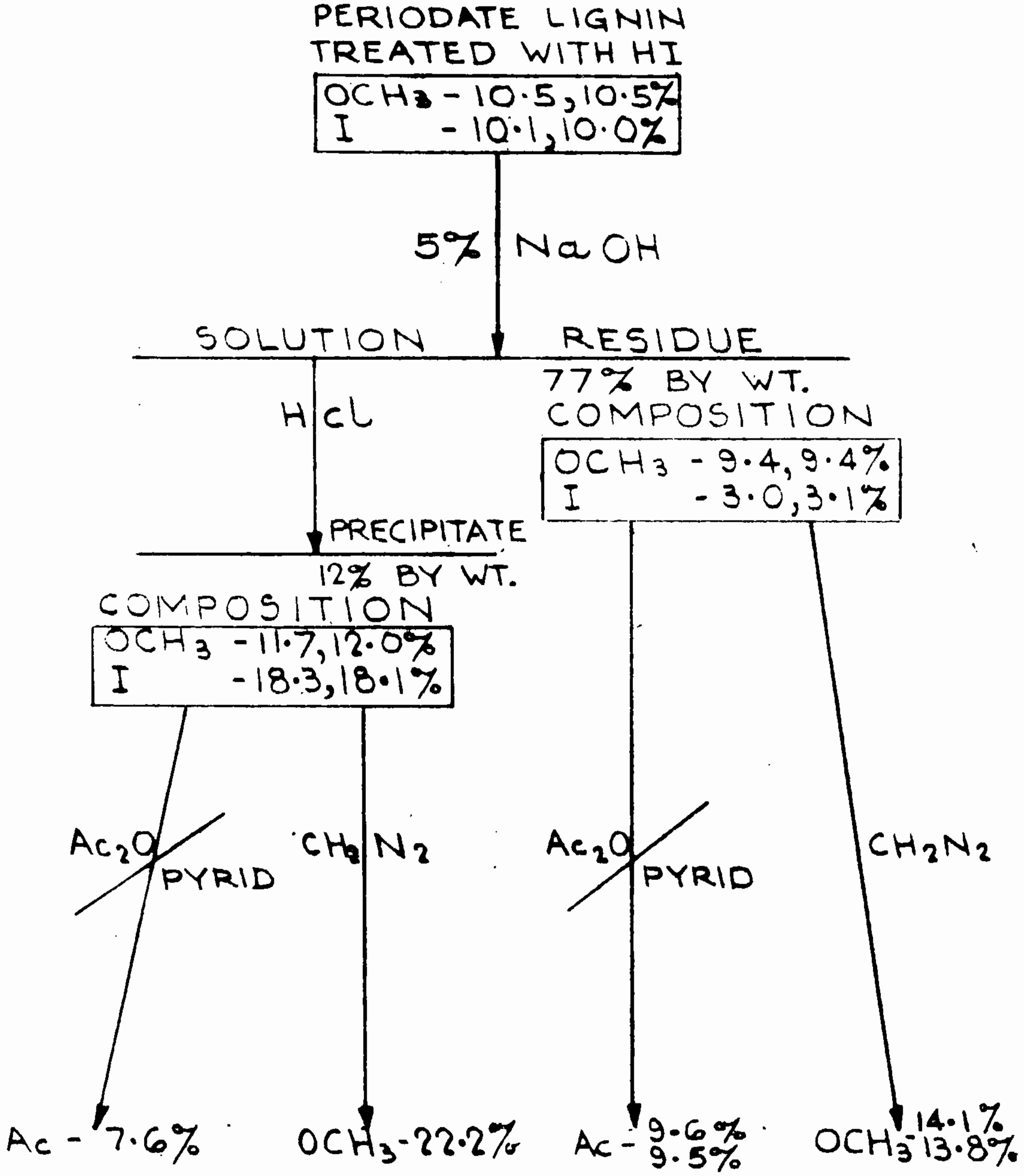
$$\frac{(77\text{x}3) + (12\text{x}18.3)}{89} = 5\%$$

indicating that about one-half of the iodine was split off and therefore was probably "aliphatic". This half corresponded to about 0.04 moles of aliphatic hydroxyl group per 100 g sample, whereas the formation of phenolic groups appeared to be about 0.2 mole. The results suggested the cleavage of an alkyl aryl ether, the aliphatic alcohol so formed being recovered partly as alkyl iodide and partly as hydrocarbon. A benzyl alcohol unit in particular would be expected to react in this way.

Hydriodic acid of the same strength maintained at 50° for 8 hours was also used to reduce the hypochlorite oxylignin, as well as its head fraction and the combined fractions 2-6 obtained from the fractionation described on page 44. Any considerable differences between the main fractions of

TABLE XII

ACTION OF ALKALI ON PERIODATE LIGNIN TREATED WITH 47% HI AT 50°



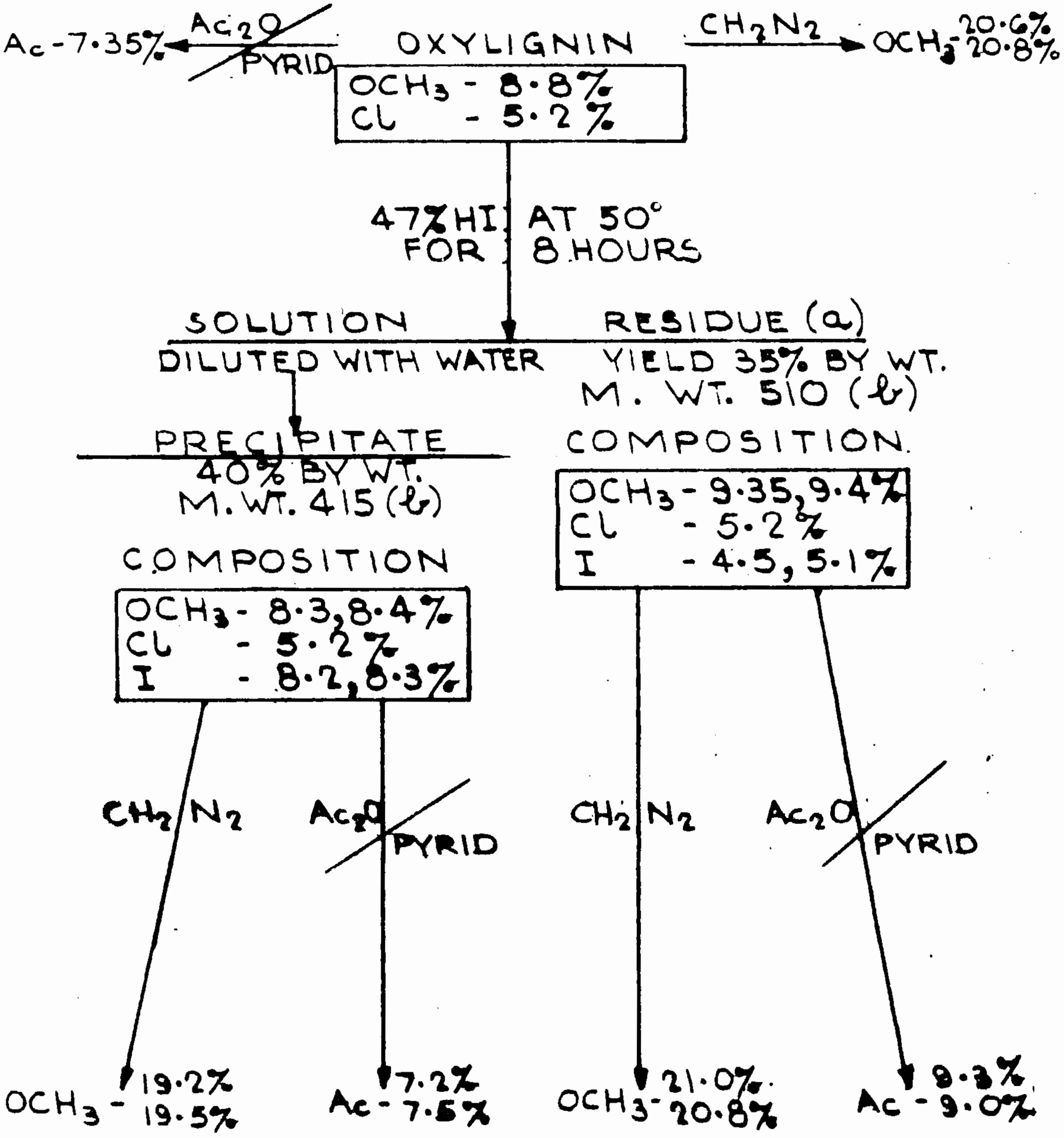
the oxylignin should be made detectable by acetylation or methylation, to which all the products of the above reactions were subjected. Experimental details for the reaction of 47% hydriodic acid with oxylignin and its fractions were the same, and the behaviour of the fractions of the oxylignin was identical. The data are summarised in Tables XIII, XIV and XV.

The most striking difference in the behaviour of oxylignin treated with 47% hydriodic acid as compared with the periodate lignin was the formation of a partial solution in the former case, while the latter remained over 90% insoluble, although changed chemically. (Table XIII) Another important difference was the almost complete retention of the methoxyl groups in the oxylignin, while the periodate lignin suffered a loss of almost 19% of the original methoxyl content. The insoluble fraction of the oxylignin, amounting to about 35% by weight, was resistant to the further action of hydriodic acid under the conditions of the experiment, showing, in this respect, the same behaviour as the corresponding fraction of periodate lignin. The molecular weights of the soluble and insoluble products, determined by Signer's isothermal distillation method, were 415 and 510 respectively, so that the hydriodic acid had brought about extensive degradation. The number average molecular weight of the oxylignin was about 2300.

Brounstein (13) showed by extensive methylation

TABLE XIII

ACTION OF HYDRIODIC ACID ON THE OXYLIGNIN

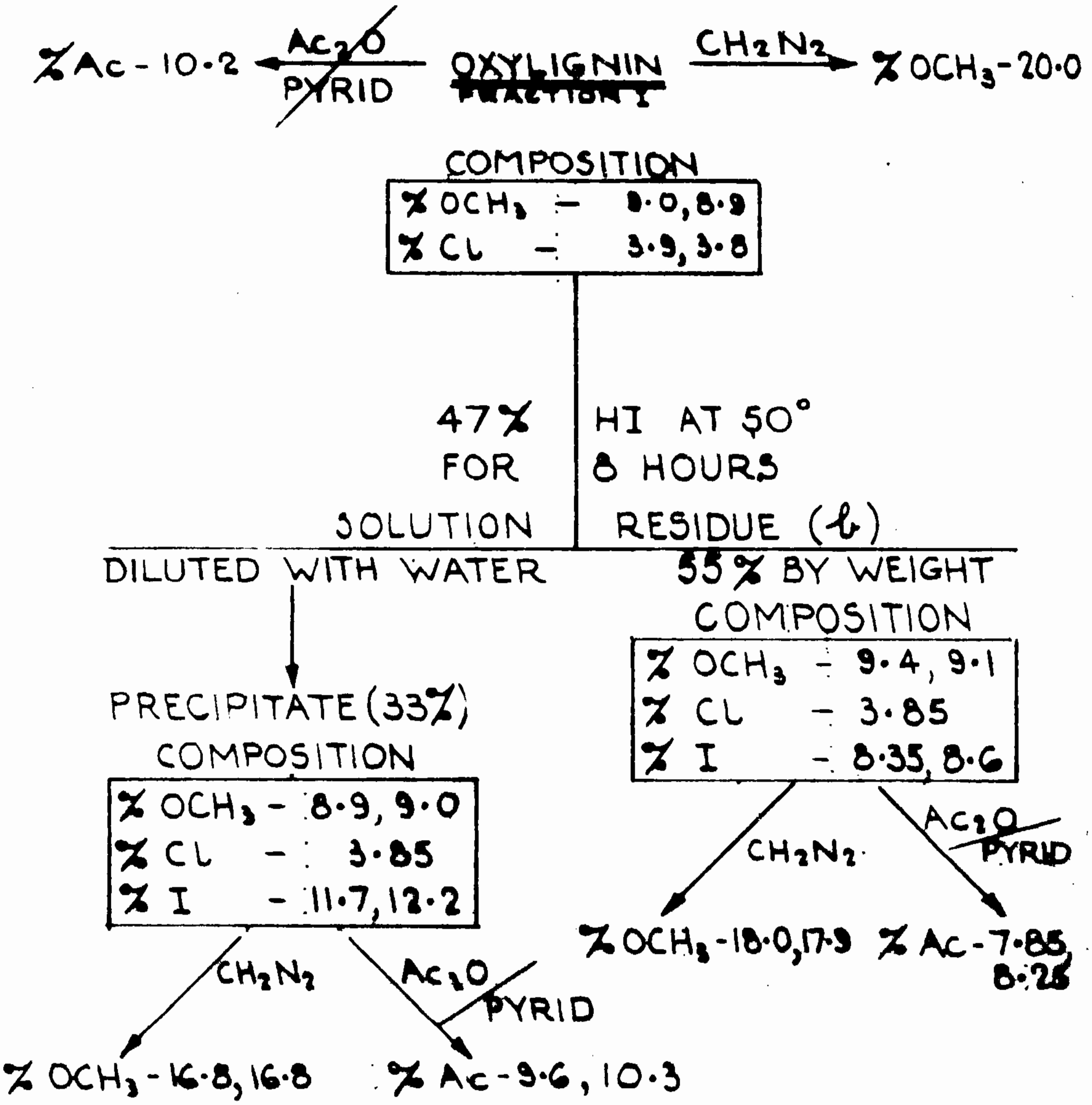


a) RESISTANT TO FURTHER ACTION OF HYDRIODIC ACID.

b) BY SIGNER'S METHOD.

TABLE XIV

ACTION OF HYDRIODIC ACID ON FRACTION I OF THE OXYLIGNIN (a)

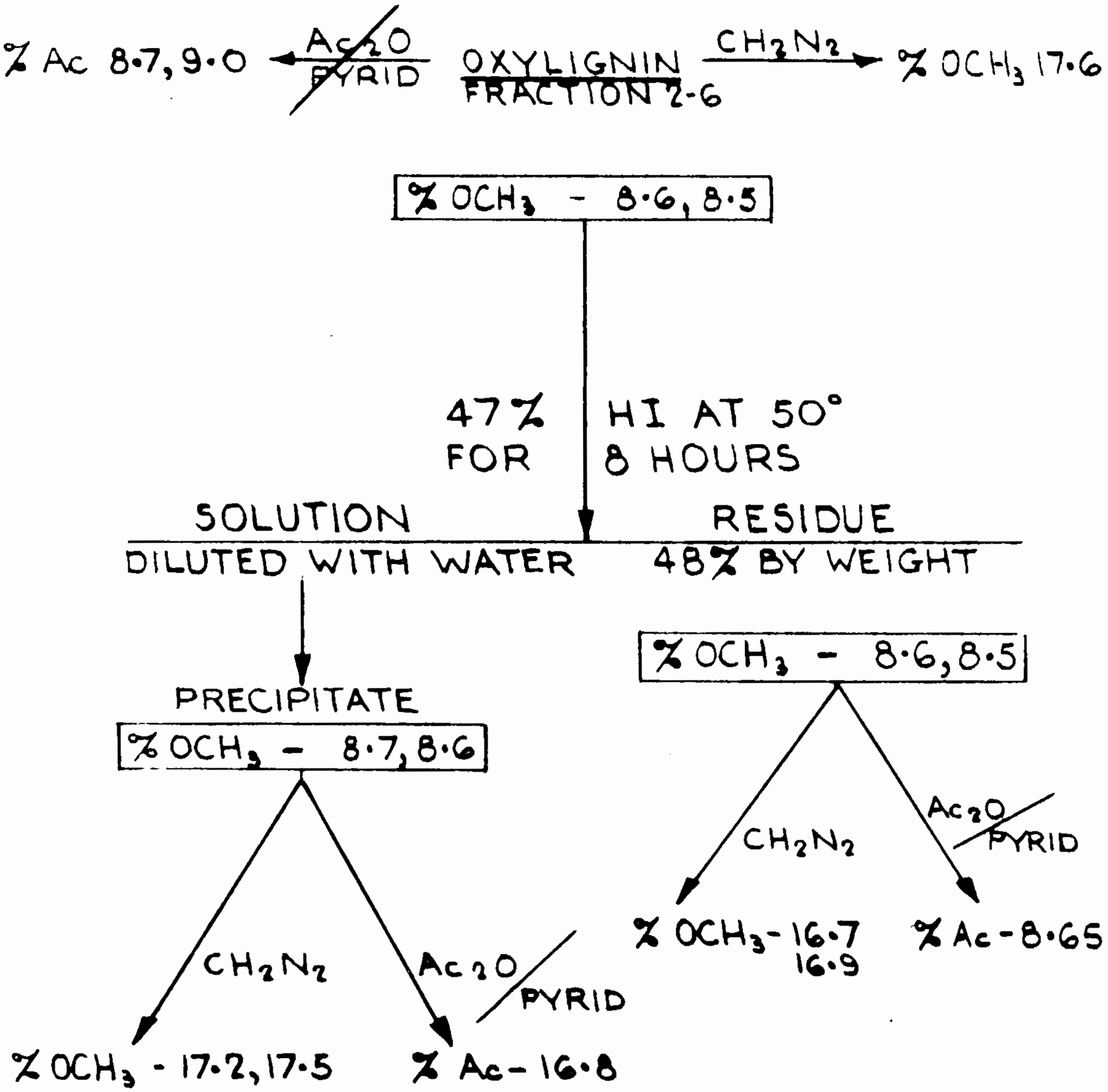


(a) SEVENTY PERCENT OF ORIGINAL OXYLIGNIN (TABLE XIII)

(b) RESISTANT TO FURTHER ACTION OF HYDRIODIC ACID

TABLE XV

ACTION OF HYDRIODIC ACID ON COMBINED FRACTIONS (2-6) OF THE OXYLIGNIN



studies, using dimethyl sulphate and also diazomethane, that the oxylignin was free of phenolic hydroxyl groups. Methylations with diazomethane therefore methylated only carboxyl groups.

A surprising similarity of methoxyl contents of both the soluble and the insoluble fractions obtained by the action of hydriodic acid on oxylignin, as well as of their methylated products, is quite apparent from Table XIII. Furthermore, the methoxyl value of the methylated oxylignin was very close to the others, and differed widely in this respect from the data on periodate lignin. Changes in acetyl content were also minor.

The same remarks were true when the major fraction of the oxylignin was treated with hydriodic acid (Table XIV) and also for the more soluble minor fractions (Table XV), although in this case one portion of the product had the anomalously high acetyl content of 16.8%. With this doubtful exception, it appeared that no new phenolic hydroxyl groups were formed when the oxylignin was subjected to the action of hydriodic acid under conditions, which apparently split ether linkages in periodate lignin. This statement has to be qualified because the accuracy of the method was low, and because the oxylignin was cleaved into two kinds of fragment each of average molecular weight 400 to 500, presumably by the scission of a very few ether bonds. If so, then the core of the oxylignin structure either had no other ether bonds, in which case only carbon-carbon linkages could exist, or all the other ether bonds, like those of the remaining methoxyl groups, were stable to hydriodic acid at 50°.

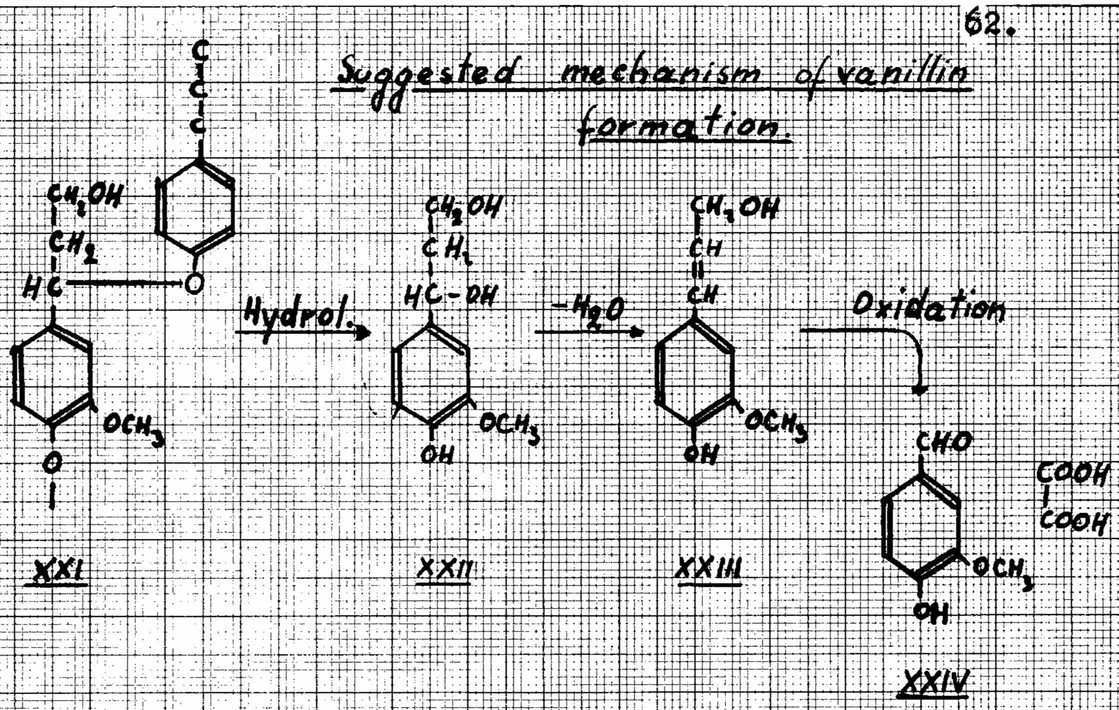
The first possibility was most unlikely, because it would then be very difficult to account for the formation of vanillin, which was isolated from the oxylignin by further oxidation with alkaline nitrobenzene.

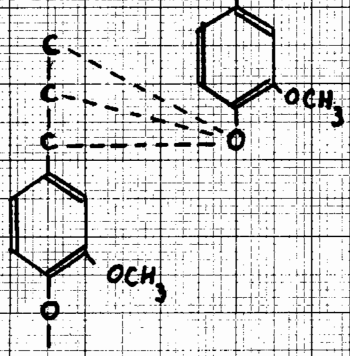
The mechanism of the above oxidation is not definitely known, but Lautsch (109) suggested that vanillin was formed by the hydrolysis of ether linkages of type XXI shown to yield α-hydroxy propanol groups XXII, which then lost water, giving an isoeugenol XXIII type of compound. This compound underwent an oxidative cleavage of the double bond giving oxalic acid and vanillin XXIV.

The question of the stability of ether bonds, which could be present in the oxylignin, toward 47% hydriodic acid at 50°, was investigated by carrying out the above reaction on synthetic model compounds. The same compounds were then subjected to the action of alkaline hypochlorite under the conditions used to oxidise the periodate lignin, as obviously only structures stable to alkaline hypochlorite could exist in the oxylignin.

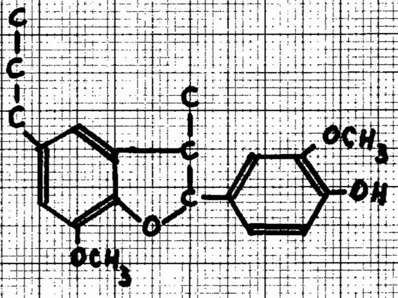
The action of alkaline hypochlorite on lignins, on phenol ethers related to lignin and on relatively simple

Suggested mechanism of vanillin formation.





XXV



XXVI

phenolic substances was investigated by Richtzenhain and Alfredsson (19) (20), while Sutherland (25) studied the behaviour of other simple phenols toward the same reagent. The phenol ethers examined in the present research were included among the compounds investigated by Richtzenhain (20); however, the temperature used in his work (95°) was much higher than that used by the author in the preparation of oxylignin, and the repetition was believed to be necessary.

The existence in lignin of a side chain of 3 carbon atoms was definitely proved by numerous workers (110) (111). Lignin can be assumed to be a compound whose ether bonds, originating from phenolic hydroxyl groups, are joined to the alpha, beta or gamma carbon atoms of the side chains, as represented by structure XXV (page 62). Another possibility is the structure where the ether bond is built into the coumarin ring XXVI (page 62). Furthermore, since the periodate lignin was proved to contain very few, if any, free phenolic groups, the model compounds had to be based on veratrole rather than on the guaiacol nucleus, especially in view of the observation by Richtzenhain (19), that free phenolic groups greatly facilitated the attack of alkaline hypochlorite. Table XVI lists the ethers which were subjected to the action of alkaline hypochlorite and summarises the results.

The formation of veratric acid from compounds (1) and

Table XVI

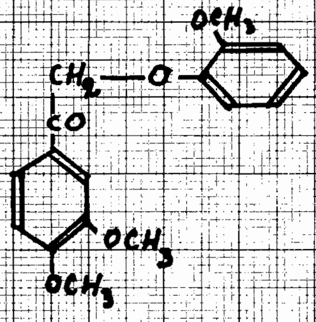
Action of Alkaline Hypochlorite on Model Ethers (a)

No.	Compound	Product of Reaction
1	ω-(2-methoxy phenoxy)-acetoveratrone	Veratric acid, 40% yield
2	2-(3:4-dimethoxy phenyl)-2-hydroxy -1-(2-methoxy phenoxy) ethane	Veratric acid, 20% yield
3	Dehydro diisoeugenol	Rapid consumption of hypochlorite. Products not identified (b)
4	Dehydro diisoeugenol methyl ether	Recovered unchanged
5	Diveratryl ether	Recovered unchanged (b)
6	p-Methoxy benzyl guaiacyl ether	Recovered unchanged (b)

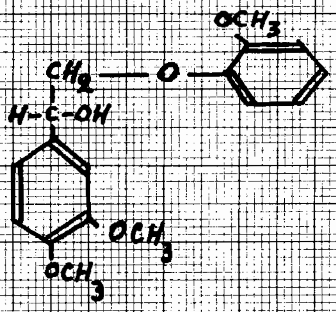
(a) Formulae tabulated in the appendix - next page

(b) Richtzenhain's experiments (20)

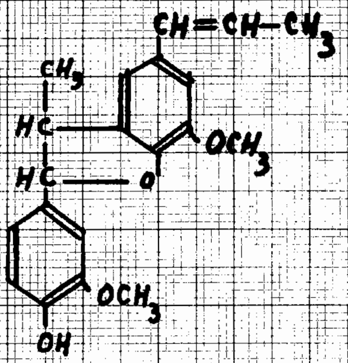
Appendix to Table XVI



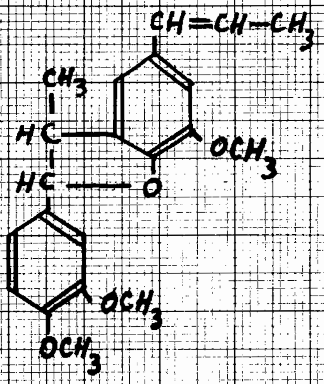
Compound 1.



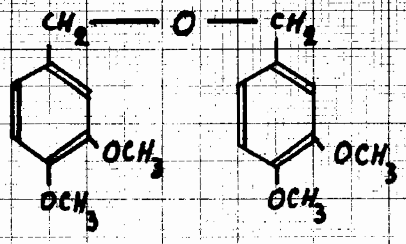
Compound 2.



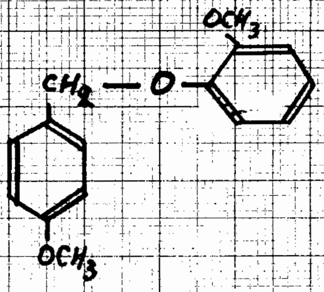
Compound 3.



Compound 4.



Compound 5.

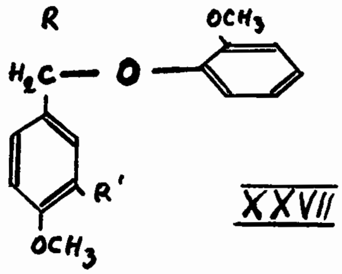


Compound 6.

(2) (Table XVI) was not due to alkaline cleavage, because sodium hydroxide solution of the same strength as used in the alkaline hypochlorite oxidation did not split the molecule even on boiling. Similarly, the reduction of the keto group in compound (1) to a secondary alcohol (2) did not affect the result. The oxidation of compounds (3) and (4) showed that the action of alkaline hypochlorite was entirely due to the free hydroxyl group in (3). In view of the absence of free phenolic groups in periodate lignin, the structure (3) could be omitted from further considerations.

A compound with the ether bond joined to the γ carbon atom of the side chain was not synthesised, because several of this type were found by Richtzenhain (20) to be resistant to alkaline hypochlorite oxidation, even at elevated temperatures.

When the same model compounds (Table XVI) were subjected to the action of 47% hydriodic acid at 50° and for 8 hours, the starting material was recovered unchanged with the exceptions of diveratryl ether and p-methoxy benzyl guaiacyl ether (compounds 5 and 6). As far as these very limited experiments went, it appeared that hydriodic acid as used was limited to the cleavage of di-alkyl ethers (compound 5) and aryl-benzyl ethers (compound 6) of the type XXVII. Such types, however, if present had adjacent groups, presumably in the side chain, which caused their



XXVII

destruction by the prolonged action of alkaline hypochlorite. The resulting oxylignin, which amounted to only 30% of the periodate lignin, was probably free, or almost free, of di-alkyl and benzyl-aryl ether groups. On this basis, the ether groups remaining in the oxylignin were almost entirely of an alkyl-aryl kind, as in phenetol, or were diaryl ether, as in diphenyl ether. An internal ether type, as in (4), Table XVI, was, of course, not excluded. The formation of vanillin in 69% yield from dehydrodiisoeugenol by Leopold (112) supported, but did not prove, the existance of such an ether bond in the oxylignin.

Attempts were made to isolate ether soluble products from the reaction of 47% hydriodic acid with oxylignin or its methyl ester in the hope that a structure determination might throw some light on the nature of the bond cleaved. The isolation of a crystalline material, melting point 128-129°, in 7.6% yield, from a single similar experiment was reported by Brounstein (13). In spite of repeated efforts and strict adherence to the experimental conditions used by the above author, no appreciable amount of an ether extract was obtained and no crystalline material was isolated.

Cleavage of Oxylignin with Sodium in Liquid Ammonia

The action of sodium in liquid ammonia on the oxylignin was studied in an attempt to split any ether groups it contained and so to degrade it to phenolic substances of lower molecular weight. The use of this powerful reducing agent to elucidate the structure of lignin, and particularly the work of Freudenberg (51) (52) and Shorygina (53) (54) (55) (56) (57) (58) (59) has already been described.

The present work originally followed the technique of Shorygina (53), who treated the cuoxam lignin with sodium in liquid ammonia, dissolved the residue in water, extracted the alkaline solution with ether and acidified the aqueous residue with acid. In the present research the partly degraded oxylignin, which precipitated on the acidification of the solution, was then subjected to repeated treatments with sodium in liquid ammonia until most of the oxylignin became soluble in the acidified liquor. The acidic liquors from all cycles were combined and extracted with ether, which failed, however, to remove all of the product. The ether-insoluble portion was successfully extracted with butanol, but this solvent was found to esterify the material. The butyl esters when hydrolysed formed an unmanageable gum.

In order to avoid the butanol extraction, and if possible to increase the solubility of the reaction products in ether, two modifications were introduced in the next reduction which employed 9.7 g of oxylignin.

First, a more concentrated solution of sodium in liquid ammonia was used, and sodium metal was added whenever the ammonia solution became colourless. Second, the acid filtrate from the precipitation of the oxylignin was diluted with a large excess of acetone to precipitate the sodium sulphate. The filtrate was concentrated to remove acetone and was then extracted at pH 1.5 with ether. After removing sulphuric acid from the aqueous residue by adding the exact equivalent of barium hydroxide, the liquor was evaporated to dryness and the residue weighed.

The loss of methoxyl groups by the oxylignin after consecutive sodium in liquid ammonia cycles, and the percentage of the oxylignin made soluble in dilute acid solution, was determined by separating the insoluble residue. Results are summarised in Table XVII.

Table XVII

Behaviour of the Oxylignin during Liquid Ammonia - Sodium Cycles

	% OCH_3 in the insoluble residue	% of oxylignin soluble in dilute acid
1st cycle	8.25,8.30	23.4
2nd cycle	8.00,7.95	54.8
3rd cycle	7.68,7.82	69.8

As seen from the above Table, after three treatments, about 70% of the oxylignin became soluble in dilute acid. The final insoluble portion differed considerably from the starting material in physical appearance and was not subjected to further degradations.

The total ether extract, amounting to 5.15% of the starting material, when chromatographed revealed 4 spots, which when sprayed with compounds forming coloured materials with phenols, clearly showed their phenolic character. Since the ether extract was completely soluble in 5% sodium bicarbonate solution, the ether soluble material must have been a mixture of phenolic acids. Molecular weight determinations of the fractions obtained from the reduction showed that considerable degradation took place, and the formation of new hydroxyl groups was indicated by the results of methylation with diazomethane which consistently gave higher methoxyl values than those obtained after treatment with 47% hydriodic acid. The results are summarised in Table XVIII.

It is the author's opinion that a more detailed examination of the products of this reductive scission, and especially of the ether soluble fraction, would add valuable information regarding the structure of oxylignin and hence the periodate lignin. In view of the relatively low yield of the ether soluble portion, it would, however, be necessary to start with a considerably larger quantity of material than was possible in this work.

Table XVIII

Analyses of Fractions from Three Liquid Ammonia - Sodium Cycles

Description of fraction	Yield, g	Yield, %	% OCH_3	% OCH_3 after methylation	M.Wt. (Signer method)
Final, insoluble residue-1	2.93	30.20	7.75	23.2	550
Ether soluble-2	0.50	5.15	8.15	26.5	350
Ether insoluble, acetone soluble-3	1.05	10.83	12.5	21.8	470
Ether insoluble, acetone insoluble-4	0.30	3.10	2.00	-	No solvent

EXPERIMENTAL SECTION

Analytical Methods

All samples prior to analysis were dried under reduced pressure over phosphorus pentoxide and analyses made at least in duplicate.

Klason Lignin

Klason lignin was determined according to the standard method recommended by TAPPI (64) using 72% sulphuric acid and 0.5 to 0.7 g samples.

Ash

Samples of about 10 mg were ignited to constant weight in a micromuffle furnace (113).

Holocellulose

Holocellulose was estimated by the Ritter-Kurth method (114) using chlorination with chlorine gas followed by extraction with ethanol-pyridine until no further solution of the sample (0.2 to 0.6 g) appeared to take place.

Methoxyl

Methoxyl contents were estimated by the method of Vieböch and Schwappach (107) using 10 to 20 mg samples and apparatus designed by Clark (115). The liberated iodine was titrated with 0.03N sodium thiosulphate solution.

Chlorine and Iodine

The method due to Elek and Hill (116) was used. This method was based upon the oxidation of the sample (10 to 15 mg) with sodium peroxide in a modified Parr bomb. The sodium halide formed was estimated by precipitation with silver nitrate. Estimation of organic iodine in the presence of chlorine, encountered in the reaction of oxylignin with 47% hydriodic acid (page 57) was calculated from the total weight of silver halide by assuming that no loss of chlorine occurred during the reaction, and that the percentage of chlorine in all analysed fractions was the same as in the starting material.

Neutralisation Equivalent (N.E.)

Method as described in Vogel (117) using about 0.2 g of the sample dissolved in water or alcohol-water mixture was used. The solution was titrated against standard sodium hydroxide solution using phenolphthalein as indicator. In case of coloured solutions, the Fisher titrometer was used.

Nitrogen

Kjeldahl's micro method, modified by Gunning (118), using 20-30 mg samples digested with a salicylic acid-sulphuric acid mixture, was used.

Molecular Weights

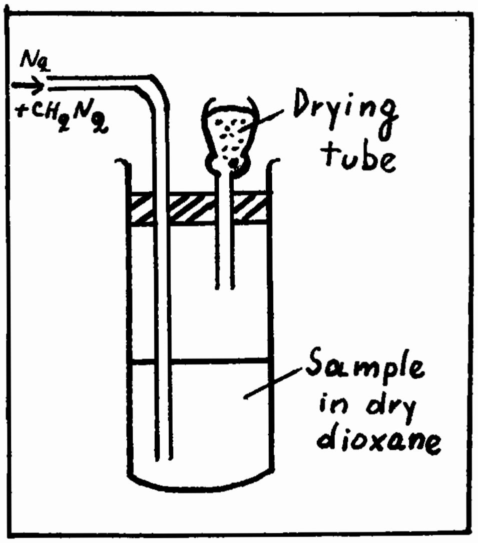
Signer's method (119) of isothermal distillation was carried out in the apparatus of Clark (120), using anhydrous acetone and a constant temperature bath (39°).

About one week was required for equilibrium to be established. Naphthalene was the reference compound.

Methylations

All methylations were carried out by means of diazomethane acting on a suspension or solution of the substance in anhydrous dioxane. About 0.3 g of the material was shaken in a stoppered test tube for 2 hours with 5 cc of anhydrous dioxane; any particles adhering to the walls of the tube were washed down with a further 5 cc of dioxane. An inlet for diazomethane and a loosely packed drying tube were inserted as shown.

Diazomethane gas, prepared by the action of 12 cc of ice-cold, 50% sodium hydroxide solution on 5 g of N-methyl nitrosourea (121) dissolved in 30 cc of ether, was passed into the tube, the entire assembly being cooled in an ice bath. The diazomethane was diluted with nitrogen, which at the same time agitated the contents of the generating mixture, and the reaction was considered complete when the ether layer became almost colourless. The methylation mixture was then stirred overnight. If the products were completely soluble in dioxane, they were precipitated by the drop by drop addition of water, were dried, redissolved in dioxane and precipitated again into ether.



Insoluble products were washed with water, acetone and finally ether. After the methoxyl content was determined in the usual way, the whole methylation procedure was repeated until constant values were obtained.

Acetylations

All acetylations, unless stated otherwise, were carried out using pyridine and acetic anhydride (122).

The material, 1 g, was dissolved in 15 cc of dry pyridine, 12 cc of acetic anhydride was added and the mixture allowed to stand at room temperature for 2 days. The mixture was then poured over cracked ice, and after recovery the precipitate was washed with distilled water and dried over sulphuric acid and solid sodium hydroxide. A solution of the product in anhydrous dioxane was centrifuged, filtered and poured drop by drop into dry ether. After washing with ether and petroleum ether, the powder was dried under vacuum.

Acetyl

Acetyl content was determined by the procedure of Clark (123) (124) which involved saponification with alcoholic sodium hydroxide and distillation of the hydrolyzate after acidification with a sulphuric acid-magnesium sulphate solution. The distillate was titrated to a permanent pink colour with 0.02N barium hydroxide, using phenol red as indicator.

Preparation of Reagents

Sodium Paraperiodate

This substance was prepared by the chlorine method due to G. F. Smith (125). One pound of sodium iodate was dissolved in 2 l of boiling water and a cold, saturated solution of 410 g of sodium hydroxide was slowly added with vigorous stirring. The mixture was then strongly heated until it boiled again, and water was added in small quantities at a time until a clear solution resulted. Chlorine gas was bubbled rapidly through a glass tube 1 cm in diameter into the boiling solution, which was stirred continuously for 15 minutes; then the passage of chlorine was stopped and the white suspension was filtered rapidly under suction through sintered glass. A suspension of the precipitate in weak sodium hydroxide solution was stirred for 5 minutes and then filtered. The precipitate was washed several times with cold water. The reactions were

$$NaIO_3 + 3\ NaOH + Cl_2 \longrightarrow Na_2H_3IO_6 + 2\ NaCl$$

$$NaIO_3 + 4\ NaOH + Cl_2 \longrightarrow Na_3H_2IO_6 + 2\ NaCl + H_2O$$

The first filtrate contained a considerable amount of unchanged sodium iodate, and was consequently treated with saturated sodium hydroxide solution, diluted with water if necessary and saturated with chlorine gas, thus yielding an additional quantity of sodium paraperiodate. An overall yield of 70.5% was obtained.

The purity of the paraperiodate was assessed by Fleury and Lange's (126) titration which depended on the liberation of iodine from alkaline potassium iodide.

Equations $Na_3H_2IO_6 + 2KI + H_2O \longrightarrow 2KOH + 2NaOH + NaIO_3 + I_2$

$I_2 + H_3AsO_3 + H_2O \longrightarrow H_3AsO_4 + 2HI$

A 1 cc aliquot of a 4% solution of potassium paraperiodate was diluted to 25 cc, and was buffered to about pH 8 (phenolphthalein) with sodium bicarbonate. Exactly 10 cc of 0.1N sodium arsenite and an excess of solid potassium iodide were added, and after waiting for at least 10 minutes the excess of arsenious oxide was titrated with standard iodine solution with starch as an indicator. The periodate was 91.7% pure.

Periodate Lignin

Spruce wood meal, 2100 g, (moisture 5.6%) obtained by slowly grinding a log about 50 years old and passing the coarse powder through a 60 mesh sieve in a Wiley mill, was moistened with a measured quantity of water and the excess of water was removed by gentle suction. The moist meal was then placed in a stoneware vessel. Sodium paraperiodate, 660 g, was placed in a large jar and 10 l of water was added, followed by glacial acetic acid until the pH reached the value of 4. The mixture was stirred during the addition of the acid, and after a few minutes and gentle heating a clear solution resulted which, after cooling, was poured into the vessel containing the wood meal. Cold water was added to bring the total volume of the solution to 16 l. The mixture was then stirred mechanically for 24 hours, light being excluded. The temperature never exceeded 26°. At the end of the 24 hour period the stirring was stopped and the solution allowed to settle for 1 hour. The supernatant liquid was carefully siphoned away as far as possible and the rest was filtered with suction. The filtrate was saved for recovery of unchanged paraperiodate. The residual partly oxidised wood meal was repeatedly washed with cold water to remove acetic acid and was then suspended in 40 l of 0.1N cold sodium hydroxide solution. This suspension was again stirred for 24 hours and allowed to settle; the liquor was siphoned and filtered away and the residual meal was repeatedly washed with water until the filtrate was almost colourless. Samples of partially oxidised wood meal were withdrawn for analysis and the paraperiodate-sodium hydroxide cycle was repeated 5 times. The changes in methoxyl and Klason lignin content after each cycle were determined experimentally and the data plotted in figures 3 and 4, pages 31 and 37. Considerable difficulty was encountered in filtering the alkaline liquor in the third and fourth cycles. The inverted sintered glass funnels became clogged with finely divided particles and the process was extremely slow. It was found that the addition of some glacial acetic acid to lower the pH was beneficial and then the process, although slow and tedious, could be completed in about 6 hours. Periodate lignin, free of holocellulose, was obtained in 20% yield based on the wood and the methoxyl content was 12.8%.

The combined filtrates from the paraperiodate-acetic acid treatments were made alkaline with sodium hydroxide and evaporated on a steam bath to about one-half of their original volume. On cooling, crystals of sodium acetate and sodium paraperiodate precipitated. Extraction with hot water gave almost pure paraperiodate which was then washed with water and dried.

Alkaline Sodium Hypochlorite

Sodium hydroxide, 320 g, was dissolved in 4 l of water and the resulting solution was cooled in ice. Chlorine gas was then bubbled into the solution for about 20 minutes. Samples of hypochlorite were withdrawn every few minutes until the solution was found to contain about 1.5 equivalents of hypochlorite per litre. To determine the equivalence, an aliquot of 1 cc was diluted with water, dilute sulphuric acid and solid potassium iodide were added and the liberated iodine was titrated immediately with standard thiosulphate.

Preparation of the Oxylignin

About 10 g of sodium carbonate was added to 1 l of 0.75N sodium hypochlorite to buffer it at pH 12 and the resulting solution was placed in a dark coloured bottle equipped with a stirrer. Sixty grams of periodate lignin was added and the mixture was stirred continuously for 160 hours, the consumption of hypochlorite being followed by withdrawing samples every 24 hours and titrating in the way just described. After 160 hours, the stirring was stopped, the solution transferred to a large beaker and a small quantity of inorganic impurity discarded. The mother liquor was treated with small volumes of acetone to destroy the excess of hypochlorite and was then carefully acidified with 6N hydrochloric acid to pH 2. The yellow, fluffy precipitate which formed at this pH was separated by centrifuge, washed with water and the mixture centrifuged again. In aqueous, neutral solution the oxylignin did not settle in the centrifuge cups even after prolonged action, and complete separation was only achieved in a slightly acidic medium. A thick, yellow paste of oxylignin, allowed to dry partially in air, followed by drying over phosphorus pentoxide _in vacuo_, yielded 22.5 g or 37.5% of the original material. The oxylignin obtained in this manner contained a considerable amount of ash and was purified by dissolving in 95% acetone. A brown, insoluble residue was extracted with 95% acetone in a Soxhlet apparatus and the slightly coloured extract combined with the other. An almost colourless solid remaining in the extraction thimble was found to be inorganic chloride, most probably sodium chloride. The combined acetone solutions were concentrated by distilling most of the acetone and were poured into a large volume of distilled water. The precipitate was centrifuged, washed with water and dried first in air and finally over phosphorus pentoxide.

Found: OCH_3, 8.85, 8.80%; Cl, 5.27, 5.24%;
Ash 0%

This was the oxylignin used in all subsequent experiments.

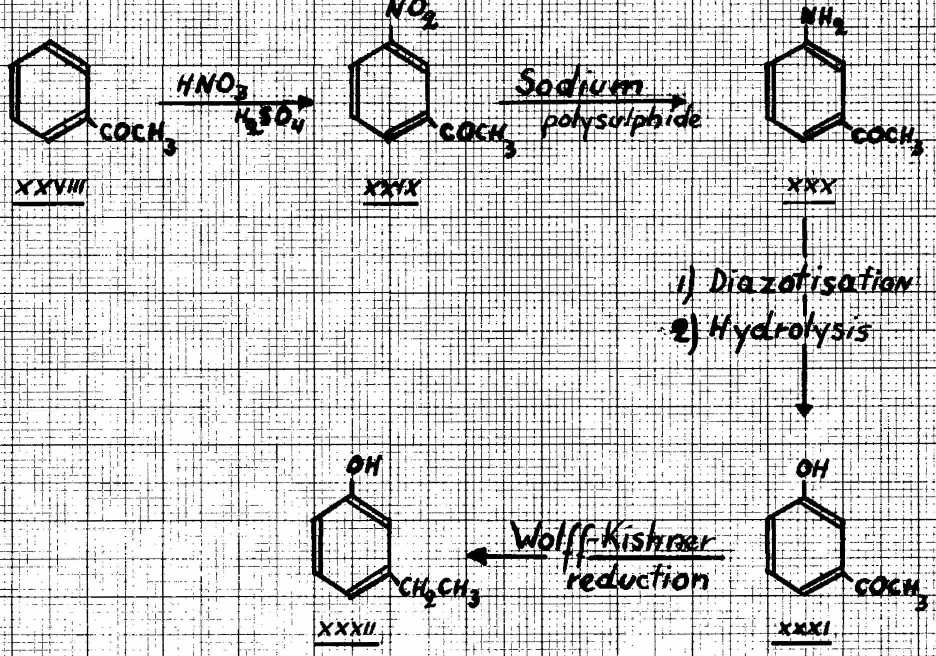
Syntheses

A) m - Ethyl Phenol

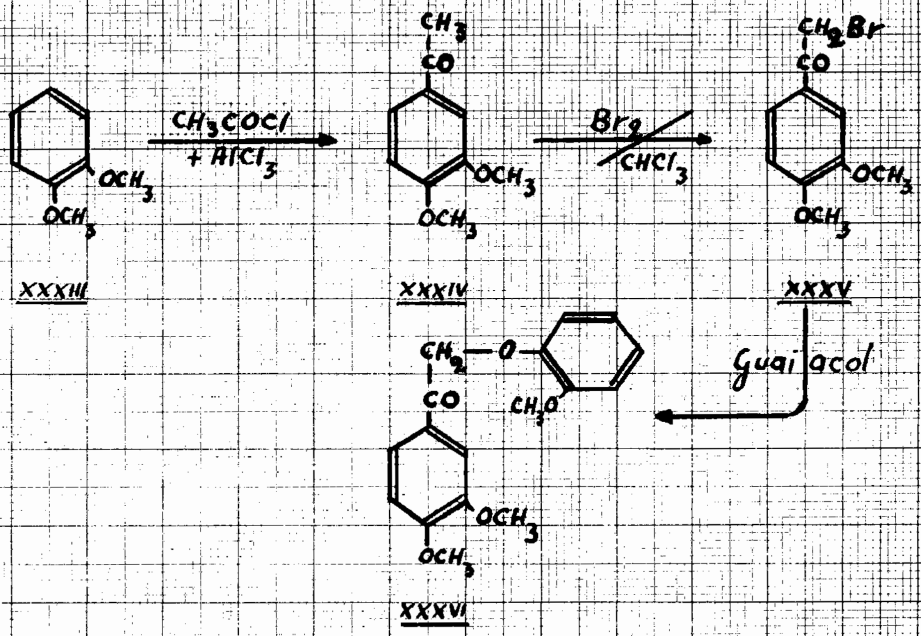
The synthesis was achieved from acetophenone (XXVIII) according to the scheme illustrated on page 81.

Nitration of acetophenone (XXVIII) dissolved in cold (-10°) sulphuric acid, with a mixture of sulphuric and nitric acid according to Kenner and Statham (127) gave, after recrystallisation from alcohol, m-nitro acetophenone (XXIX) in 88% yield. Melting point 80-81°. Reduction of (XXIX) with sodium polysulphide gave a poor yield (30%) of m-amino acetophenone (XXX) as olive-green crystals, melting point 95°. The amine was dissolved in dilute sulphuric acid and was diazotised with sodium nitrite according to E. G. Cobb (128). The resulting diazo compound was dropped into water and boiled, giving a brown tar, which after clarification with charcoal and recrystallisation from water gave m-hydroxy acetophenone (XXXI) in 45% yield. Melting point 95°. The reduction of (XXXI) to m-ethyl phenol (XXXII) was achieved by the Wolff-Kishner method modified by H. Minlon (129) using 80% hydrazine solution and triethylene glycol as the solvent. Crude m-ethyl phenol was purified by distillation under reduced pressure. Boiling point 218° (darkening).

m- Ethyl phenol



ω-(2-Methoxy phenoxy) acetoveratrone



B) ω-(2-Methoxy phenoxy) Acetoveratrone

The above compound was prepared according to the scheme illustrated on page 81.

Veratrole (XXXIII) was subjected to the Friedel-Kraft reaction with acetyl chloride in carbon disulphide according to Koepfli and Perkin (130), yielding acetoveratrone (XXXIV) almost quantitatively. Compound (XXXIV) was brominated in chloroform solution according to Erdtman and Leopold (131). The excess of bromine was removed by bicarbonate solution and the evaporation of the chloroform yielded a light brown oil which crystallised on standing. Recrystallisation from methanol yielded 76% of ω-bromo acetoveratrone (XXXV). Melting point 80°. Compound (XXXV) was converted to ω-(2-methoxy phenoxy) acetoveratrone (XXXVI) by condensation with guaiacol in dry acetone solution and in the presence of anhydrous potassium carbonate as described by Adler, Lindgren and Saeden (24). Yield, after two recrystallisations from methanol, was 67% and melting point was 91°.

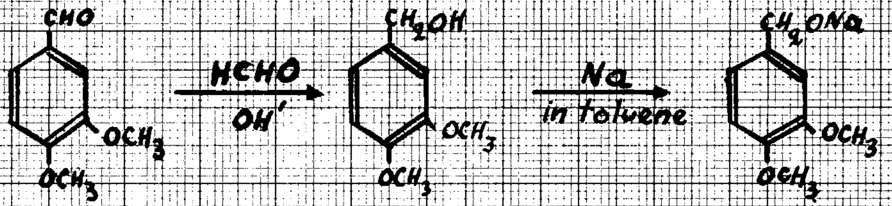
Found: OCH_3, 30.6, 30.9%. Calc.: OCH_3, 30.8%

C) Diveratryl Ether

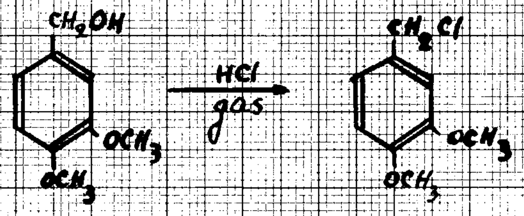
Veratraldehyde (XXXVII) was used as the starting material for the synthesis illustrated on page 83.

Veratryl alcohol (XXXVIII) was prepared in a good yield (about 70%) using the crossed Cannizzaro reaction as described by Davidson and Bogert (132). Veratryl chloride (XL) was obtained in 60% yield by passing dry

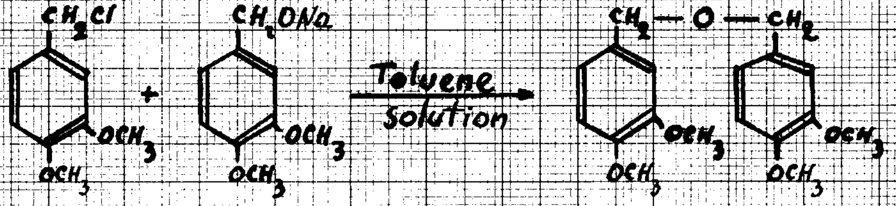
Diveratryl ether



XXXVII XXXVIII XXXIX

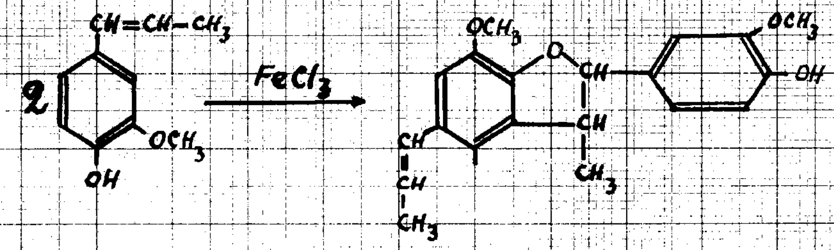


XXXVIII XL



XL XXXIX XLI

Dehydro diisoeugenol



XLII XLIII

hydrogen chloride gas into a cold ethereal solution of (XXXVII) according to Tiffeneau (133) and recrystallising the product from petroleum ether (b.p. 30-60°). Compound (XL) and sodium veratrylate (XXXIX) were condensed in toluene according to Lindgren (134), yielding 47% of diveratryl ether (XLI) which, after recrystallisation from methanol, melted at 69°.

Found: OCH_3, 39.6, 39.4%. Calc.: OCH_3, 39.0%

D) Dehydro Diisoeugenol

Dehydro diisoeugenol (XLIII) was prepared by self-condensation of isoeugenol (XLII) in aqueous alcohol solution in the presence of ferric chloride, as described by Leopold (112). Recrystallisation of the crude product from alcohol yielded 31% of (XLIII), melting point 131°.

Found: OCH_3, 19.1, 18.7%. Calc.: OCH_3, 19.0%

E) 2-(3:4 Dimethoxy phenyl)-2-hydroxy-1-(2-methoxy phenoxy) Ethane

The above compound (XLIV),(page 85) was prepared in 60% yield by the reduction of (XXXVI) with sodium borohydride in ethanol solution, according to Adler, Lindgren and Saeden (24).

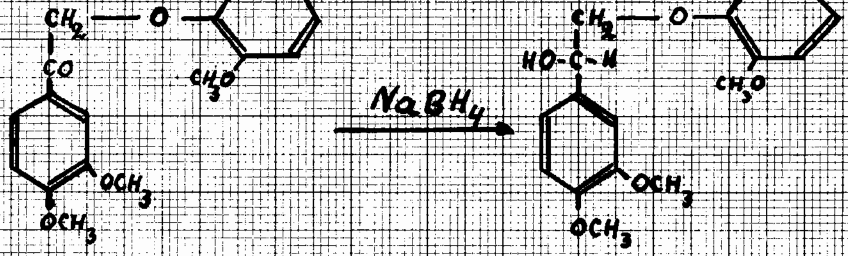
F) Dehydro Diisoeugenol Methyl Ether

This compound (XLV), illustrated on page 85, was prepared in a small yield by direct diazomethylation of XLIII. Method due to Richtzenhain (20).

G) p-Methoxybenzyl Guaiacyl Ether (LV)

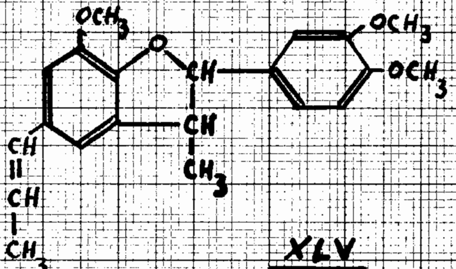
This compound was prepared by the method of

2-(3:4-Dimethoxy phenyl)-2-hydroxy-1-(2-methoxy phenoxy)ethane



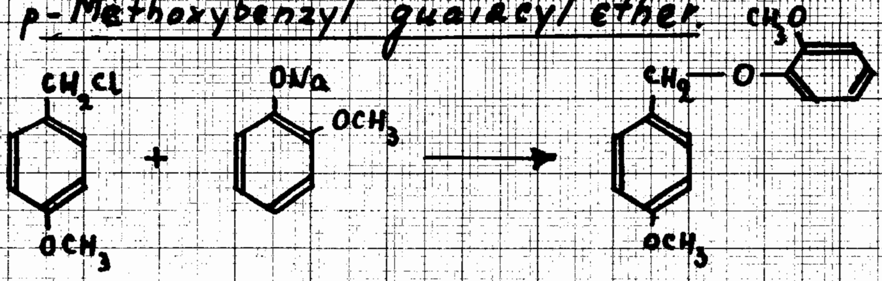
XXXVI XLIV

Dehydro diisoeugenol methyl ether



XLV

p-Methoxybenzyl guaiacyl ether.



LV

Richtzenhain (135) which consisted of condensation of sodium salt of guaiacol with anisyl chloride (LVI), the latter of which was synthesised from anisyl alcohol according to Quelet and Allard (136).

H) 6-Chloro Vanillin

Vanillin (XLVI) was acetylated with an acetic anhydride-sulphuric acid mixture to yield 3-methoxy-4-acetoxy benzal diacetate (XLVII), which was then chlorinated in glacial acetic acid-sodium acetate mixture to give the chlorobenzal acetate (XLVIII). Alkaline hydrolysis of (XLVIII) yielded 6-chloro vanillin (IL), melting point 167°. The method was due to Raiford and Lichty (137).

I) 6-Chloro Vanillic Acid

The preparation of 6-chloro vanillic acid (LII) was achieved from 6-chloro vanillin (IL) by its conversion to an oxime (L) followed by dehydration to the cyanide (LI). Alkaline hydrolysis of (LI) afforded (LII) in 60% yield. The method was due to Raiford and Lichty (138).

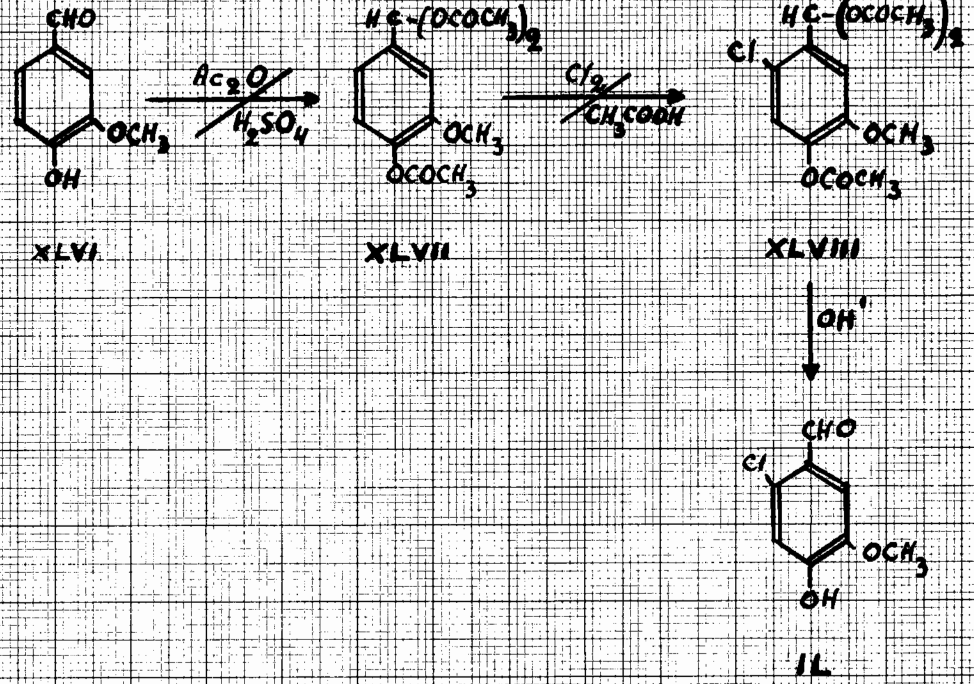
J) 5-Chloro Vanillin

This compound was prepared by direct chlorination of vanillin (XLVI) in chloroform solution. Method was due to Raiford and Lichty (137).

K) 5-Chloro Vanillic Acid

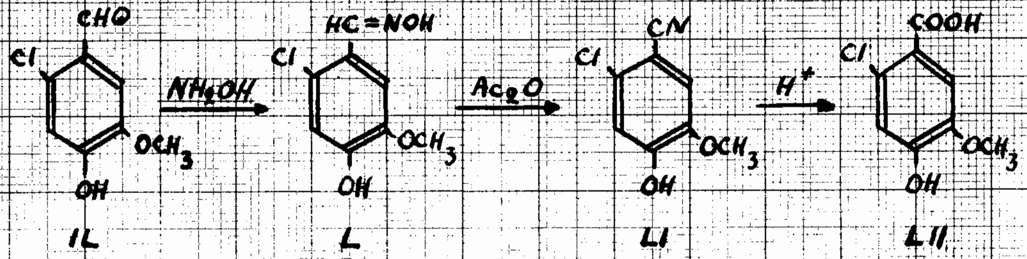
Vanillic acid (LIII), prepared by the oxidation of vanillin (XLVI) with silver oxide according to Pearl (139), gave a good yield of 5-chloro vanillic acid (LIV)

6-chloro vanillin



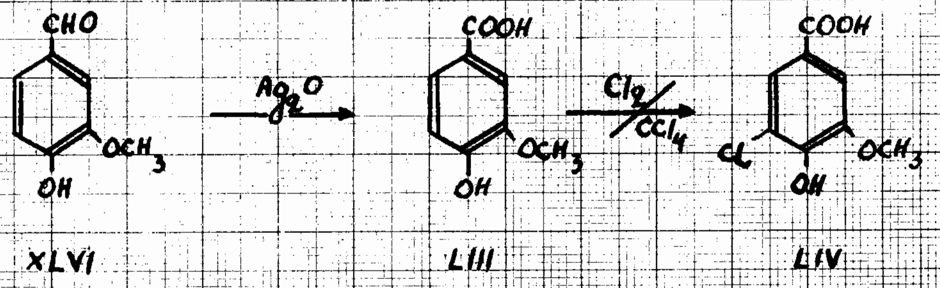
XLVI XLVII XLVIII IL

6-chloro vanillic acid



IL L LI LII

5-chloro vanillic acid



XLVI LIII LIV

when chlorinated in carbon tetrachloride solution. Method was due to Raiford and Lichty (138).

Purification of Tetrahydrofuran

The available commercial grade of "purified tetrahydrofuran" was not suitable for the reduction described on page 106 because of its high moisture content. An initial attempt to employ the liquid "as was" led to an explosion and considerable loss of material. Distillation alone was insufficient; tetrahydrofuran distilled as an azeotropic mixture.

The method of Hrubesch and v. Kupetow (140), where the wet tetrahydrofuran was mixed with a hydrocarbon having a higher boiling point than itself and the aqueous layer was removed followed by distillation of the organic layer, was not believed to be satisfactory for a laboratory preparation.

To prepare pure, moisture-free tetrahydrofuran, the following method was devised. Commercial "purified" tetrahydrofuran was shaken for 6 hours with anhydrous calcium chloride, filtered and distilled. The fraction passing into the receiver at 66-67° was collected and left over sodium wire for 2 days. After removing the excess of sodium, the liquid was redistilled in a nitrogen atmosphere over fresh sodium. The fraction boiling at 66° was collected and stored in an air-tight container.

Preliminary Experiments

Rate of Chlorine Substitution in Periodate Lignin

Ten grams of periodate lignin were treated with alkaline hypochlorite as described under the preparation of oxylignin. Samples of about 20 cc were withdrawn at intervals during a period of 144 hours and any solid present in the sample dissolved on addition of acetone, which was added immediately to decompose the hypochlorite. The oxylignin, which precipitated on adding 6N hydrochloric acid, was carefully purified as described under the preparation of oxylignin to eliminate any inorganic chloride. Results are given in Table VI, page 34.

Reaction of oxylignin with cyanide ions

Two grams of oxylignin, 50 cc of ethyl alcohol and 5 g of potassium cyanide were heated under reflux for 8 hours. The oxylignin dissolved completely forming a wine-red solution which, after cooling and diluting with slightly acidified water, yielded a precipitate. This precipitate was dissolved in alcohol and reprecipitation in water was repeated twice, the final precipitate being named cyanolignin. Yield 1.5 g.

Found: Cl, 3.3, 3.5%; N, 1.16, 1.18%; N.E. 471

The cyanolignin, 0.75 g, was then hydrolysed by heating under reflux with 50 cc of 10% sodium hydroxide solution for 2 hours. The hydrolysed product was precipitated by acidification followed by purification as just described.

Found: Cl, 2.35, 2.50%; N, 0.0%

Tests for Functional Groups in Oxylignin

The oxylignin was insoluble in water, dilute mineral acids and anhydrous organic liquids like pyridine, acetone, dioxane, chloroform, ether, alcohols, but dissolved in them upon addition of even small quantities of water. Dilute sodium hydroxide and 5% sodium bicarbonate solution dissolved the oxylignin readily, the latter with evolution of carbon dioxide. The neutralisation equivalent was 277.

Routine laboratory tests for carbonyl groups, unsaturation and phenolic hydroxyl groups were inconclusive. The oxylignin, however, slowly liberated iodine from acidified potassium iodide solution and exhibited a slight change of colour on heating with sodium hydrosulphite.

(1) Esterification followed by reduction

About 1 g of oxylignin was added to 25 cc of absolute methyl alcohol containing a small quantity of anhydrous zinc chloride. On heating to boiling point, the solution became almost clear except for a fine suspension. The mixture was refluxed gently for one-half hour and most of the alcohol was then distilled under reduced pressure, leaving behind a gum which did not possess free carboxylic acid groups. The material was dried and dissolved in pure tetrahydrofuran. Lithium aluminium hydride, 5 g, dissolved in tetrahydrofuran, was added slowly, with stirring, and under a nitrogen atmosphere, to this solution. The vigourous reaction was allowed to proceed until it subsided and the solution was then heated under reflux for an additional 15 minutes. After decomposing the excess of hydride by the careful addition of moist tetrahydrofuran, the heavy precipitate which had formed during the reaction was removed on a filter. The filtrate contained only tetrahydrofuran, while attempts to extract the residue with ether failed. The residue was suspended in water and dilute sodium hydroxide was added to precipitate aluminium and zinc hydroxides, which were discarded. Acidification of the filtrate with dilute hydrochloric acid caused the separation of a light brown precipitate which was centrifuged, washed repeatedly with water, but even after 5 washings still contained much ash. The precipitate was dried and extracted with acetone in a Soxhlet apparatus, reprecipitated by dilute hydrochloric acid, washed and dried.

Found: Ash, 1.5%; OCH_3, 10.8, 10.0%

(2) Addition of dry hydrogen chloride gas

Dry hydrogen chloride gas was passed for 10 minutes into 60 cc of freshly distilled anhydrous chloroform. Oxylignin, 0.5 g, was suspended in the resulting saturated solution, which was then heated under reflux for 45 minutes. The solution, which never became clear, was acidified with 6N hydrochloric acid, the two layers were separated and centrifuged. A yellow solid, 0.35 g, resembling the original oxylignin, was separated and purified in the usual manner.

Found: Cl, 5.84, 6.00%

(3) Acetylations with a variety of reagents

Method a Oxylignin, 280 mg, was treated with 15 cc of acetic anhydride, but even on heating failed to dissolve. The suspension was cooled and 5 drops of pyridine were added. The resulting solution was stirred for 1 hour, was allowed to stand overnight and was poured into a large volume of water. A light brown powder (225 mg) was collected and dried in the usual manner.

Found: Acetyl, 7.35, 7.45%

Method b Oxylignin, 276 mg, dissolved in a small quantity of 5N sodium hydroxide solution, was cooled with crushed ice, and acetic anhydride was immediately added. The mixture was shaken vigourously for a few minutes, allowed to stand overnight and poured into a large quantity of acidified water. Yield 180 mg.

Found: Acetyl, 5.10, 4.80%

Method c Oxylignin, 250 mg, went completely into solution on adding 3 drops of concentrated sulphuric acid to its suspension in acetic anhydride. The solution, which darkened somewhat, was left overnight and poured into a large volume of acidified (pH 2.5) water. The tan powder, obtained in 65% yield, was centrifuged and dried. This product was soluble in acetone and lower alcohols.

Found: Acetyl, 9.80, 9.60%

Method d A suspension of 110 mg of oxylignin in acetic anhydride was treated with 0.2 g of zinc dust and 6 drops of concentrated sulphuric acid. The mixture was stirred magnetically for 48 hours. Undissolved zinc was removed, the filtrate was boiled for a few minutes and poured into acidified water. The dark brown powder, obtained in 55% yield, was purified and dried.

Found: Acetyl, 9.50%

Alkaline Nitrobenzene Oxidation of Oxylignin

The oxylignin, 7.5 g, was dissolved in 285 cc of 2N sodium hydroxide and the resulting solution was poured into three stainless steel bombs, each of about 125 cc capacity. Freshly distilled nitrobenzene was added (5 cc per bomb) and the bombs closed tightly. The rotating autoclave, immersed in oil, was heated as rapidly as possible to 175° and maintained at this temperature for 2 hours (78). At the end of this period, the bombs were mechanically raised from the oil bath and cooled by a powerful jet of air while they were still rotating. The contents, when sufficiently cooled, were poured into a separatory funnel and a small quantity of oil was separated from the bulk of the solution. This oil, 0.6 g, contained nitrogen and was discarded. The remaining solution was steam-distilled to remove any unchanged nitrobenzene and then concentrated under reduced pressure to its original volume (about 300 cc). The solution was then extracted with benzene to remove any possible traces of nitrobenzene and other neutral materials and the extract combined with the nitrobenzene obtained from the steam distillation. Fractional distillation of the benzene-nitrobenzene mixture yielded 13.5 cc of unchanged nitrobenzene and a small quantity of oil, both of which were discarded. The aqueous residue was carefully acidified with 40% sulphuric acid to pH 2 and repeatedly extracted with ether. The ether extract, which amounted to 16.4% of the starting material, was extracted twice in succession (77) with each of the following reagents:

Sodium bisulphite - 21% solution
Saturated sodium bicarbonate solution
Sodium hydroxide - 5% solution

yielding respectively 6.4%, 7.3% and 2.7% based on the weight of the starting material.

Recrystallisation of the crude product from the extraction with bisulphite yielded crystals with the characteristic odour of vanillin.

Found: OCH_3, 19.8%; Cl, trace; melting point 78°

Calc. for vanillin: $C_8H_8O_3$: OCH_3, 20.4%; Cl, 0%; melting point 80° (141)

To confirm the presence of vanillin, the 2:4 dinitro phenylhydrazone was prepared. The compound melted at 268° undepressed by admixture with an authentic sample with the recorded (142) melting point of 267-268°.

The sodium bicarbonate extract was acidified and extracted with ether, the extract being evaporated under reduced pressure. When the residue was dissolved in dilute alcohol and a small quantity of dilute hydrochloric acid was added, an impure substance precipitated, which after recrystallisation from very dilute acetic acid melted at 202-204°. Repeated recrystallisation increased the melting point to 205-206°, the value recorded for vanillic acid being 210° (143).

Found: OCH_3, 17.2%; Cl, trace; N, (by sodium fusion) not detected; N.E., 177

Calc. for vanillic acid $C_8H_8O_4$: OCH_3, 18.4%; Cl, 0%; N, 0%; N.E., 168.

The acetate was prepared by shaking acetic anhydride with the cold solution of the material in dilute sodium hydroxide solution (144) and after two recrystallisations melted sharply at 146°, undepressed by the addition of an authentic sample of O-acetyl of vanillic acid with the recorded (145) melting point of 146°.

The sodium hydroxide extract was acidified and extracted with ether, the extract being evaporated under reduced pressure. Chromatographic analysis of this crude fraction indicated the absence of carboxylic acids, but vanillin and possibly a minute quantity of 6-chloro vanillin were detected. At least three other phenolic compounds were present. Sodium fusion revealed the presence of nitrogen and of chlorine. Found: Cl, 1.70%.

In view of the small amount of material available, the presence of nitrogen and the results from previous fractions, no further work was carried out on the phenolic fraction.

A portion of the aqueous residue from the ether extractions was analysed for chloride ion, which amounted to about 30% of the chlorine originally present in the oxylignin. The remaining solution was extracted with butanol, the inorganic salts were removed and the butanol removed under reduced pressure. About 5 g of a thick, undistillable oil remained and was not examined any further.

<u>Chromatographic analysis of products from alkaline nitrobenzene oxidation.</u>

Butanol saturated with 2% aqueous ammonia (77) was used as a solvent system for phenols and phenolic acids (page 86) which could have been formed from the oxylignin by the alkaline nitrobenzene oxidation. Vanillin at R_f 0.36 and 5-chloro vanillin at R_f 0.46 could be readily distinguished by using an aniline hydrogen phthalate spray, which gave a fluorescent spot with vanillin. The spot corresponding to 6-chloro vanillin at R_f 0.66 was not fluorescent but the difference in R_f value permitted the differentiation from the corresponding 5-chloro compound.

Vanillic acid and mono chloro derivatives of vanillic acid (page 86) moved rather slowly on the chromatogram. Vanillic acid and 6-chloro vanillic acid had very similar R_f values. Attempts to remedy this deficiency by introducing new solvent system failed. It was found that, if the chromatograms were allowed to run for 60 hours, then the spots separated sufficiently to permit identification.

During this period the solvent front ran off the paper and therefore no R_f values could be calculated.

Ether extracts from the acidified sodium bisulphite and sodium bicarbonate fractions (page 94) were chromatographed against vanillin and its mono chloro derivatives, and against vanillic acid and its mono chloro derivatives, respectively, using the solvent system and conditions just described. The first of the above extracts gave a fluorescent spot whose R_f value was identical to that of vanillin. The second (ie acidic) extract, when chromatographed, strongly suggested the presence of a large proportion of vanillic acid and left the presence of a little 6-chloro vanillic acid doubtful. The possibility of 5-chloro vanillic acid being present was definitely excluded.

<u>Chromatography of oxylignin.</u>

The method developed by A.Bailey (146) was applied to the oxylignin. This method consists of placing an 18 cm square of Whatman No. 1 filter paper between somewhat larger glass plates, one of which had a hole (about 1 cm in diam.) drilled through the centre. Butyl acetate was applied through the hole dropwise until capillary action carried the liquid to the rim of the filter paper. A drop of a solution of the oxylignin in dioxane was then applied slowly to the centre of the paper. As soon as this was absorbed, butyl acetate was applied slowly with a dropper to a cotton ball in the centre and the application was continued while the capillarity carried the oxylignin forward. In this way a total of about 20 mg of the oxylignin was applied. A small portion of the oxylignin moved to the outer portion of the paper but the bulk travelled only a short distance, forming a many-cornered star near the point of application. The overall picture was very similar to that obtained on butanol lignin by A. Bailey (146).

The chromotographic method on a strip of Whatman No. 1 filter paper, using the ternary solvent system butanol: acetic acid : water (50:2:48) with ammoniacal silver nitrate, ferric chloride or bromocresol green as spraying agents, developed by Bate-Smith and Westall (147), (148) for separation of phenolic substances, failed to separate the oxylignin.

Another attempt to chromatogram the oxylignin using a butanol : ethanol : formic acid : water solvent system in the ratio of 10:2:2:2 and a three stage spraying with potassium ferrocyanide, ferric ammonium sulphate and ammonium hydroxide in the order given (149), resulted only in heavy "tailing".

Fractionation of the oxylignin.

Fifteen grams of oxylignin (OCH_3, 8.82%; Cl, 5.25%) was dissolved in 300 cc of acetone containing a few cc of water. Petroleum ether (boiling range 30-60°) was added in portions and the oily precipitate formed after each addition was recovered, dried and analysed. The data are summarised in Table VIII, page 44.

Further fractionation of the fraction 1.

The oxylignin, 4.99 g, was dissolved in 100 cc of acetone to which 1 cc of water had been added. Petroleum ether, 25 cc, (boiling range 30-60°) was added and 3.45 g or 69% of the oxylignin (fraction 1 of the previous fractionation) precipitated. Found: Cl, 3.82, 3.77%. This fraction after drying was redissolved in 70 cc of moist acetone and petroleum ether was added as shown in Table IX, page 45.

Action of selective reducing agents on oxylignin.

(1) Reduction of oxylignin by Clemmensen method (90), (91), (92).

The dioxane used in this experiment was purified by boiling with dilute acid to hydrolyse any acetal, followed by the addition of excess of solid potassium hydroxide to remove water and finally by refluxing over sodium to resinify the acetaldehyde as described in Vogel (150).

Method a One gram of the oxylignin suspended in 5 cc of this dioxane was added in small portions over a period of 3 hours to a boiling mixture of 10 cc of water, 4 cc of dioxane, 25 cc of concentrated hydrochloric acid, and the amalgamated zinc prepared from 3.5 g of the metal and 0.2 g of mercuric chloride. The mixture was heated under reflux for 10 hours, during which time a further 20 cc of hydrochloric acid was added.

Method b A suspension of 1 g of oxylignin in a mixture of 7.5 cc of water, 17 cc of concentrated hydrochloric acid and 10 cc of toluene was added to an excess of amalgamated zinc. The mixture was refluxed briskly for 20 hours, during which time a 5 cc portion of concentrated hydrochloric acid was added every 6 hours.

Method c The method was the same as in b) above, except that the oxylignin was first dissolved in dioxane.

At no time did the oxylignin go into solution, and in all cases a sticky, rubber-like resin was formed to the extent of 46-67%. After the removal of the resin, zinc was precipitated as the hydroxide but the separation was never quantitative. Subsequent ether extracts were always contaminated with zinc salts, which were fairly soluble in ether. These extracts yielded pungent red oils in such small quantities that no purification or fractionation was possible. Spot tests on the products from the extractions indicated phenolic material (solubility in sodium hydroxide, reaction with benzoyl chloride, ferric chloride test). In view of the 50-60% loss of the original material by the formation of insoluble resins, and the low yields, contaminated with zinc salts, further work on the Clemmensen reduction of oxylignin was discontinued.

Wolff-Kishner Reductions (95) (96)

Method a Pilot experiment at a lower temperature.

Pyridine (boiling point 117°) was chosen as the solvent on account of its comparatively low boiling point, its basic character and its stability, while benzophenone was selected as the reducible compound. Eighteen grams of benzophenone, 100 cc of dry pyridine, 7 g of potassium hydroxide and 12 cc of 85% hydrazine hydrate were heated under reflux for 7 hours. The solution turned red almost immediately. After cooling and acidifying, the mixture was extracted with ether, the ether washed several times with dilute hydrochloric acid and finally with water. Evaporation of the dried ether extract yielded yellow crystals, from which traces of pyridine were removed by repeated washings with dilute acid. The product was finally recrystallised from alcohol yielding 12 g of pale yellow crystals, melting point 162°. The presence of nitrogen was proved by a sodium fusion and a search of the literature (151) revealed that diphenyl ketazine melted at 162-164°. A higher temperature was thus an essential part of the Wolff-Kishner reduction if the production of nitrogen-containing intermediates was to be avoided.

Method b Reduction of oxylignin

The modified method due to Huang-Minlon (129) was used.

Triethylene glycol, 90 cc, solid potassium hydroxide, 5 g, hydrazine hydrate (85% solution), 7 cc, and 5 g of oxylignin were boiled under reflux for 1½ hours in a 3-necked flask equipped with a stirrer, air condenser and a thermometer. The initial boiling point of the mixture was 173° and reached 176° at the end of the refluxing time. After this period, the condenser was removed and the more volatile matter allowed to evaporate by heating the contents up to 190° for about 5 minutes. The condenser was replaced and the mixture again heated under reflux for 3.5 hours. The solution was allowed to cool and 20% hydrochloric acid was added, followed by sodium bicarbonate until the pH reached the value of 3. After being extracted with 3 portions of ether, the acidic liquor was made more acid and again extracted with ether. The combined ether extracts were treated with two portions of 1N sodium hydroxide (which became strongly coloured) and the alkaline extract was acidified, whereupon a yellow, fine suspension separated. This acidic solution was then repeatedly extracted with ether, the ethereal extract washed with water and dried with anhydrous sodium sulphate. When the evaporation of ether from the resulting golden yellow solution was almost complete, some needles separated in amount too small for purification. These needles melted over the range 165-170° and micro tests suggested that they were phenolic. The small remainder was benzoylated with benzoyl chloride in alkaline solution yielding a crude product, which could not be purified and which melted over the range 190-205°. The above data, although far from accurate, suggested the presence of hydroquinone. The rest of the ethereal extract could not be fractionally distilled because of its small volume, but chromatographic analysis by the method of Hossfeld (100) revealed spots, whose R_f values and colour were identical to those given by authentic samples of guaiacol, phenol and eugenol (the last one only in a very small amount). The presence of guaiacol was confirmed by its isolation through distillation in vacuo and identification as a crystalline tri-bromo derivative. No phenol or eugenol could be obtained in a pure state.

The acidity of the aqueous residue was adjusted to pH 1 and a solid which separated was recovered on the centrifuge. This precipitate, which contained inorganic chloride, was extracted in a Soxhlet apparatus with acetone. The concentrated extract when poured into acidified water yielded 600 mg (14%) of a dark brown powder.

Found: Cl, 8.45, 9.20%; OCH_3, 10.2, 9.7%; N, 0%; Ash, 0%.

On the gradual addition of strong potassium hydroxide to the mother liquor, a precipitate began to form almost immediately; the addition was stopped at pH 6.5, the precipitate was isolated and washed repeatedly with water to remove potassium chloride. The remaining brown solid amounted to 350 mg or 8% of the original material.

Found: OCH_3, 9.6, 9.8%; Cl, 0.94, 1.10%

The residual liquor was still strongly coloured and after acidification was extracted with butanol. The extract was washed 3 times with water to remove triethylene glycol and was finally distilled under reduced pressure almost to dryness, the residue proving to be a thick, undistillable tar, 2.5 g, insoluble in ether or acetone and partially soluble in boiling dioxane. Attempts to cause partial precipitation by adding organic liquids to the dioxane-soluble fraction invariably caused reprecipitation of the tar in bulk (about 1.5 g). Altogether, the fractions amounted to about 80% of the starting material.

Method c Reduction of periodate lignin

Periodate lignin, 20 g, triethylene glycol, 200 cc, solid sodium hydroxide, 12 g and 15 cc of 85% hydrazine hydrate solution were placed in a 3-necked flask and were treated as described for oxylignin, page 101.

The solution, after diluting with 100 cc of water, was acidified to pH 2 with hydrochloric acid and was extracted for 48 hours with ether in a liquid-liquid extractor. The ethereal layer was treated with 1N sodium hydroxide, which became strongly coloured. This alkaline solution was acidified to pH 2 and was again extracted with ether, water being removed from the extract by anhydrous sodium sulphate. After removal of ether, the remaining dark liquid was distilled under high vacuum. A slightly coloured liquid was collected and its boiling point under normal pressure, determined by a micro method, was 214-216° with darkening. Standard tests revealed the phenolic character of the liquid. The aryloxyacetic acid derivative was prepared as described in Vogel (152) by boiling the sodium salt of the compound with 50% chloroacetic acid solution, then acidifying to Congo red, extracting with ether and separating the derivative as the sodium salt by shaking the ether extract with sodium carbonate solution. After acidification of the sodium carbonate layer, 110 mg of white crystals were recovered which, after recrystallisation from dilute alcohol, melted sharply at 75°.

Carbon and hydrogen analyses, determined with a newly assembled and tested micro combustion train, gave the following results:

Found: C, 66.60, 66.55%; H, 6.72, 6.63%

Calc. for ethyl phenyl oxoacetic acid: $C_{10}H_{12}O_3$:
C, 66.7%; H, 6.67%

The melting point of the aryloxyderivative 75°, agreed with that reported (153) for the same derivative of m-ethyl phenol, and a mixed melting point with the specially synthesised sample was not depressed.

On diluting the original aqueous residue with a large quantity of acidified water, a considerable amount of solid was precipitated. This solid was purified by dissolving in acetone, separating and discarding a small amount of insoluble tar, and pouring the solution into a large volume of water. After thorough drying, the product weighed 2 g.

Found: OCH_3, 7.85, 7.80%; N.E. 181, 183

The remaining solution was extracted with a large quantity of butanol.

The butanol extract was washed with water in an attempt to remove the triethylene glycol and then was fractionally distilled under reduced pressure. When all the water and butanol were removed, the only product distilling over was triethylene glycol (proved by refractive index). At this point the distillation was stopped and the remaining thick, heavy liquid was treated with water in a repeated effort to remove the triethylene glycol. A large amount of solid (7 g) precipitated on addition of water. This solid was readily soluble in acetone, alcohol and partially so in dilute sodium hydroxide solution. When treated with 5% sodium hydroxide solution, 3 g remained undissolved and were separated by a centrifuge. The alkaline extract was benzoylated with benzoyl chloride and the product was recrystallised from dilute alcohol.

Found: N.E., 145, 143; OCH_3, trace; Melting point, 112-116°

The portion insoluble in sodium hydroxide solution was reprecipitated by acid forming a tar, which was not examined.

Reduction with Lithium Aluminium Hydride (154)

Powdered lithium aluminium hydride, 10 g, suspended in 150 cc of tetrahydrofuran, was placed in a 4-necked round bottom flask equipped with a condenser, mercury sealed stirrer, separatory funnel and nitrogen inlet. During the whole experiment the moisture was rigidly excluded from the system by passing dry nitrogen and attaching calcium chloride tubes on top of the condenser and separatory funnel. Oxylignin, 4 g, previously shaken for 6 hours with 200 cc of tetrahydrofuran, was gradually added to the lithium aluminium hydride suspension. During this operation, a considerable evolution of gas and heat was observed and the contents of the flask became gelatinous. After the initial vigour of the reaction had subsided, the mixture was heated under reflux for 3 hours, and then the excess of hydride was destroyed by the very cautious addition of wet tetrahydrofuran, the flask being cooled in ice and the hydrogen diluted by passing nitrogen. The resulting slurry was treated with dilute sulphuric acid and heated under reflux for one hour, whereupon two layers formed; the upper one being wine-red while the lower was light yellow in colour. On cooling, large amounts of white crystals formed, initially at the interface and then in both layers. These crystals were removed, together with a small quantity of brown powder, and were readily identified as aluminium sulphate. The brown powder, 0.27 g, fraction 1 was separated from the inorganic salts by dissolving them in water.

Found: Cl, 2.4, 2.5%; OCH_3, 4.8, 4.9%.

The organic layer was separated from the aqueous portion and each part was extracted with ether. Evaporation of the ether extracts yielded no products. The ether-extracted aqueous portion was concentrated to about one-half of its original volume by evaporation under reduced pressure, and a large excess of acetone was added. The bulky precipitate was rejected, the acetone evaporated and the theoretical amount of barium hydroxide was added to the aqueous residue to precipitate sulphate ion. The remaining sulphate-free solution was evaporated to dryness and extacted in a Soxhlet with acetone. When the extract was diluted with ether, fraction 2, 0.80 g, was precipitated.

Found: Cl, 2.6, 2.3%; OCH_3, 8.05, 8.15%.

The portion insoluble in acetone remained as a dark tar, completely insoluble in ether. This tar, when boiled with acetone, yielded an extract which was slowly diluted with ether. A thick, brown oil separated. Attempts to distill this oil under vacuum yielded about 1 g of a pungent, colourless liquid, apparently not phenolic in character, which could not be identified. The rest of the material formed a hard layer adhering to the walls of the distilling flask and continously blocking the capillary tube. Further investigation of this residue was discontinued. The residue insoluble in boiling acetone, fraction 3, 0.53g, was analysed.

Found: Cl, 1.73, 1.85%; OCH_3, 3.4, 3.0%.

Reduction of Oxylignin with Sodium Borohydride (106)

Method a Reduction of sodium salt

Oxylignin, 5 g, was dissolved in 25 cc of 0.1 N sodium hydroxide, and 5 g of powdered sodium borohydride was carefully added. No evolution of heat was observed but the solution acquired a lighter colour. The mixture was heated gently under reflux for 1 hour, allowed to cool, and dilute hydrochloric acid was added drop-wise with stirring and cooling. A yellow-white precipitate was recovered on the centrifuge and was washed, but on drying over phosphorus pentoxide turned dark brown. The precipitate was then purified by extraction in a Soxhlet apparatus with acetone, yielding 3 g (60%) of a light yellow powder whose UV spectrum was very similar to that given by untreated oxylignin.

The Soxhlet thimble retained a sticky, unmanageable oil, about 1 g, which was insoluble in all organic liquids but dissolved partially in hot sodium hydroxide solution. The mother liquor was extracted at pH 2 with ether and 0.1 g of a light brown powder was obtained.

Found: OCH_3, 14.0, 14.5%; Cl, 9.3, 10.0%

Method b Reduction of methyl ester

A stirred solution of 0.1 mole of sodium borohydride in 50 cc of the dimethyl ether of diethylene glycol and 1.2 g of methylated (OCH_3 - 20.2%) oxylignin was treated slowly with a 2M solution of anhydrous aluminium chloride in the same solvent. The methyl ester dissolved almost completely in the dimethyl ether of diethylene glycol, and on adding aluminium chloride a white gelatinous precipitate formed. The reaction was allowed to proceed as in a). Upon completion, dilute acid was added and the mixture was boiled to hydrolyse the complex. The gelatinous mass dissolved, leaving in the flask a brown powder (0.3 g).

Found: OCH_3, 10.5, 11.0%

After aluminium ion had been removed by adding sodium hydroxide solution and the precipitate had been discarded, the solution was adjusted to pH 2 and extracted with ether. Evaporation of the extract left a small (0.1 g) residue.

Found: OCH_3, 3.36, 3.50%

The remaining liquor was evaporated under reduced pressure to about one-half of its volume, a large quantity of ethanol was added and a white inorganic precipitate was discarded. The excess of ethanol and water was then distilled and the residue cooled. The heavy precipitate was washed in acetone and, on evaporation, the acetone-soluble portion separated as 0.5 g of a slightly coloured substance. This fraction was soluble in water, 5% sodium hydroxide solution, 5% sodium bicarbonate solution, insoluble in 5% hydrochloric acid and in ether.

Found: OCH_3, 2.2, 2.6%; N.E., 354

The acetone-insoluble fraction was not investigated.

Reduction with Zinc in Alkali

Oxylignin, 1 g, was dissolved in 75 cc of 0.1 N sodium hydroxide and 2 g of zinc dust was added. Initially the reaction was quite violent and strong external cooling was applied. The solution was heated under reflux for 2 hours, (abundant foaming) then acidified, whereon a large (0.7 g) precipitate, resembling the original oxylignin, settled. Purification was achieved by an acetone-water cycle.

Found: Cl, 1.35%; OCH_3, 10.0%; N.E., 168

The remaining solution when extracted with ether yielded a negligible extract.

Reduction with Zinc in Glacial Acetic Acid

Oxylignin, 1 g, was dissolved in 50 cc of glacial acetic acid containing a few drops of water. Zinc dust, 2 g, was added and the mixture heated under reflux for 6 hours. The resulting solution was almost clear when hot but became cloudy on cooling. After the hot solution had been filtered, the filtrate was made alkaline with sodium hydroxide solution and was treated with hydrogen sulphide until the precipitation of zinc sulphide was complete. The filtrate was then acidified and the excess of hydrogen sulphide removed by boiling. The resulting solution was golden yellow, and extraction with ether failed to yield any product. The acidic mother liquor was concentrated under reduced pressure, an inorganic precipitate was discarded, and the remaining solution was evaporated over solid potassium hydroxide. The residue was treated with hot acetone and the extract evaporated to yield 0.75 g (75%) of a yellow gum free of sulphur and labile chlorine.

Found: OCH_3, 6.75, 7.00%; Cl, 1.40, 1.55%

A similar reduction of the oxylignin with magnesium in glacial acetic acid resulted in a 75% yield of resinous material which was insoluble in organic liquids and was for this reason discarded.

Oxidation of Ether Model Compounds with Alkaline Hypochlorite (19) (20)

A suspension of .76 g of ω-(2-methoxy phenoxy) acetoveratrone (XXXVI) in 250 ml of 0.4 N sodium hypochlorite, buffered at pH 12, was stirred mechanically for 21 hours at room temperature. The consumption of the hypochlorite was followed by withdrawing 2 cc aliquots and titration as described under the preparation of the oxylignin. At the end of the period, the suspension was allowed to settle and the precipitate amounting to 0.3 g (39.5%) was removed on a filter and recrystallised from methanol.

Found: OCH_3, 30.7%

Calc. for $C_{14}H_9O_2(OCH_3)_3$: OCH_3, 30.8%

The melting point, 91°, was undepressed by the admixture of synthetic ω-(2-methoxy phenoxy) acetoveratrone.

The filtrate was treated with an excess of acetone to destroy any residual hypochlorite, was then acidified to pH 1 and extracted with ether. The evaporation of the ether yielded crude acidic material, 0.4 g, melting range 155-170°, which when recrystallised twice from very dilute acetic acid melted at 181°. This melting point agreed with that reported for veratric acid (155) and a mixed melting point with pure veratric acid showed no depression.

The same procedure with 2-(3:4-dimethoxy phenyl)-2-hydroxy-1-(2-methoxy phenoxy) ethane (XLIV) yielded 20% of veratric acid; the starting material was recovered in 55% yield.

In the identical treatment of methylated dihydrodiisoeugenol (XLV) no consumption of hypochlorite was observed and the starting material was recovered in an almost quantitative yield.

Action of 47% Hydriodic Acid on Ether Model Compounds

Hydriodic acid (47%), 21 cc, was poured over 1.33 g of ω-(2-methoxy phenoxy) acetoveratrone (XXXVI) kept in a flask immersed in a thermostat maintained at 50°. The mixture was stirred mechanically for 8 hours but never became homogenous, although it acquired a wine colour. After 8 hours, the mixture was diluted with water, the solid residue removed by filtration and washed with water, 5% potassium iodide and again with water. The washings were discarded. The filtrate was continuously extracted with ether, but on drying and evaporation, only a negligible residue was obtained.

The washed residue from the original filtration, which was dark in colour and weighed 1.35 g, was insoluble in water, dilute acids or alkali and soluble in ether or acetone. A standard test revealed the presence of a keto group. The crude material was recrystallised four times from dilute alcohol, yielding 0.7 g of slightly yellow material, melting point 90-92°. This material readily gave an oxime, which after recrystallisation melted sharply at 119.5°, while the oxime of the original substance melted at 119°.

Found (on oxime): OCH_3, 29.2%

Calc. for $C_{14}H_9O\ (NOH)\ (OCH_3)_3$: OCH_3, 29.3%

The melting points of the above compounds were unchanged by the admixture of authentic samples of ω-(2-methoxy phenoxy) acetoveratrone (24) and of its oxime, respectively.

When 1.01 g of dehydro diisoeugenol (XLIII) was treated in the same manner with 47% hydriodic acid, 93% of the starting material was recovered unchanged. This was confirmed by mixed melting point and methoxyl determinations.

Found: OCH_3, 19.1, 18.8%

Calc. for dehydro diisoeugenol $C_{20}H_{22}O_4$: OCH_3, 19.0%

Similarly, the methylated dehydro diisoeugenol (XLV), and 2-(3:4-dimethoxy phenyl)-2-hydroxy-1-(2-methoxy phenoxy) ethane (XLIV) were recovered unchanged.

When a similar reaction was carried out with 1.02 g of diveratryl ether (XLI), a brown layer was formed on the surface of the hydriodic acid solution, but this layer gradually disappeared during the first 2 hours. After another 2 hours, a gum-like lump of brown solid was formed, which gradually and partially dissolved in the hydriodic acid solution turning it dark brown in colour. Sticky particles adhered to the walls of the reaction vessel for the remainder of the reaction time. After the period of 8 hours, the solution was cooled and diluted with water. A light brown precipitate, 0.7 g, was removed by filtration and was recrystallised twice from 1:1 methanol-acetone mixture. This material melted over a range of 90-110° and had a methoxyl content of 27.8%. No phenolic properties were detected. The ether extract of the mother liquor, after drying and evaporating, failed to yield any appreciable residue.

p-Methoxy guaiacyl ether (1 g), when treated in a similar way, dissolved in hydriodic acid completely and almost immediately. After 6.5 hours the reaction was stopped, water was added but no precipitate was formed. The resulting solution was extracted with ether yielding a wine-red extract, which was washed with 5% potassium iodide solution, water and then treated with 5% sodium hydroxide solution. The alkaline solution was extracted with ether, the ether-soluble material being discarded. The remaining aqueous phase was acidified to pH 2 and again extracted with ether. After drying and evaporation of the ether, about 0.3 g of an oily material remained. This material exhibited definite phenolic properties, as shown by standard tests.

Reductions with Hydriodic Acid

A) Periodate Lignin

Periodate lignin, 1 g, and 21 cc of 47% hydriodic acid were heated under reflux for 6.5 hours in a carbon dioxide atmosphere. The apparatus employed was identical to that used in a methoxyl determination except for the still, which was of 100 cc capacity as compared with 20 cc normally used. The bromine-acetic acid-sodium acetate absorbing solution was changed at the time intervals shown in the Table X, page 50, and the loss of methoxyl groups from the sample was followed by titration as in methoxyl determinations. Fresh absorbing solution was connected to the apparatus as soon as possible. At the end of 6.5 hours, the resulting suspension of demethoxylated lignin was recovered on a filter and was washed repeatedly with 5% potassium iodide solution followed by water. The washings were discarded. The residue, almost black in colour, amounted to 80% of the starting material.

Found: OCH_3, 1.49, 1.56%; I, 10.7, 10.5%

The filtrate, which was highly coloured, was treated with potassium iodate solution to oxidise all hydriodic acid to iodine, which in turn was combined with sodium thiosulphate. During the last step, a considerable amount of sulphur precipitated and the solution had a strong odour of sulphur dioxide, which was removed by boiling. Sulphur was allowed to coagulate and was subsequently removed by filtration. Continuous ether extraction of the filtrate yielded 8% of a wine-red tar which was heavily iodinated. At this point the experiment was abandoned in order to search for better conditions of carrying out the reduction.

B) Reduction in Presence of Phosphorus (108)

Periodate lignin, 5 g, potassium iodide, 20 g, phosphorus, 5g, phosphoric acid, 100 cc, and water, 20 cc, were boiled under reflux for 5 hours, the temperature rising to about 140°. The resulting mixture was cooled, diluted with 50 cc of water and filtered with difficulty. The filtrate was repeatedly extracted with ether but only a very small quantity of material was obtained after the evaporation of the ether.

The residue, when treated with dilute (5%) sodium hydroxide solution, dissolved partially; the insoluble portion consisted mainly of unchanged phosphorus and lignin debris and was discarded; the soluble portion, which was dark brown in colour, was acidified with dilute hydrochloric acid. Contrary to expectation, no precipitate was formed. The acidic solution was evaporated to about one-third of its volume and was slowly poured into a large quantity of ether. The thick paste which separated was dried over phosphorus pentoxide and then extracted with acetone in a Soxhlet apparatus. The acetone-soluble portion (1.3 g) was reprecipitated by the gradual addition of ether.

Found: I, 0.0%; OCH_3, 2.6%

Although iodination of the lignin was avoided in this experiment, the product did not seem amenable to further study and milder conditions were sought for the reduction.

C) Reduction of Periodate Lignin at 50°

Periodate lignin, 1 g, was covered with 21 cc of 47% hydriodic acid and the mixture was stirred for 8 hours at 50°. The resulting suspension was cooled to room temperature, diluted with about 20 cc of water and filtered. The filtrate contained only traces of free iodine, which were destroyed by sodium thiosulphate solution. The ether extract, which turned golden yellow owing to the gradual decomposition of hydriodic acid, was decolourised with a small quantity of aqueous potassium iodide followed by aqueous thiosulphate. On drying and evaporation, only a negligible residue remained which was discarded.

The product insoluble in ether was resistant to the further action of 47% hydriodic acid at 50°. After washing with 5% potassium iodide solution and water, followed by drying, this residue amounted to over 90% of the original lignin.

Found: OCH_3, 10.5, 10.5%; I, 10.0, 10.1%

Treatment of the above residue with 5% sodium hydroxide solution resulted in partial solution, giving alkali-soluble and alkali-insoluble fractions.

The alkali-soluble portion was wine-red in colour and the organic material it contained was readily reprecipitated by the addition of mineral acid. The product was dried and freed from inorganic material by extraction in a Soxhlet apparatus with acetone; the final purified material amounted to 12% of the original lignin.

Found: OCH_3, 11.7, 12.0%; I, 18.3, 18.1%

The alkali-insoluble product, 77% by weight of the original lignin, was repeatedly washed with water, dried and analysed.

Found: OCH_3, 9.4, 9.4%; I, 3.0, 3.1%

All the fractions described above, including the periodate lignin, were methylated with diazomethane. Other portions of the same fractions were acetylated with acetic anhydride and pyridine. The results of both processes were included in Tables XI and XII, pages 53 and 55 respectively.

D) Reduction of Oxylignin at 50°

Oxylignin, 2 g, was placed in a 2-necked flask equipped with a mechanical stirrer and a reflux condenser. Hydriodic acid (47%), 42 cc, was poured over the oxylignin, the flask immersed in a thermostat maintained at 50° and the mixture stirred for 8 hours. Partial solution of the oxylignin in the hydriodic acid was observed. At the end of the 8 hour period, the resulting suspension was filtered through a sintered glass crucible. Free iodine was removed from the residue by repeated washing with 5%

potassium iodide solution followed by water. The dried residue, 35% by weight of the original oxylignin, which was insoluble in ether, was dissolved in acetone and any inorganic material removed by filtration. The acetone solution was added drop by drop to an excess of ether, the resulting precipitate was centrifuged, washed with ether and dried under vacuum. This material was resistant to the further action of hydriodic acid at 50°.

Found: OCH_3, 9.35, 9.40%; Cl, 5.2%; I, 4.5%

M. Wt.: 510 (in acetone solution, Signer's method)

The filtrate from the original reduction was diluted with 3 times its volume of water, and the resulting precipitate, 40% of the oxylignin, was recovered and purified in the manner just described.

Found: OCH_3, 8.3, 8.4%; Cl, 5.2%; I, 8.2%

M. Wt.: 415 (in acetone solution)

The above two fractions, and also the original oxylignin, were methylated with diazomethane. Other portions of the same fractions were acetylated with acetic anhydride and pyridine. The results of both processes, which were also carried out on the two hydriodic acid reductions described below, are summarised in Table XIII, page 57.

E) Reduction of Fractionated Oxylignin at 50°

Fraction 1 of the oxylignin, obtained as described on page 98, and the combined fractions 2-6 from the same source, were subjected to the action of hydriodic acid under conditions described in detail for oxylignin (page 119). The analytical and quantitative data were summarised in Tables XIV and XV, pages 58 and 59.

Reduction with Sodium in Liquid Ammonia
(50) (51) (52) (53) (54) (55) (56)

A) General Procedure

All reactions involving sodium and liquid ammonia were carried out with the utmost care (rubber gloves and goggles and in a fume-hood). A thermos flask of about 1 l capacity, equipped with a drying tube filled with potassium hydroxide pellets, served as the reaction vessel. Ammonia gas from a cylinder was dried by passing through a 3 foot long glass tube of about 1 inch in diameter filled with potassium hydroxide pellets. Condensation of the ammonia gas was achieved in an ordinary 1 l capacity Ehrlenmeyer flask securely clamped and immersed in an acetone-solid carbon dioxide bath. As soon as a sufficient volume of liquid ammonia was collected, it was carefully poured through a wide-mouth funnel into the thermos flask containing the material to be reduced. Sodium metal, stored under toluene, was cut into small cubes, which were then dropped rapidly into the liquid ammonia, forming a dark blue solution. After completion of the reaction, the ammonia was allowed to evaporate overnight and the last traces were removed by passing nitrogen. Any unused sodium in the residue was very carefully decomposed by the slow addition of wet ether.

B) Small-scale Reduction of Oxylignin

Liquid ammonia, about 250 cc, was carefully poured over 5 g of oxylignin, and 3.2 g of sodium cubes were added. The rubber stopper, equipped with a drying tube, was quickly and tightly pushed into the neck of the thermos flask. The reaction mixture was shaken occasionally during the next 48 hours and additional amounts of liquid ammonia were added as required to keep the volume approximately the same. At the end of the reaction, the residual ammonia was allowed to evaporate and the sodium was destroyed as just described. When the residue was treated with 150 cc of water, it dissolved completely. The alkaline solution was continuously extracted with ether for 24 hours, but after drying and evaporation of the ether, no extract was obtained.

Acidification of the aqueous residue with 10% sulphuric acid caused the separation of a large precipitate 1, which was isolated by centrifuging. This precipitate was purified by solution in acetone, filtration from a small amount of insoluble matter and precipitation by the slow addition of the solution into an excess of ether. The purified product amounted to 72% by weight of the original oxylignin.

Found: OCH_3, 8.35, 8.40%

The aqueous residue was extracted for 24 hours with ether, and the solid recovered from the extract 1, amounting to about 2% of the original oxylignin, was combined with the ether extracts of subsequent liquid ammonia-sodium cycles. The solution remaining after the extraction with ether was still strongly coloured, but all coloured matter could be extracted with butanol, yielding extract 2.

The purified and carefully dried precipitate 1 (72% of the oxylignin) was then subjected four times to identical treatments with sodium and liquid ammonia. The ether extracts from each cycle were combined; the same procedure applied for the butanol extracts. After the fourth treatment, the insoluble residue had lost its light-brown colour and had become dark brown. The tar-like material after the fifth treatments amounted to only 6.8% of the oxylignin.

Found: OCH_3, 7.75, 7.95%

The combined ether extracts were unfortunately lost by an accident.

The combined butanol extracts became esterified while the solution was being concentrated under reduced pressure. Attempts to recover free carboxylic acids by heating the residue for 1 hour under reflux with 10% sodium hydroxide solution, followed by acidification, yielded an unmanageable gum.

The insoluble residue, left over after 5 consecutive treatments, (6.8% of the oxylignin) was fully methylated with diazomethane.

Found: OCH_3, 23.0%

C) Reduction of Oxylignin - Modified Procedure

A mixture of liquid ammonia, 250 cc, oxylignin, 9.7 g, and 4.5 g of sodium metal were kept for 48 hours; additional quantities of sodium metal and of liquid ammonia being added during the reaction time as the sodium was used up. The precipitate obtained on acidification of the alkaline solution with 20% sulphuric acid was subjected to subsequent sodium-liquid ammonia cycles as described previously.

The residue from the 3rd cycle was black in colour and was separated as a tar which, when dried, dissolved only partially in 10% sodium hydroxide solution. This tar (fraction 1) was soluble with difficulty in organic liquids which readily dissolved the untreated oxylignin. Fraction 1 was not subjected to further action of sodium in liquid ammonia.

The alkaline solution obtained after each cycle was acidified with 20% sulphuric acid to pH 1.5 and the filtrate was combined with others obtained in the same manner from subsequent liquid ammonia-sodium cycles.

The combined acidified solution was evaporated under reduced pressure (60 mm) and at 40° until its volume was reduced to 250 cc. About 1 l of acetone was added and the resulting precipitate was isolated, washed with acetone and the washings combined with the bulk of the mother liquor. The inorganic precipitate was discarded.

Almost all of the acetone was removed by evaporation under reduced pressure and the resulting solution (pH about 1.5) was extracted with ether for 24 hours. The ether extract, after drying with sodium sulphate and evaporation, yielded a coloured material which passed completely into 5% aqueous sodium bicarbonate solution. No free phenolic material was present. The bicarbonate extract, when worked up as previously described, yielded 0.5 g or 5.15% by weight of the original oxylignin as a light brown, thick oil - fraction 2.

The acidified solution left over after the ether extraction was neutralised by barium hydroxide solution and the precipitate of barium sulphate was discarded. The remaining solution was evaporated to dryness and the residue was divided into acetone-soluble and acetone-insoluble fractions 3 and 4, respectively.

The analytical results are summarised in Table XVIII, page 70. Fractions 2 and 3 of the above Table were analysed by paper chromatography, using butanol saturated with 2% ammonium hydroxide as the solvent (77) and ferric chloride-potassium ferricyanide solution as the spraying agent. Both fractions were found to be a mixture of at least 4 phenolic compounds.

The various fractions shown in the Table XVIII, page

70, amounted to almost 50% of the starting material; undoubtedly appreciable mechanical losses were incurred during the long procedures just described.

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

Previous workers had observed that the exhaustive oxidation of spruce periodate lignin with sodium hypochlorite at pH 12 produced a "limit" oxylignin that was stable to the oxidant. This oxylignin contained aliphatic hydroxyl and carboxylic acid but no free phenolic groups, about 5% of chlorine, and possibly some quinone groups. The present thesis added the following major items of information concerning the oxylignin.

1. The ash-free oxylignin was reacted with cyanide ions, hydrogen chloride gas, methyl alcohol followed by lithium aluminium hydride and a variety of acetylating agents; all these reactions were indicative of the quinone groupings and all were positive. The content of quinone groups was roughly estimated at 2%.

2. The original periodate lignin acquired its small percentage of chlorine from the alkaline hypochlorite far more rapidly than it became oxidised. Fractionation of the oxylignin from acetone solution yielded 10 fractions, all of which contained chlorine. The halogen was therefore fairly uniformly dispersed throughout the oxylignin. Only about half of the chlorine was readily removed by alkali, and its stability in this respect suggested that both aromatic and aliphatic chlorides were present in approximately the same proportion. When the oxylignin was oxidised with nitrobenzene and alkali, chromatography revealed traces of a

chlorinated derivative which was not 5-chloro vanillic acid. The nature of the supposedly aromatic chlorine was not determined.

3. Attempts were made to reduce the oxylignin with sodium borohydride, lithium aluminium hydride, zinc and hydrochloric acid, and with hydrazine and sodium hydroxide. The last of these agents gave a product which included about 1% of m-ethyl phenol but with this exception no chemical individual was identified among any of the products. m-Ethyl phenol was a new degradation product of lignin.

4. After a search for the best experimental conditions, periodate lignin was treated with 47% hydriodic acid at 50° for 8 hours. Previous authors had shown that this reagent was capable of cleaving tetramethyl glucose to glucose in a high yield, but this was the first application to a lignin. Since periodate lignin yielded 90% of a product which retained over 80% of the original methoxyl groups, the reagent had little effect on methyl phenyl ethers. Nevertheless, the product contained many free phenolic groups which must have arisen from the cleavage of an aryl ether grouping.

5. Under the same conditions, hydriodic acid failed to produce free phenolic groups in the oxylignin although the average molecular weight was reduced from 2300 to 400-500. Hence the aryl ether unit cleaved in the original lignin was destroyed during the oxidation with alkaline hypochlorite. Another type of aryl ether linkage was

present and survived the action of alkaline hypochlorite, because re-oxidation of the oxylignin with nitrobenzene yielded vanillin and vanillic acid, as well as the trace of an unidentified chloro compound already mentioned.

6. Six compounds were synthesised to serve as models of various types of ethers. These compounds were subjected to the action of 47% hydriodic acid at 50° for 8 hours and to alkaline hypochlorite. The results indicate that the oxylignin is free, or almost free, of di-alkyl and benzyl-aryl ethers.

7. The oxylignin was submitted in preliminary experiments to the action of sodium in liquid ammonia, a reagent previously used only with unoxidised lignins. Degradation to compounds of lower molecular weight and the formation of new phenolic hydroxyl groups occurred, but most of the original methoxyl groups were retained.

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