Acknowledgments

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General Introduction

Several years ago the Wood Chemistry Committee of the Technical Section, Canadian Pulp and Paper Association, observed that chlorine dioxide was becoming of importance as a bleaching agent for pulps, and that more detailed knowledge concerning its chemical action on the lignin of wood was desirable. This recommendation led to a thesis by N. Levitin on the oxidation of Black Spruce periodate lignin with aqueous chlorine dioxide under various conditions. He showed that the initial product of this oxidation, called oxylignin A, progressively yielded oxylignins B and C.

The present thesis expands Levitin's preliminary study of oxylignins B and C, and greatly improves the isolation of these labile substances, which have a great tendancy to condense to unmanageable, dark tars. Sustained efforts were made to degrade oxylignin B (which was technically the more amenable of the two) to compounds of molecular weight low enough to be identified structurally, yet high enough to be of significance for the structure of lignin. Two degraded fractions of oxylignin B, called oxylignins G and H, were eventually prepared. These oxylignins had apparent molecular weights of several hundred, which were in the desired range, but both were mixtures whose detailed examination had to be left for future research.

HISTORICAL INTRODUCTION

Isolated Lignins

The study of lignin has been complicated by the fact that no method exists for its isolation in high yield and in unchanged form. Brauns (1) has summarized most of the useful methods of isolation and these may be loosely grouped into lignins isolated by dissolving them from the wood and those isolated by dissolving the holocellulose from the wood.

The first type, the soluble lignins, consist of those which have been leached from the wood by solvents such as alcohols, aldehydes, phenols, ethers such as dioxane and organic acids. A little mineral acid is usually added to facilitate solution of the lignins. The yields were never quantitative and considerable evidence has been accumulated which indicates these lignins have been altered.

The most outstanding member of this class of lignin derivatives is Brauns' "Native Lignin" (2) which was isolated in 3% yield by leaching wood at room temperature with neutral methanol. This lignin was completely soluble in calcium bisulphite solutions in the conditions of a standard cook and exhibited, except for its solubilities, most of the properties and analysis of lignin *in situ*. No molecular weight determinations were undertaken by Brauns although the analysis of the functional groups of "Native Lignin" indicated a repeating unit of 840. Quite recently, Hess (3) found that the molecular weight of a series of

fractions from "Native Black Spruce Lignin" varied from 700 to 2800. Brauns (4) showed that "Native Lignin" formed a green solution in 43% hydrochloric acid and that a solution in methanolic hydrogen chloride was wine red in colour. When heated for several hours under reflux with the latter reagent, 35% of the lignin precipitated as a condensation product, and the soluble fraction was found to be similar to methanol lignin isolated under identical circumstances. This result would tend to indicate that methanolysis could lead to condensation as well as to degradation of the lignin.

Biological methods of isolating lignin have also been investigated. Ploetz (5) employed the digestive juices of snails for the selective dissolution of the holocellulose portion of wood, but the incidental extractions with cupriethylene diamine rendered the method of questionable value. Nord (6) increased the yield of Brauns' "Native Lignin" by rotting the wood with a cellulose destroying fungi before extracting with methanol. Since the fungi might have produced some unpredictable change in the lignin, this method did not lend itself particularly well for further lignin studies. In keeping with this suspicion, was the discovery by Harris (7) that freshly cut wood would not yield any appreciable quantity of "Native Lignin" when it was extracted with methanol.

Examples of the insoluble lignins are very common and it is only necessary to mention Klason lignin (8), Willstätter lignin (9) and Freudenberg lignin (10).

Evidence has been accumulated which shows that these lignins have undergone varying degrees of condensation, some of which will be mentioned later in the Introduction.

A major step towards the isolation of lignin resembling lignin *in situ* and yet maintaining a high yield was made by Ritchie and Purves (11). The method was based upon the selective oxidation at room temperature of the 1,2-glycol groups of the holocellulose portion of wood by means of sodium paraperiodate. The oxidation was followed by boiling the oxidized, iodine-free wood meal in water at pH 6. After several such oxidation cycles, a lignin was isolated in almost quantitative yield which was free of residual holocellulose. These authors prepared periodate lignins from spruce, birch, maple and beech woods and analysis revealed that the oxygen content of spruce periodate lignin was greater than that of lignin *in situ*, while the carbon and methoxyl contents had been decreased. The hardwood lignins reflected degradation by a decrease in the yield of the corresponding periodate lignin and not by a loss in methoxyl content.

Hydrogenation of spruce periodate lignin using copper-chromite as catalyst according to the procedure of Harris (12) yielded 4-n-propylcyclohexanol and 4-n-propyl-1, 2-cyclohexanediol in addition to the usual tars. The ethanolysis of spruce periodate lignin produced fractions resembling those from spruce lignin isolated by Hibbert (13), while nitrobenzene oxidations gave oils and vanillin in

approximately the same yield as spruce lignin (14). Since the yield of vanillin was close to that obtained from lignin in situ, Ritchie and Purves concluded that oxidation by means of trisodium paraperiodate had not destroyed the precursor of vanillin, and that the loss in methoxyl groups incurred in the preparation of periodate lignin was not connected with the vanillin yielding portions of the complex. It was pointed out that since Pennington and Ritter (15) had shown that phenolic compounds were oxidized by periodic acid, the progenator of vanillin must have been protected by chemical combination of the phenolic hydroxyl groups. Quite recently, Brickman (16) showed that veratraldehyde was oxidized by nitrobenzene to vanillin and thus that the substitution in the para position did not interfere seriously with this reaction.

Most lignin preparations exhibit poor solubility and low sulphonation rates during a standard cook with calcium bisulphite. Under these circumstances, only 2.9% of the periodate lignin remained insoluble, although the rate of cooking was found to be somewhat slower than that reported by Corey and Maass (17) for spruce wood itself. The results were, nevertheless, superior to those reported for any other lignin preparation and have been duplicated by Cabot (19), Brickman (16) and Levitin (18) in this laboratory. Recently a spruce periodate lignin was prepared by Smith and his collaborators (20) whose elementary analysis corresponded very closely to that given by Ritchie and Purves (11). The carbonyl content of this lignin was measured under drastic

conditions in an attempt to show that its structure did not resemble that proposed by Russel (21) for lignin.

The Degradation of Lignin

A vast amount of work has been carried out on the degradation of lignin. In this report only some of the more important investigations which lead to an understanding of the structure of lignin will be considered, as well as those degradations which have some bearing on the present investigation.

The early experiments on lignin degradation were usually carried out with strong oxidizing agents under drastic conditions. Even in those experiments where mild oxidants were employed, investigators found that the lignin was either only slightly degraded or shattered to carbon dioxide and simple aliphatic acids which gave no clue concerning their origin. Typical oxidants were ozone (22), hydrogen peroxide (23), potassium permanganate (24) and oxygen (25). Doree and Cunningham (22) for example, oxidized beech wood with ozone and in the organic debris identified carbon dioxide, acetic, formic and oxalic acids. The investigation of the high molecular weight tars from such oxidations was difficult, many of them being highly water soluble and impossible to purify. An early attempt at handling these products was made by Grüss (26) who oxidized pine wood tracheid cells with hydrogen peroxide and isolated the amorphous degraded lignin as a copper salt. This salt was purified by dialysis and separated into ethanol-soluble and ethanol-insoluble fractions.

At the present time, interest in the amporphous portions from lignin degradations is becoming greater as investigators have begun to direct their attention not only to determining what changes were wrought in the lignin, but also to the isolation of lignin "dimers" and "trimers".

As techniques were devised for the isolation of low molecular weight products, the drastic oxidations of lignin began to yield more information. Although the isolation of vanillin from waste sulphite liquor was reported by Grafe (27) in 1913, it was not until a generation later that the oxidations were repeated on a systematic basis by Kurschner (28). Phillips and Goss (29) prepared ethylated and methylated corn cob lignins and after either nitric acid or ozone oxidations, were able to isolate p-methoxybenzoic acid and p-ethoxybenzoic acid. Heuser and Winswold (30) confirmed these indications of the aromatic nature of lignin by isolating 12% of protocatechuic acid from hydrochloric acid lignin fused in sodium hydroxide. By varying the conditions of the experiment, they were able to isolate as much as 21% of catechol, formed by the decarboxylation of protocatechuic acid. Freudenberg (31) modified the alkali fusion of methylated spruce wood by methylating the fused product with dimethyl sulphate and by oxidizing it with potassium permanganate. He was able to isolate as much as 20% of veratric acid and some isohemipinic acid. These fragments confirmed the aromatic nature of lignin and indicated a second possible position where the non-aromatic

portion of lignin might be joined to the phenolic residue.

Freudenberg and Lautsch (32) discovered that nitrobenzene in alkaline conditions and at 135°C would oxidize spruce wood and lignosulphonic acids to almost 27% of vanillin, a few percent of phenols of the guaiacol type and 10% of a mixture of vanillic acid and vanillin-5-carboxylic acid. Control experiments led Freudenberg (33) to believe up to 50% of lignin was aromatic in nature. Creighton, McCarthy and Hibbert (34) confirmed Freudenberg's work and discovered in addition that maple wood could yield 31.8% of syringaldehyde and 3.4% of vanillin under the same conditions. Creighton, Gibbs and Hibbert (14) determined the yields of aromatic aldehydes from a large number of woods, ferns and grasses and found that those woods which gave the Maule test for hardwoods contained syringaldehyde. Grasses were found to contain small amounts also of p-hydroxybenzaldehyde (35).

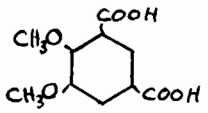
The alkali degradations were modified by Lautsch (36) who bubbled oxygen through a heated alkaline mixture of ligneous material to which was added a small amount of cobaltic hydroxide to act as an oxygen carrier. These experiments yielded vanillin, while Pearl (37) who substituted silver oxide as the oxygen carrier isolated vanillin, vanillin-5-carboxylic acid, acetoguaiacone and guaiacol from waste sulphite liquors. From a bromolignin prepared by the bromination of cuproxam lignin, Lautsch (38) isolated 6-bromovanillin. Since vanillin normally brominated in the 5 position unless acetylated, Lautsch claimed he had proof

that the phenolic group of the vanillin progenator was blocked. Several arguments were subsequently presented (39) refuting the conclusion on the evidence offered.

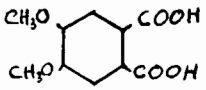
Further information concerning the structure of lignin was obtained by Harris, D'Ianni and Adkins (12) when they found 4-n-propylcyclohexanol, 4-n-propyl-1,2-cyclohexanediol and 3-(4-hydroxycyclohexyl)-1-propanol were formed when aspen wood was hydrogenated under high pressure in the presence of copper chromite. These products showed the presence of a three carbon side chain on the aromatic nucleus. Hibbert and his co-workers (40) repeated the hydrogenations at a lower temperature using Raney-nickel as catalyst, and isolated 3-guaiacyl-1-propanol and 3-syringyl propane from maple wood. The isolation of these benzene derivatives verified the assumption that Harris' products had originally been aromatic and were in accordance with the theory that syringyl and guaiacyl groups were connected by ether linkages, although some guaiacol groups might be linked by carbon-carbon bonds.

The oxidation of lignin with strong reagents may also reveal chemical changes that occur as a result of harsh chemical treatment. Recently, Richtzenhain reinvestigated Freudenberg's oxidation of methylated hydrochloric acid lignin with neutral potassium permanganate solution (41) and extended it to an investigation of the degradation products from a series of isolated lignins. In addition to veratric acid and isohemipinic acid (I) he was able to isolate meta-

hemipinic acid (II). Since the yield of isohemipinic acid (I) was increased when wood was heated with alkali, he concluded it was formed in part at least by condensation of liberated guaiacyl propane units to form the isohemipinic acid progenator. On the other hand, acid caused lignin to condense to form the structural units which gave metahemipinic acid (II) on further degradation.



I



II

Read and Purves (42) oxidized a variety of lignin preparations by the technique employed by Bone and his collaborators (43) for the investigation of coal, and isolated benzene penta- and hexacarboxylic acids. Since spruce wood yielded only 0.14% of these acids, periodate lignin 0.8%, Willstätter lignin 1.0%, Klason lignin 2.4% and alkali lignin 3.8%, the precursors of these products were not all present in the original wood, but were the result of condensations caused by the conditions and the reagents employed to isolate the lignins. These data were also used as evidence to show that periodate lignin, although somewhat oxidized, had not undergone as extensive a condensation as the other lignin derivatives which were investigated. Cabot (19) continued this investigation and showed that periodate lignin lost its solubility in calcium bisulphite when pretreated with acid at 160°C. for 4 hours and when pretreated in this way gave 5.5% of benzene polycarboxylic acids when oxidized with

potassium permanganate.

The less drastic oxidation techniques have also been used to investigate the nature of lignin. Traynard and Robert (44) suspended poplar wood in glacial acetic acid and oxidized it at 40°C. with nitric acid. From the acetic acid solution, they isolated a lignin derivative in 12% yield. An additional fraction could be isolated if a second oxidation was carried out at 60°C. on the partially extracted wood meal. The product was claimed to be crystalline from its X-ray diffraction pattern and its behaviour under the polarizing microscope. From elementary analysis, from methoxyl, equivalent weight, carbonyl and molecular weight determinations they represented the poplar nitro-lignin by the following formula:

$$\begin{matrix}(NO_2)_3\\(COOH)_4\\(C{=}O)_2\end{matrix}\Bigg[\quad C_{34}\ H_{38}\ O\quad\Bigg]\begin{matrix}(OCH_3)_2\\(COOCH_3)_2\\(COOR)_2\end{matrix}$$

where R represented an ethoxyl group introduced by an incidental extraction with ethanol. They were unable to isolate any structurally significant degradation product by oxidizing the nitro-lignin with potassium permanganate or chromic acid.

Sodium hypobromite, employed by Holmberg (45) to oxidize extracted sprucewood, produced an acid insoluble product whose composition was constant within the limits expressed by the following repeating unit:

$$(C_9\ H_{7.4}\ O_{3.9\ -\ 4.2}\ (OCH_3)_{0.68\ -\ 0.73}\ Br_{0.29\ -\ 0.45})_x$$

Direct titration of this derivative with sodium hydroxide gave an equivalent weight of 312 to 334, but if the substance was kept in aqueous sodium hydroxide for 24 hours, the equivalent weight decreased to 211 to 230. Holmberg claimed these results indicated the presence of lactone rings and tried to prove his claim by methylation studies. Gralen (46) found by the ultracentrifuge method that this spruce hypobromite lignin had a molecular weight of 7000 and the molecular weight decreased to 3500 when heated in acid or base.

Whittal (47) began a study of the effects of sodium hypochlorite on periodate lignin and concluded that the oxidation was most amenable to control at pH 12. The oxidations were continued by Brounstein (48) who isolated three major fractions of varying methoxyl content. The largest fraction, oxylignin A, was repeatedly methylated with dimethyl sulphate but with no temperature control. No constant methoxyl content could be achieved and the molecular weight was estimated to be 3000. From further methylation studies, Brounstein showed aliphatic hydroxyl groups were present as well as acidic groups. Repetition of the dimethyl sulphate experiments at 35°C. gave a product of lower molecular weight having the following composition:

$$C_{32.8}H_{28}\ Cl\ (OH)_2\ (COOH)_2\ (OCH_3)_{2.7}$$

Fractionation of oxylignin A gave fractions of varying methoxyl content.

Numerous attempts were made to degrade oxylignin A

and its methylated derivative. These attempts included acid methanolysis, autohydrolysis with water or methanol, partial demethylation, low pressure hydrogenation, oxidation with silver oxide and chromium trioxide, reduction with zinc in sodium hydroxide or glacial acetic acid, and treatments with methanolic hydrogen chloride and sodium ethoxide. In no case was it possible to isolate fractions with a molecular weight less than 800 and in many instances condensation seemed to have occurred.

Methylated oxylignin A was found to be unstable to sodium hypochlorite and several additional fractions were isolated when samples were re-oxidized. The major fraction had a molecular weight of 1000 and the empirical formula:

$$C_{45}\ H_{34}\ O_9\ Cl_{1.5}\ (OCH_3)_2\ (COOH)$$

This product appeared to have a greater molecular weight than oxylignin A itself.

The second fraction from the hypochlorite oxidation of periodate lignin, oxylignin B, when re-oxidized with sodium hypochlorite gave a volatile oil with a molecular weight of 200. Another fraction, oxylignin C, was investigated chromatographically using "Magnesol" and three fractions, badly contaminated with ash, were collected.

Sodium chlorite has been extensively employed in recent years as an oxidant to remove the non-cellulosic portions of wood from the holocellulose. Although its action on holocellulose has been rather thoroughly investigated, its effect on lignin has not. The first attempts in

this direction were made by Sohn and Reiff (49) who digested wood with acidic solutions of sodium chlorite and then acidified the mother liquor strongly. They isolated a flocculent white precipitate which they believed to be hemicellulosic in nature. Jayme and Hanke (50) recovered up to 50% of the sodium chlorite extract as an amorphous substance containing 8.6% to 9.8% of methoxyl, 1.4% to 4.5% of chlorine, about 25% of reducing sugars and 10% of aldonic acids. Barton (51) oxidized solvent extracted Slash Pine wood at 80°C. with an acetic acid solution of sodium chlorite and isolated several fractions called chlorite lignins A, B and C by solvent precipitation. Chloracetic acid, oxalic acid, fumaric acid, and a crystalline substance $C_{26}H_{53}OH$ were isolated from the residual liquors. The largest lignin fraction, chlorite lignin A, was studied by methylation and acetylation methods and was represented by the following formula:

$$\begin{matrix}(CH_3O)_2\\(OH)_2\end{matrix}\Big[\quad C_{22}H_{18}O_6\quad\Big]\begin{matrix}(COOH)_2\\Cl\end{matrix}$$

No molecular weight determinations were undertaken. It was especially interesting to observe that this oxylignin did not yield the absorption spectra near 280 m in the ultraviolet which is characteristic for phenolic units and therefore for unoxidized lignins. In addition, the chlorite lignins did not give a distinct end point when titrated electrometrically with sodium hydroxide.

Bublitz (52) repeated Barton's oxidation on Black Spruce wood and isolated a spruce chlorite lignin after electrodialysing the concentrated liquor. This fraction amounted to 17% of the lignin and contained 12.5% of methoxyl groups and 4.0% of chlorine atoms. Wacek and Schroth (53) isolated acidic lignins from a tropical wood and from spruce wood by acidifying the alkali extract from a chlorite holocellulose determination. Subsequent oxidations with nitrobenzene yielded vanillin, syringaldehyde, and syringic acid from the tropical wood extract.

The investigation of Slash Pine and Black Spruce chlorite lignins was continued by Jayne (54) at Appelton. Nitrobenzene oxidations followed by careful chromatographic studies showed that 3.7% of vanillin, 2.3% of 6-chlorovanillin, and 5.8% of vanillic acid could be isolated from spruce chlorite lignin, while slash pine chlorite lignin yielded 0.9% of vanillin, 2.3% of 6-chlorovanillin and some vanillic acid. No 5-chlorovanillin could be detected among the oxidation products nor was any appreciable quantity of 6-chlorovanillic acid present. The same worker also oxidized vanillin, 6-chlorovanillin and vanillic acid with alkali and nitrobenzene and found that 80% of the starting materials could be recovered unchanged. The aldehydes underwent simple oxidation and demethylation but the degradation products of vanillic acid did not contain any simple product.

Oxidations with Chlorine Dioxide.

Several workers in this laboratory have employed

chlorine dioxide to oxidize both lignin model compounds and periodate lignin. Since oxidations with sodium chlorite were also carried out at the same time, the chlorite oxidations will be described in this section.

The chemistry of chlorine dioxide has been reviewed by Mellor (55) and excellent summaries have been given by Husband (56) and Logan (57).

Furst (58), the first to study the effect of chlorine dioxide on organic compounds, discovered that the gas would react with unsaturated substances such as ethylene.

$$CH_2{=}CH_2 + 2ClO_2 \longrightarrow CH_2ClCOCl + H_2O + O_2 \longrightarrow CH_2ClCOOH + HCl + O_2$$

Forty years later Schmidt and his co-workers (59 to 64) used aqueous chlorine dioxide to oxidize a wide variety of organic substances and to remove the lignin or incrusting substances from plant materials. Schmidt and Graumann (59) showed that cellulose, mercerized cellulose, oxycellulose, cellulose from fungi, mannan, xylan, glucose, mannose, galactose, xylose, arabinose, maltose and glucosamine were unaffected by chlorine dioxide. From the oxidation of wood with chlorine dioxide they were able to isolate the so-called "Skelletsubstanzen", carbon dioxide, maleic acid and small amounts of chlorinated tars.

Schmidt and Braunsdorf (60) studied the reactivity of chlorine dioxide towards natural proteins and other substances. Of twenty-five amino acids and their derivatives none were attacked, nor were imides, amides, aliphatic

alcohols or hydroxy acids, free or esterified acids, nitrite groups, ring systems such as benzene, naphthalene, cyclohexane or salts of pyridine, piperidine, quinoline or β-nitroanethole. On the other hand, the carbon-sulphur bonds of mercaptans and thiourea, as well as the disulphide bonds of cystein were attacked. They found that unsaturated compounds reacted as Furst had claimed, but not without exception as α-crotonic acid, fumaric acid, maleic acid and maleic anhydride were unaffected. A variety of phenolic compounds including o-nitrophenol, resorcinol, pyrogallol, phloroglucinol, salicylic acid, m-hydroxybenzoic acid, gallic acid, and furfural were oxidized by Schmidt, Haag, Abele and Sperling (63) to oxalic acid, maleic acid, and traces of organic gums by chlorine dioxide. The oxidations were carried out with an excess of 3% aqueous chlorine dioxide using vanadium chloride as catalyst. Fuchs and Honsig (65) repeated much of Schmidt's work on wood and phenolic materials. By oxidizing wood with chlorine dioxide and dialysing the aqueous liquors, they obtained an amorphous residue which had only 40% of carbon and almost no methoxyl groups. Willstätter lignin under similar circumstances was converted to water soluble materials, and after evaporation of the solvent, a dirty white substance, claimed to be a polysaccharide, was extracted from the crude residue.

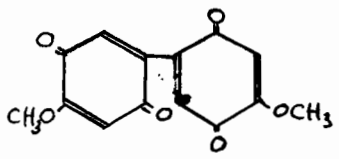
In 1935, Sarkar (66) showed that although phenols were readily attacked by chlorine dioxide, alkylated or esterified phenols were much less reactive. Aliphatic

hydroxyl groups in the position of the side chain of an aromatic ring were oxidized to a carbonyl group, except when protected by etherification or esterification. Those functional groups, such as the nitro group, which tended to decrease the electrophylic reactivity of the aromatic ring caused a great decrease in the ease of oxidation.

In order to explore the oxidative ability of chlorine dioxide discovered by Schmidt, Husband (56) and Logan (57) carried out extensive degradation studies on several phenolic compounds but under less drastic conditions than those employed previously. Methoxyhydroquinone was oxidized at various pH levels and under suitable conditions gave up to 94% of methoxyquinone. The high yield enabled Logan to establish a chlorine balance from which he was able to construct the following equation.

$3ClO_2$ + 3methoxyhydroquinone $\longrightarrow$ 3HCl + 3O + 3H + 3methoxyquinone

The oxidizing equivalent of chlorine dioxide in this case was found to be 2 instead of 5 as was previously thought. Although the chlorine balance was complete, the incomplete redox balance suggested that hydrogen peroxide was formed as a by-product. Sodium chlorite at pH 2.2 gave coloured condensation products of methoxyhydroquinone, but at pH 1 the oxidation led to the following diquinone (III) in 91% yield.



III

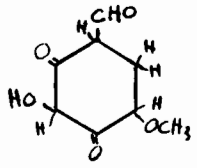
Veratraldehyde was found to be rather stable to chlorine dioxide, but under very acid conditions veratric acid, 5,6-dichloroveratric acid and oils were isolated. Since at a higher pH (3 to 6) veratraldehyde was stable to chlorine dioxide, Logan (57) was able to study the effect of sodium chlorite on veratraldehyde without interference from the chlorine dioxide formed as a by-product. The high yield of veratric acid from the oxidation enabled him to verify the equation of Jeans and Isbell (67) for the oxidation of aldehydes.

$$RCHO + 3HClO_2 \longrightarrow RCOOH + 2ClO_2 + HCl + H_2O$$

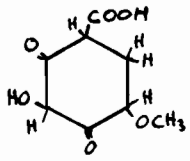
Unlike veratraldehyde, acetylvanillin was stable to chlorine dioxide at a low pH, but at pH 6 acetylvanillic acid was isolated in 25% yield. Sodium chlorite at pH 4 gave 78% of acetylvanillic acid and a trace of chlorinated acids. Chlorine dioxide was found to convert p-hydroxybenzaldehyde and pyrogallol to unmanageable tars at all pH levels.

Husband (56) showed that vanillin was unaffected by sodium chlorite at pH 6, but that chlorine dioxide was consumed at a rate that decreased with decreasing pH. An aldehydic substance was isolated with the formula $C_8H_8O_5$ (IV)

which was unstable to alkali. Logan (57) discovered that sodium chlorite was able to oxidize vanillin to the compound $C_8H_8O_5$(IV) in about 20% yield and to 20% of 5-chlorovanillin at pH 5, while at pH 1, an acidic substance $C_8H_8O_6$(V) was isolated. The latter compound could be obtained from the substance $C_8H_8O_5$(IV) by oxidizing the aldehydic group to a carboxyl group with acidulated sodium chlorite. Several derivatives of these two compounds were prepared and their structures were thought to be the following:



IV



V

Levitin (18) extended the study of the chlorine dioxide and sodium chlorite oxidations to periodate lignin. The oxidation of lignin with sodium chlorite was shown to be complicated by a simultaneous oxidation originating with the chlorine dioxide by-product. The experiments with sodium chlorite were therefore abandoned in favour of others using unbuffered chlorine dioxide, and such experiments had an additional advantage in that a relatively ash free lignin degradation product could be readily isolated. In the present discussion, any future reference to an oxylignin will be that obtained from the degradation of spruce periodate lignin by means of chlorine dioxide.

In general, the oxidations were carried out by suspending spruce periodate lignin in an aqueous solution of chlorine dioxide for 8 hours. After this interval, the pH of the solution had decreased from 4 to about 1.5. The insoluble lignin fraction was isolated and leached immediately with methanol, the extract was centrifuged to remove the insoluble oxylignin A and poured into ether to precipitate oxylignin B. From the aqueous oxidation liquors a third fraction, called oxylignin C, was isolated by extraction with butanol. Since periodate lignin was found to yield oxylignin A when gently oxidized, and since oxylignin A gave B, and B gave oxylignin C under similar circumstances, Levitin proposed the following relationships:

Periodate Lignin → Oxylignin A → Oxylignin B → Oxylignin C.

Oxylignin A was shown to be phenolic, not acidic, in nature and was insoluble in organic liquids. From a large number of oxidations, the methoxyl content of oxylignin B lay between 5.7 and 7.3%, while the chlorine content was found to be between 7.3 and 9.3%. This oxylignin was freely soluble in sodium bicarbonate (with the evolution of carbon dioxide), sodium hydroxide, alcohols, acetone, dioxane, and pyridine, but when it was dried only a portion re-dissolved in those reagents. Oxylignin B was insoluble in benzene, ether, petroleum ether and acidic aqueous solutions. Because of the ease with which oxylignin B was isolated, and because of its convenient solubilities, it was chosen for further investigation. Methanol was used

as the solvent to separate oxylignin B from oxylignin A, but contamination of the isolated product with this solvent seemed to have occurred. For example, a crude mixture of oxylignins A and B averaged 4.8% of methoxyl groups and of this mixture after extraction with methanol, 7.1% contained 3.9% of methoxyl groups and the remaining 92.9% had 6.9% of methoxyl groups corresponding to an average of 6.7%. Whether the increase in methoxyl content was caused by a tenacious adsorption of methanol or by esterification was a question Levitin's work (18) did not answer.

Neither butanol nor ether extracted oxylignin B from an alkaline solution, but 90% could be removed by butanol extraction when the solution was acidified. Subsequent analysis of the products showed that no appreciable change in methoxyl or chlorine contents had occurred. A freshly prepared methanol solution of oxylignin B was fractionally precipitated into ether and no noticeable change in the chlorine content was observed. A decrease in methoxyl content from 7.8 to 7.1% occurred when an aqueous solution of oxylignin B was boiled under reflux for several hours. Dialysis of an alkaline solution of oxylignin B, followed by a quantitative recovery of the fractions, showed a slight overall loss of chlorine and it would seem that chlorine atoms had been removed from the oxylignin. Dialysis in methanol gave fractions whose chlorine content decreased slightly but progressively, and it was possible that some esterification of the oxylignin had occurred. None of

Levitin's results (18) positively showed that oxylignin B was inhomogeneous, but as he pointed out, neither was any proof of its homogeneity found.

Oxidation of oxylignin B with nitrobenzene and alkali did not yield any vanillin or its 2,4-dinitrophenylhydrazone, but a small quantity of crystals which were thought to be 5- or 6-chlorovanillin were isolated. However, the inability of these crystals to form a 2,4-dinitrophenylhydrazone would tend to eliminate the possibility that they were an aldehyde. The absence of vanillin from such an oxidation was not surprising because the chlorine dioxide would tend to destroy the phenolic nuclei in lignin as it did in model substances.

An unsuccessful attempt was made to measure the molecular weight of oxylignin B by the isothermal method of Blank and Willard (68) which depended upon the attainment of equal vapor pressures between solutions of the oxylignin and a standard, the equilibria being attained at atmospheric pressure and elevated temperature. The isothermal method of Signer (69) was also tried and molecular weights of 895 and 980 were obtained for oxylignin B. The latter method was carried out at reduced pressure and at room temperature and hence avoided the harmful effects of higher temperatures. In setting up the apparatus, Levitin assumed a molecular weight of 895 to 980, and since little change occurred, he concluded the molecular weight of the oxylignin was in that range.

TABLE I

Neutralization Equivalents of Oxylignin B

Determined by Levitin (18)

Method	pH of Assumed End Point	Neutralization Equivalent
(a) Direct Titration with Sodium Hydroxide	9.0	346
	9.0	376
	7.9	432
(b) Back Titration with Hydrochloric Acid	7.5	175
	9.0	165
(c) Direct Titration with Barium Hydroxide	9.0	364
	7.1	440
(d) Calcium Acetate Method (70)		243
(e) Ashing of Calcium Salt		344

The neutralization equivalent of oxylignin B could not be obtained by a direct potentiometric titration with sodium hydroxide as no point of inflection produced by a sudden change in pH was observed. This result was similar to that reported by Barton (51) for a sodium chlorite lignin. By allowing oxylignin B to remain in alkali for an unstated length of time, and by back-titrating with acid, fairly reproducible neutralization equivalents were obtained. The results of these and other methods are summarized in Table I.

A number of experiments were carried out to determine the effect of sodium hydroxide on oxylignin B. After 24 hours, a caustic solution of the oxylignin yielded an acid insoluble fraction which contained 4.7% of methoxyl,

but a butanol extract of the residual liquors gave a fraction which apparently had 9% of methoxyl groups. Levitin concluded that oxylignin B had been cleaved by alkali into two fractions of higher and lower methoxyl content and possibly of lower molecular weight.

A tentative empirical formula was proposed for oxylignin B as a result of elementary, methoxyl and chlorine analyses.

$$C_{32.8}\ H_{32.9}\ O_{20.2}\ Cl_{2.3}\ (OCH_3)_2$$

This particular oxylignin was methylated with diazomethane to a constant methoxyl content (17%). However, a satisfactory interpretation of the analysis of the methylated oxylignin B could not be obtained and Levitin (18) proposed that one of the two following formulae might represent it:

$$C_{34.7}\ H_{35.8}\ O_{14.0}\ N_{1.36}\ Cl_{1.60}\ (OCH_3)_5$$

$$C_{41.6}\ H_{42.9}\ O_{16.9}\ N_{1.63}\ Cl_{1.94}\ (OCH_3)_6$$

Since the number of carboxylic acid groups on oxylignin B could not be determined accurately, Levitin (18) hoped that the methylation had stabilized the substance to alkali and that subsequent saponification would give the information desired. No consistent results, however, could be obtained. From one of these experiments, acidification of the alkaline solution gave an insoluble fraction with 9% of methoxyl groups and a soluble fraction, isolated by evaporation of the aqueous liquor, containing 4.3% of methoxyl groups. This observation was claimed to be addi-

tional evidence that oxylignin B was cleaved into two fragments of unequal methoxyl content by alkali, although the fractions had solubilities which were the reverse of those found for the corresponding fractions obtained when oxylignin B itself was treated with alkali. Since the methoxyl values of the corresponding fractions from the two experiments were thought to be the same, it was concluded that no phenolic groups were present.

Dimethyl sulphate was employed in an effort to detect aliphatic hydroxyl groups in oxylignin B, but the poor yields of slightly methylated product rendered the method useless. When oxylignin B was methylated with diazomethane and then with dimethyl sulphate, a product with a lower methoxyl content than the diazomethane methylated product was isolated. Since this result could be due to saponification of esterified methoxyl groups in the caustic solution, the product was remethylated with diazomethane. The resulting methoxyl content was only equal to that of the original diazomethane methylated product, and it was concluded that no aliphatic hydroxyl groups were present. Methylation of oxylignin B with methanolic hydrogen chloride increased the methoxyl content to 16.3% and the slight difference between this value and that of the diazomethane methylated product indicated that only a trace of phenolic hydroxyl groups were present.

An attempt was made to measure the carbonyl content of the oxylignin by the hydroxylamine hydrochloride

method of Gladding and Purves (71) but a negative result was obtained. Several attempts were made to adapt the cyanohydrin reaction (72) to the estimation of carbonyl groups, but only a trace of these groups could be detected because of technical difficulties which will be mentioned later in the Discussion of Results. Hydrogenation of the oxylignin at low pressure using either Adam's platinum catalyst or palladium oxide, showed about one double bond in 1000 grams.

DISCUSSION OF RESULTS

The method of Ritchie and Purves (11) was employed for the preparation of the periodate lignin used in this investigation. After seven oxidations of the Black Spruce wood meal with sodium paraperiodate, analysis showed that the wood residue had not been sufficiently oxidized; only 85% Klason lignin was present and 20% of the crude periodate lignin remained undissolved after a standard cook with calcium bisulphite. The batch was set aside to be combined with the last oxidation of a second batch. The final oxidation gave a periodate lignin with a satisfactory analysis. For comparative purposes the analysis of spruce periodate lignins prepared by other workers has also been given in Table II. The difference between the methoxyl content of the present preparation and that obtained by other workers might be attributed to a difference in the wood samples or to somewhat more adventitious oxidation. However, the solubility of this periodate lignin in a standard cook with calcium bisulphite (97.9%) was in no way impaired.

Oxylignins A and B

Chlorine dioxide was prepared from sodium chlorate, oxalic acid, and sulphuric acid according to the conditions specified by Schacherl (73) and other workers (56) (57). Because of the explosive nature of the gas, it was generated in small batches and the reaction flask was heated with water only to 60°C. Even under these conditions the reaction

TABLE II

The Analysis of Spruce Periodate Lignin Prepared By Several Investigators

	Thompson-Brickman	Ritchie Purves (11)	Whittal (47)	Levitin (18)	Smith et al (20)
Wood Meal (gm.)	4479	-	-	-	-
Yield of Periodate Lignin (gm.)	730	-	-	-	-
Carbon (%)	61.8 (a)	61.4	-	-	60.0
Hydrogen (%)	5.8 (a)	6.0	-	-	5.2
Methoxyl (%)	10.3 (a)	12.2	9.92	11.4	11.1
Moisture (%)	9.3	-	-	-	-
Ash (%)	3.4 (b)	-	1.7	2.3	-
Klason Lignin (%)	91.1 (a)	93.7	91.5	88.6	-
Holocellulose (%)	trace (c)	trace	trace	4.0	-
Residue from Calcium Bi-Sulphite cook (%)	2.1 (c)	2.9	9.6	9.0	-

(a) Corrected for ash and moisture
(b) No halogen present
(c) Analysis by Dr. W. J. Brickman

TABLE III

Oxidations of Spruce Periodate Lignin with Unbuffered Chlorine Dioxide at Room Temperature (a).

Oxidation	Grams Lignin	Chlorine Dioxide			Percentage Yields of		
		Volume (cc.)	Concentration Moles/Liter	(mM./Gm.)	Oxylignin A	Oxylignin B	Oxylignin C
1	1	80	0.330	26	22.1	28.0	13.1
2	1	95	0.460	44	3.0	30.0	(b)
3	4	375	0.267	25	1.1	45.0	11.0
4	4	362	0.270	24	1.4	49.2	9.3
5	8	768	0.262	25	6.1	51.7	(b)
6	5	400	0.260	21	(b)	52.0	(b)
7	7	540	0.260	20	(b)	48.0	10.1
8	7	500	0.238	17	1.7	49.3	25.0
	17	1300	0.237	18			
	17	1200	0.247	18			
	20	1200	0.242	14			
9	20	1000	0.322	16	(b)	55.0	23.8
	30	1000	0.285	9.5			
	12	800	0.214	14			
10	40	1300	0.285	9.3	(b)	64.8	(b)
	40	1260	0.285	9.0			
	40	1300	0.285	9.3			
	40	1330	0.285	9.5			
	28	930	0.285	9.5			

(a) All oxidations were carried out for 18 hours except the first oxidation which was carried out for 8 hours.
(b) Not isolated.

TABLE IV

Analysis of the Products from the Chlorine Dioxide Oxidation of Spruce Periodate Lignin [a]

Oxylignin B

Oxidation	Methoxyl (%)		Chlorine (%)		Ash (%)		Carbon (%)		Hydrogen (%)	
3[b]	7.57		-		-		-		-	
4[b]	5.58	5.58	-		2.8	2.7	-		-	
5[b]	6.67	6.80	-		-		-		-	
6[b]	6.08		-		-		-		-	
7[b]	7.05	7.09	-		-		-		-	
7[c]	5.31	5.14	-		-		-		-	
8[c]	4.98	5.00	8.6	8.9	1.3	0.7	48.81	48.69	4.23	4.03
9[c]	4.60	4.78	8.9	9.0	0.5	0.7	-		-	
10[c]	4.81	4.92	-		0.5	0.5	-		-	
Oxylignin C										
3[d]	9.37	9.41	-		-		-		-	
5[d]	9.48		-		-		-		-	
7[d]	10.4	10.5	-		-		-		-	
8	1.1	1.1	16.9	17.4	0.2	0.3	39.93	39.85	3.78	3.90
9	1.2	1.2	16.8		2.3	2.6	-		-	
9[e]	1.2	1.2	15.6	15.7	1.7	1.7	-		-	

a None of the results were corrected for ash.
b Isolated using methanol.
c Isolated using acetone.
d Isolated using butanol.
e Excess halide ion removed before isolation.

started so violently that the glass joints of the generating apparatus had to be held together with wire springs in order to prevent the loss of the gas. All the data pretaining to the oxidations of periodate lignin with chlorine dioxide were collected in Table III, together with the yields of the oxylignins. No attempt was made to follow the progress of the oxidation, since Levitin (18) had shown that the loss of oxidant from the blank quickly became equal to the consumption of oxidant in the reaction vessel.

Although Levitin (18) had found suitable conditions for an 8 hour oxidation, the concentration of chlorine dioxide used here was reduced in order that a longer oxidation time might be employed. This arrangement permitted the chlorine dioxide to be prepared during the day and used while still fresh. The first 6 oxidations (Table III) were carried out under a variety of conditions, but the last 3 oxidations were carried out with decreasing amounts of chlorine dioxide in an attempt to increase the yield of oxylignin B by decreasing the yield of oxylignin C. In a typical run, the brown periodate lignin powder was suspended in the oxidant and stirred for 18 hours. No liberation of heat was detected, but small bubbles of gas were continuously evolved in addition to the greenish clouds of chlorine dioxide. At the conclusion of the experiment, the lignin had been converted to a bright orange mass which still retained the physical structure of the periodate lignin while wet, but became a dusty

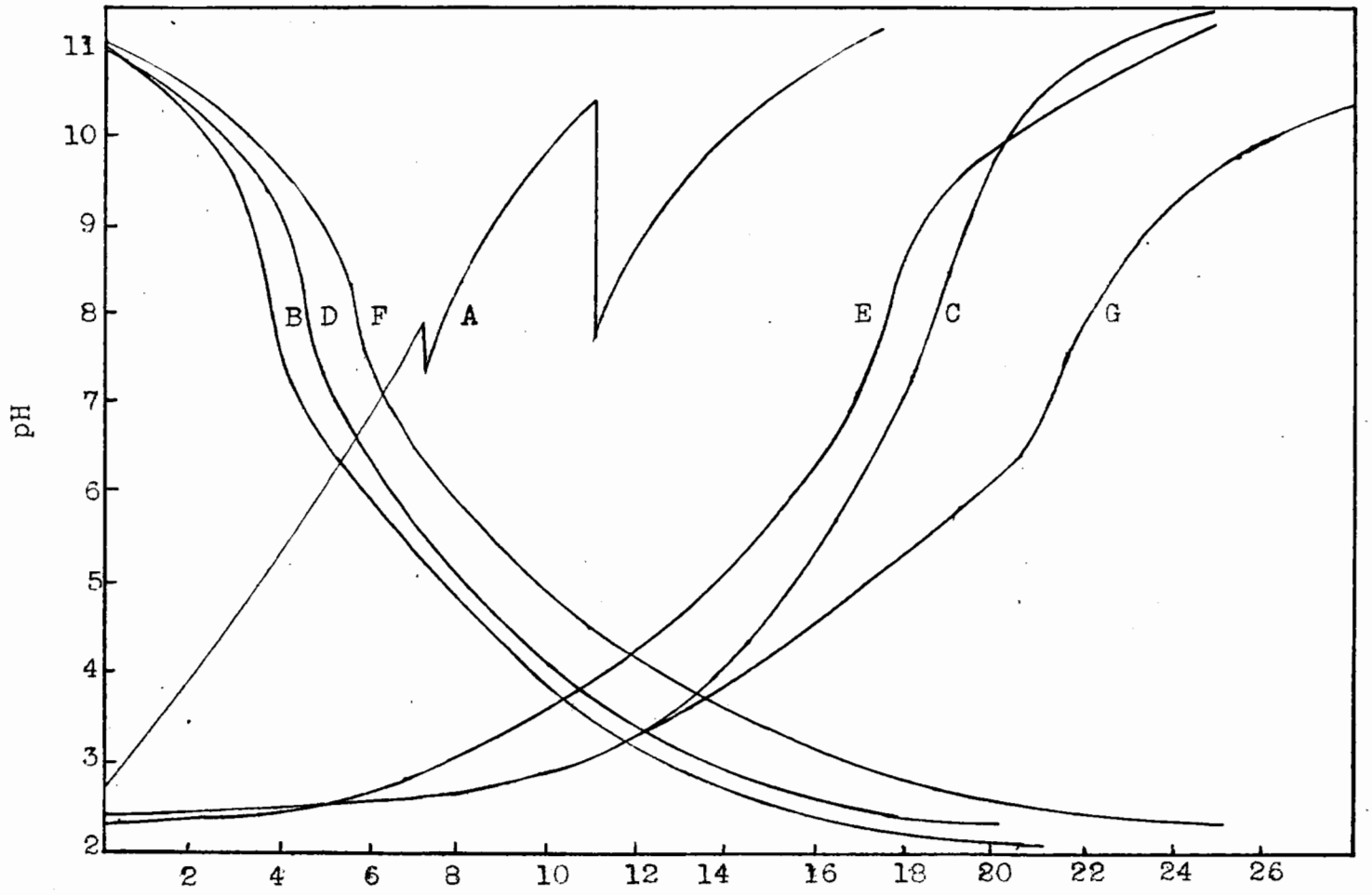
yellow powder when dry. This insoluble portion was separated by extracting it with a suitable organic solvent into the insoluble oxylignin A and the soluble oxylignin B. The latter fraction could be isolated by pouring the extract into ether or petroleum ether. The chlorine dioxide was removed from the aqueous oxidation liquors by reducing the pressure to 2 cm. and by passing a stream of nitrogen gas through them for a least 8 hours, after which time an aliquot failed to give a positive test for iodine when added to an acidified potassium iodide solution.

A small amount of oxylignin A was isolated from the first oxidation (Table III) and was found to be insoluble in organic liquids and in aqueous sodium bicarbonate. Oxylignin A would dissolve in sodium hydroxide, but acidification of the solution gave a flocculent precipitate which, when dried, was soluble in 5% sodium bicarbonate (without the evolution of carbon dioxide) and in acetone. The crude oxylignin A contained 12.4% of ash and 6.4% of methoxyl groups on an ash free basis. Oxylignin B was chosen as the fraction most worthy of further investigation because of its solubilities in a variety of organic liquids and because of the ease with which it could be separated from ash and slightly oxidized periodate lignin. In the early experiments, the crude residue from the oxidation was extracted with ethanol and the red brown extract was added to petroleum ether to precipitate oxylignin B. The mechanical losses in handling the

oxylignin were large, since thin films which were impossible to remove quantitatively formed on the glassware containing it. These losses became less as manipulative skill was developed.

Table IV shows that the methoxyl content of oxylignin B from oxidations 3 to 6 fluctuated from 5.6% to 7.6% or over the range previously found by Levitin (18). This fluctuation might have been due to unequal degrees of demethylation brought about by the oxidation, or to contamination of the oxylignin by residual quantities of alcohol employed for its isolation. A seventh oxidation was therefore carried out to compare the analysis of the same product after portions of it had been isolated with methanol and with acetone. Oxidations $7^{(b)}$ and $7^{(c)}$ (Table IV) showed quite clearly that the methoxyl content depended upon the solvent used to isolate the oxylignin. Since special care was used to remove the last traces of solvent from both portions of the seventh oxidation, it was unlikely that product $7^{(b)}$ was contaminated with adsorbed methanol and therefore esterification had probably occurred.

An outstanding feature of the previous work was the abnormal behaviour of oxylignin B toward sodium hydroxide whether in titrations, in solution or in methylation with dimethyl sulphate. For this reason, Levitin's efforts to ascertain the neutralization equivalent of oxylignin B with caustic soda were repeated. Figure I,



Volume (cc.) of 0.0768 N. Sodium Hydroxide and 0.0225 N Hydrochloric Acid

Fig.1. Potentiometric Titration of 0.1094 gm. of Oxylignin B-6 with Sodium Hydroxide and Hydrochloric Acid.

plot A was almost identical with that obtained by Levitin (18), the sharp discontinuities in the otherwise smooth plot being obtained when the titration was stopped for a few minutes. Disregarding the breaks, it was apparent that oxylignin B did not exhibit the neutralization plot which would be expected when an organic acid was titrated with sodium hydroxide. Carboxylic acid groups were present however, because oxylignin B liberated carbon dioxide from a 5% sodium bicarbonate solution. The last break in plot A was produced when the solution was allowed to stand in the presence of base overnight. Plots B, C, D, E, F and G were obtained by titrating the solution back and forth with acid and base. The solution was made strongly alkaline after titration G and allowed to stand for several days at room temperature, then the cycling process was repeated giving plots H and I (not shown in Figure I).

TABLE V

The Apparent Neutralization Equivalents of Oxylignin B from Figure I

Plot	pH	Neutralization Equivalent[a]	Plot	pH	Neutralization Equivalent[b]
A	7 to 8	481 to 371	B	7.9	199
C	7.6	243	D	8.0	225
E	7.4	246	F	7.7	229
G	7.6	255	H[c]	7.9	116
I[c]	7.6	125			

(a) Approached from the acid side.
(b) Approached from the alkaline side.
(c) Not shown in Figure I.

Disregarding the initial plot A, the data (Table V) showed that rapid titration back and forth produced nearly the same neutralization equivalent (plots C, E and G). The same phenomenon was observed by Brounstein (48) when hypochlorite spruce periodate oxylignins were titrated in this manner. Standing in alkali caused a decrease in the neutralization equivalents (plots B, D, F, and H) and acid appeared to increase it somewhat.

Several samples of oxylignin B were dissolved in sodium hydroxide to investigate the consumption of alkali, aliquots being removed at suitable intervals and titrated with acid. Figure 2 records the variation of neutralization equivalent with time for oxylignins B-7[b] and B-7[c]. The plots were divided into a fast initial and a slower second portion which appeared to be a straight line, within experimental error, up to one week. The last portion of this straight line was not shown in

Figure 2. It was assumed that the neutralization equivalent of about 120, found by extrapolating to zero time, was proportional to the acidic hydrogen atoms which were capable of reacting during a prolonged exposure to sodium hydroxide.

In another experiment, oxylignin B-6 was dissolved in sodium hydroxide and the neutralization equivalent was allowed to decrease to 114. An aliquot of this solution was added to a known excess of hydrochloric acid, and sub-aliquots were back-titrated with sodium hydroxide. After 4, 24, 48 and 72 hours exposure to the acid, the neutralization equivalents increased to 210, 256, 233 and 250. These data suggested that the neutralization equivalent due to free carboxylic acid groups was about 237, or 5.03 such groups in a molecular weight of 1193. Prolonged exposure to alkali increased the number to 9.94 groups. Several explanations might be postulated for the decrease of the neutralization equivalent in sodium hydroxide and its subsequent increase in hydrochloric acid. It will be shown later that the chlorine atoms on oxylignin B were stable to sodium hydroxide and therefore could not account for the decrease during alkali treatment. The number of phenolic groups in oxylignin B was almost negligible and the presence of a keto-enol system was doubtful.

As was mentioned in the Introduction, Levitin (18) claimed that sodium hydroxide cleaved oxylignin B into two fragments of unequal methoxyl content. The methoxyl

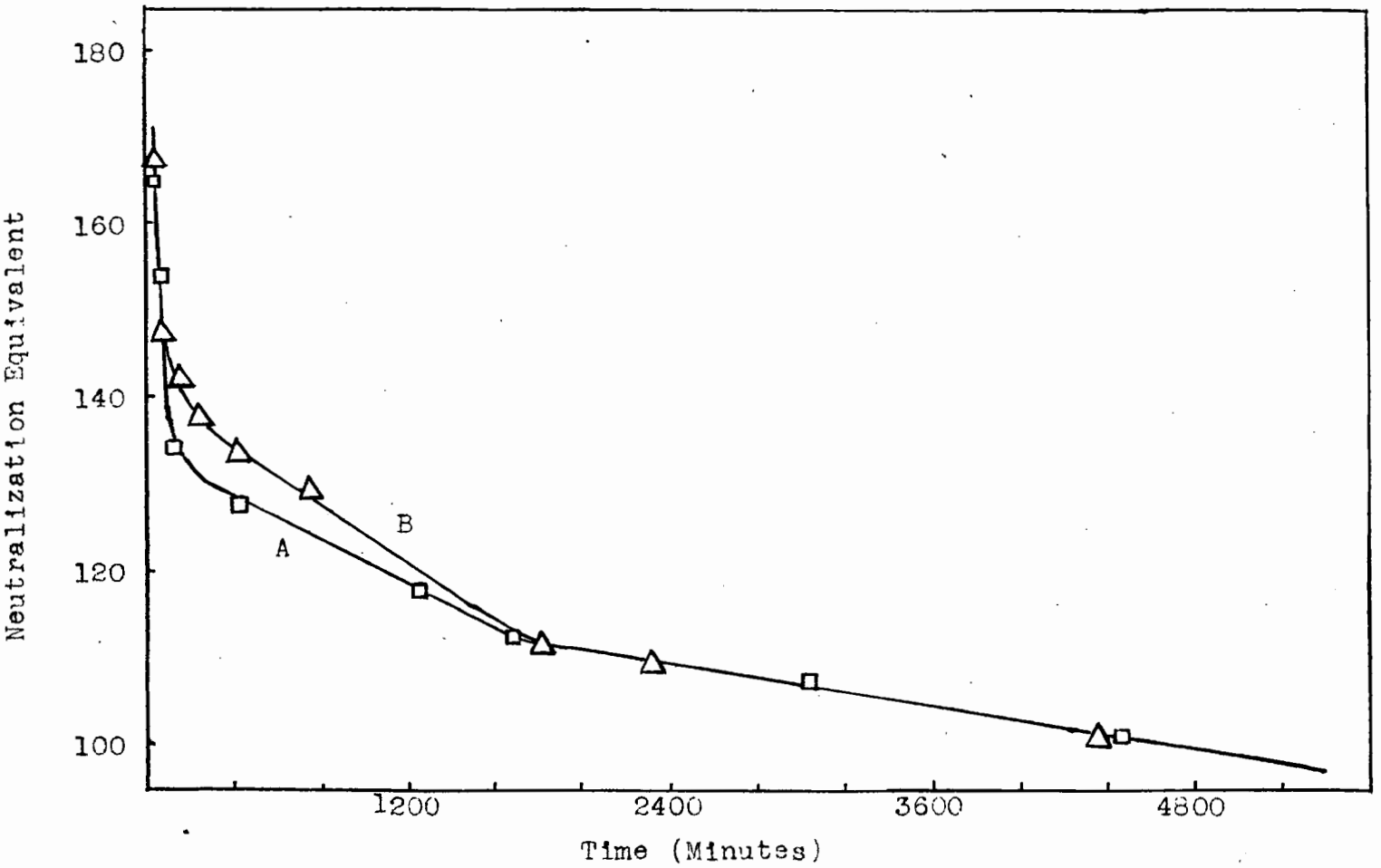


Fig.2. The Change of the Neutralization Equivalent of Oxylignin B in 0.0865 N Sodium Hydroxide at Room Temperature.
Plot A Oxylignin B-7(b), 0.1435 gm., in 30 cc. of Sodium Hydroxide.
Plot B Oxylignin B-7(a), 0.1586 gm., in 50 cc. of Sodium Hydroxide

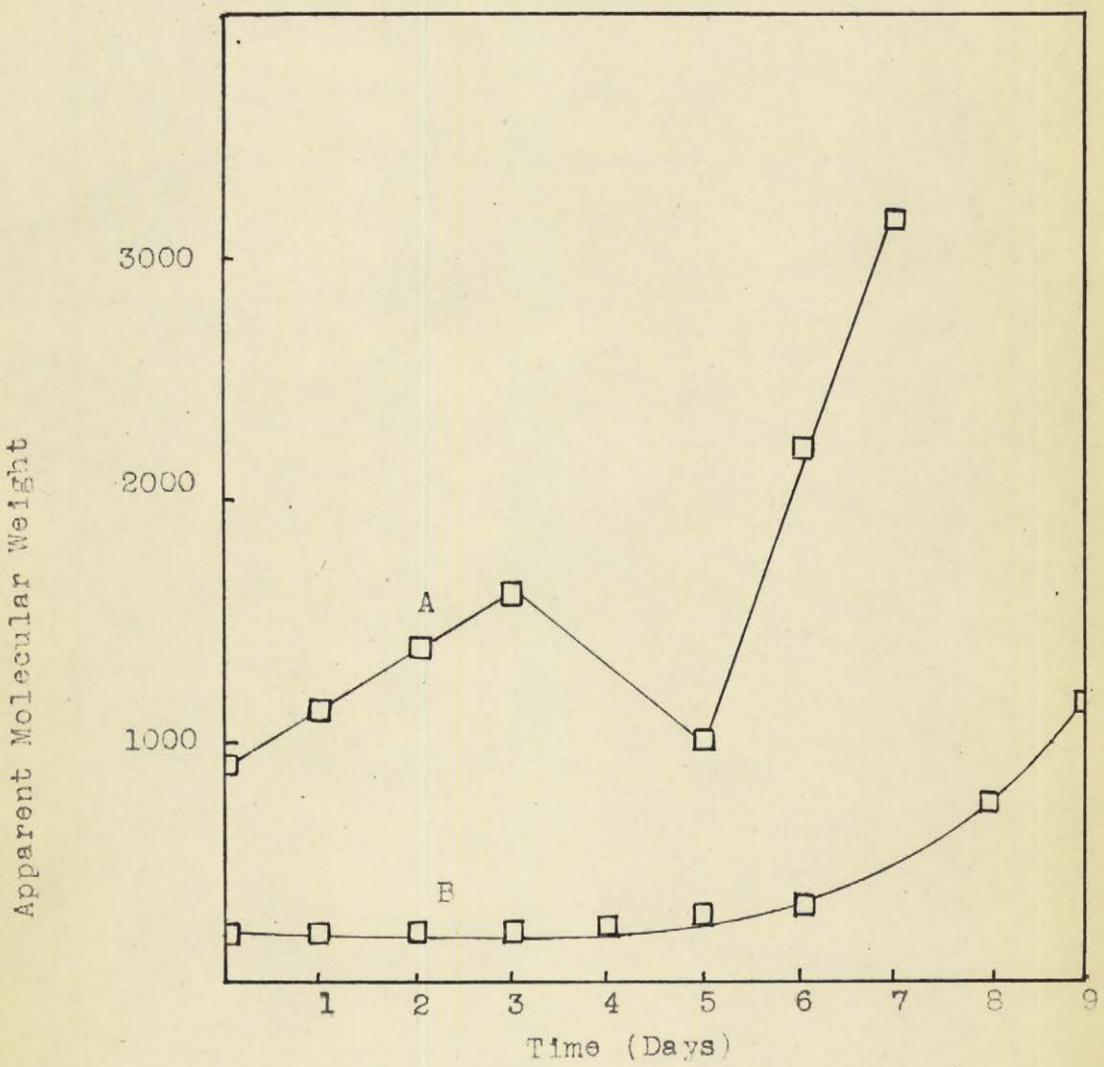


Fig.3. The Apparent Molecular Weights of Oxylignins D-2 and E-1 by the Signer Method.

Plot A Oxylignin D-2, 1.63 mg., in 1.050 cc of Methanol and 1.17 mg. of Sucrose Octaacetate in 1.020 cc of Methanol at 28.7°C.

Plot B Oxylignin E-1, 1.00 mg., in 0.98 cc of Methanol and 1.51 mg. of 2,3-Dimethyl Methyl Glucoside in 1.34 cc of Methanol at 28.7°C.

contents of his products were confirmed with the similar oxylignin B-6, which after standing in sodium hydroxide overnight gave an acid insoluble oxylignin D-1 (OCH_3 4.9%) and an acid soluble, butanol extracted oxylignin E-1 with an apparent methoxyl content of 9.6%. The molecular weight of oxylignin E-1, by the Signer method (69) was in excess of 1000.

To verify the opinion that esterification had occurred during the extractions of oxylignin E with butanol, a modified experiment was carried out using oxylignin B-8. An acid insoluble fraction, isolated in the usual manner and called oxylignin D-2, contained a somewhat lower methoxyl content than the starting material (4.5% vs. 5.0%) and a somewhat lower chlorine content (8.3% vs. 8.9%), but these differences could not account for the large decrease in neutralization equivalent mentioned above. The acidic liquors from the isolation of oxylignin D-2 were evaporated to dryness and leached with acetone to remove the organic material. Addition of the acetone solution to petroleum ether gave a light brown powder in 28% yield (oxylignin E-2). Since this fraction had only 4.8% of methoxyl instead of 9.6% for oxylignin E-1, it was likely that esterification had occurred in the latter case. The loss in methoxyl content from about 7% to 5% reported by Levitin for the acid insoluble component D was probably the result of de-esterification of the partially esterified oxylignin B

which he had inadvertently prepared, while the increase in "methoxyl" content for the acid soluble portion was the result of butoxyl groups inadvertently introduced as esters during the butanol extraction. A molecular weight determination of oxylignin D-2 by the Signer method (69) (Figure 3) gave a result in excess of 3000, while Levitin (18) had claimed the molecular weight of his oxylignin B was about 980. Since oxylignin E-1 had a molecular weight of at least 1000 it would appear that sodium hydroxide had not degraded oxylignin B into two fragments of lower molecular weight.

The neutralization equivalents of oxylignin D were determined alternately by direct titration with sodium hydroxide and by back-titration with acid after the alkaline solution had stood overnight (Table VI). A further indication that the two fractions derived from oxylignin B were not stable to sodium hydroxide may be seen in Figure 4. The neutralization equivalents of both oxylignins D-2 and E-2 decreased with time when they were dissolved in sodium hydroxide, this decrease proceeding rapidly at first and then more slowly. The maximum neutralization equivalent of 243 for oxylignin D-2 (Table VI) compared closely to 237 for oxylignin B under similar circumstances. Since the results were similar to those for oxylignin B, treatment of the latter with sodium hydroxide did not stabilize the substance toward alkali.

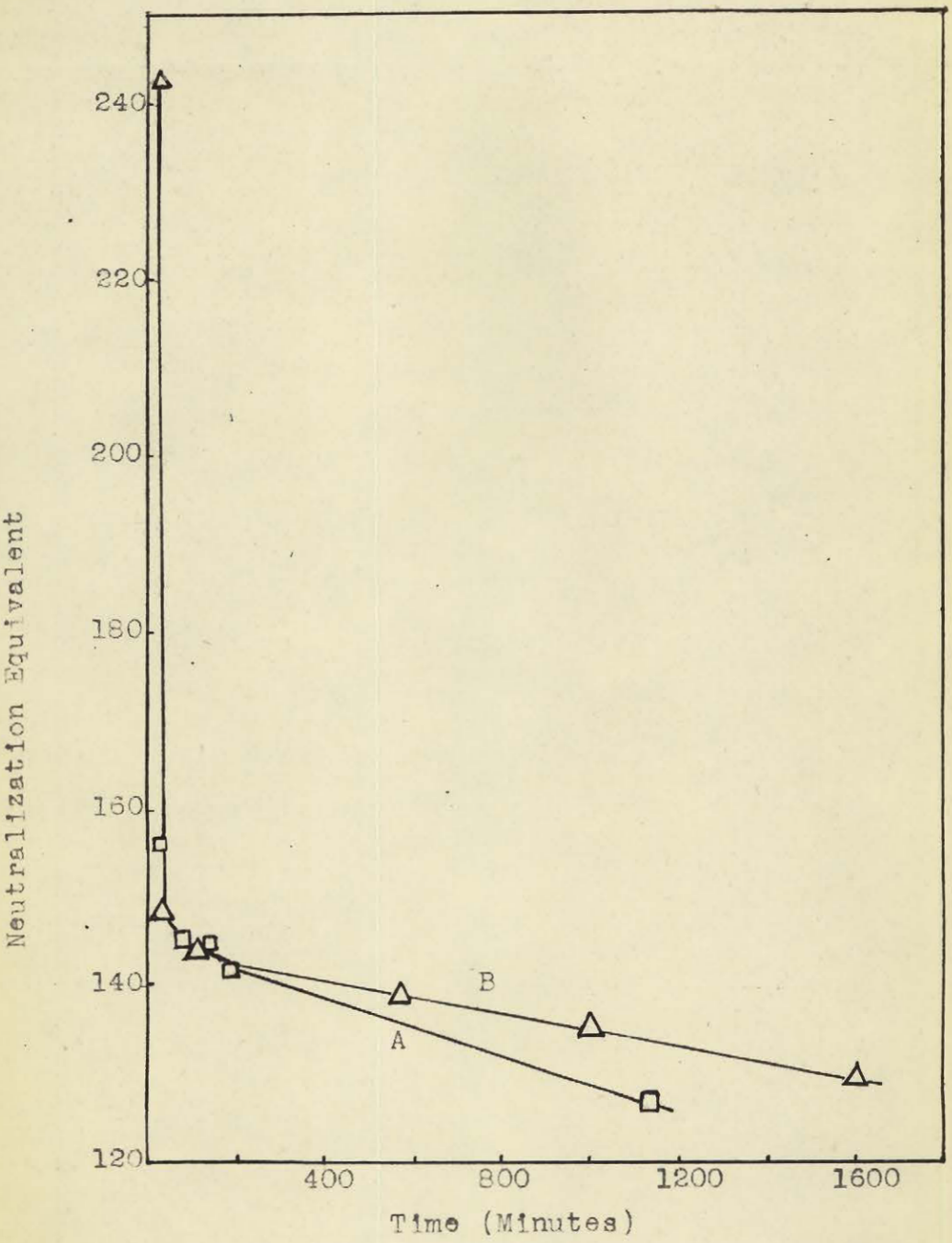


Fig.4. The Reaction of Oxylignins D-2 and E-2 with Sodium Hydroxide at Room Temperature.

Plot A Oxylignin E-2, 0.0961 gm., with 50 cc of 0.0865 N Sodium Hydroxide.

Plot B Oxylignin D-2, 0.1500 gm., with 50 cc of 0.0788 N Sodium Hydroxide.

TABLE VI

The Apparent Neutralization Equivalents of Oxylignin D-2.

Neutralization Equivalent (a)	Neutralization Equivalent (b)
242	171
221	220
243	245

(a) Approached from the acid side.

(b) Approached from the alkaline side.

To sum up, the action of alkali on oxylignin B was not to cleave it into two fragments each of smaller molecular weight and differing in alkoxyl content, as Levitin (18) had supposed, but rather to condense it to substances perhaps of higher molecular weight, differing markedly in solubility but not in methoxyl content.

Since the methanol used to isolate oxylignin B probably introduced methyl ester groups, acetone was selected as the extractant in later preparations. Acetone had an effective solvent power, a low boiling point, and was supposedly inert towards the oxylignin. Oxylignin B-8 was the first product which could be considered free of solvent influences.

Although Levitin (18) had shown that his oxylignin B was not obviously inhomogeneous, an attempt was made here to fractionate oxylignin B-8 by solution of oxylignin B and its acid-insoluble fractions in sodium bicarbonate, followed by acidification and analysis of the soluble and insoluble portions. Disregarding the loss due to splashing in the

second fractionation, the yield of the acid-insoluble portion (Table VII) remained fairly constant at 80% and was probably low due to the difficulty of minimizing mechanical losses.

TABLE VII

The Fractionation of Oxylignin B-8 with Aqueous Sodium Bicarbonate.

Precipitation	Grams Used	Acid Insoluble Fraction				Acid Soluble Fraction	
		Yield (%)	Methoxyl (%)		Chlorine (%)	Yield (%)	Methoxyl (%)
None	-	-	5.0	5.0	8.6 8.9	-	-
First	1.001	84.2	5.6	5.5	-	11.2	4.7
Second	0.742	71.0[a]	5.4	5.4	-	5.2	5.2
Third	0.465	80.0	5.2	5.7[b]	8.6 8.9	insoluble in acetone	

(a) Low due to splashing.

(b) Methoxyl was 6.1% when first analysed, continued washing decreased it to those given.

The average methoxyl content was somewhat higher than the starting material but the chlorine content remained unchanged. The acid-soluble portion decreased in yield as the fractionation proceded but the methoxyl content increased slightly. The last fractionation gave a product insoluble in acetone, perhaps due to the cumulative effect of the alkali or to standing in acidic solution for several days. These results indicated that oxylignin B might be

contaminated with a little material of perhaps 0.2% smaller methoxyl content, but in general supported Levitin's result.

The average of the analyses carried out on oxylignin B (Table IV) corresponded closely to the composition:

$$C_{47}\ H_{45}\ Cl_{3.0}\ O_{26}\ (OCH_3)_2$$

with a molecular weight of 1193, although of course this calculation made the improbable assumption that oxylignin B was a pure compound and not a mixture.

If this empirical formula was the same as the molecular formula, the molecular weight would be within the range measurable by some of the classical methods. An estimation by the ebullioscopic method of Menzies and Wright (74) was attempted in methanol solution, but the limited solubility of the oxylignin after being dried rendered the determination highly inaccurate. When most of the oxylignin had dissolved, the change in boiling point indicated a continuous decrease in molecular weight from 900 to 700, at which point the experiment was discontinued. The decrease might have been due to the solution of more oxylignin (although no change in the amount could be observed visually), to the formation of some low molecular weight substance such as water of esterification, or by a trace of thermal decomposition.

Several attempts were then made to estimate the molecular weight of oxylignin B-8 by the Signer method (69) which in the hands of Levitin (18) had given values

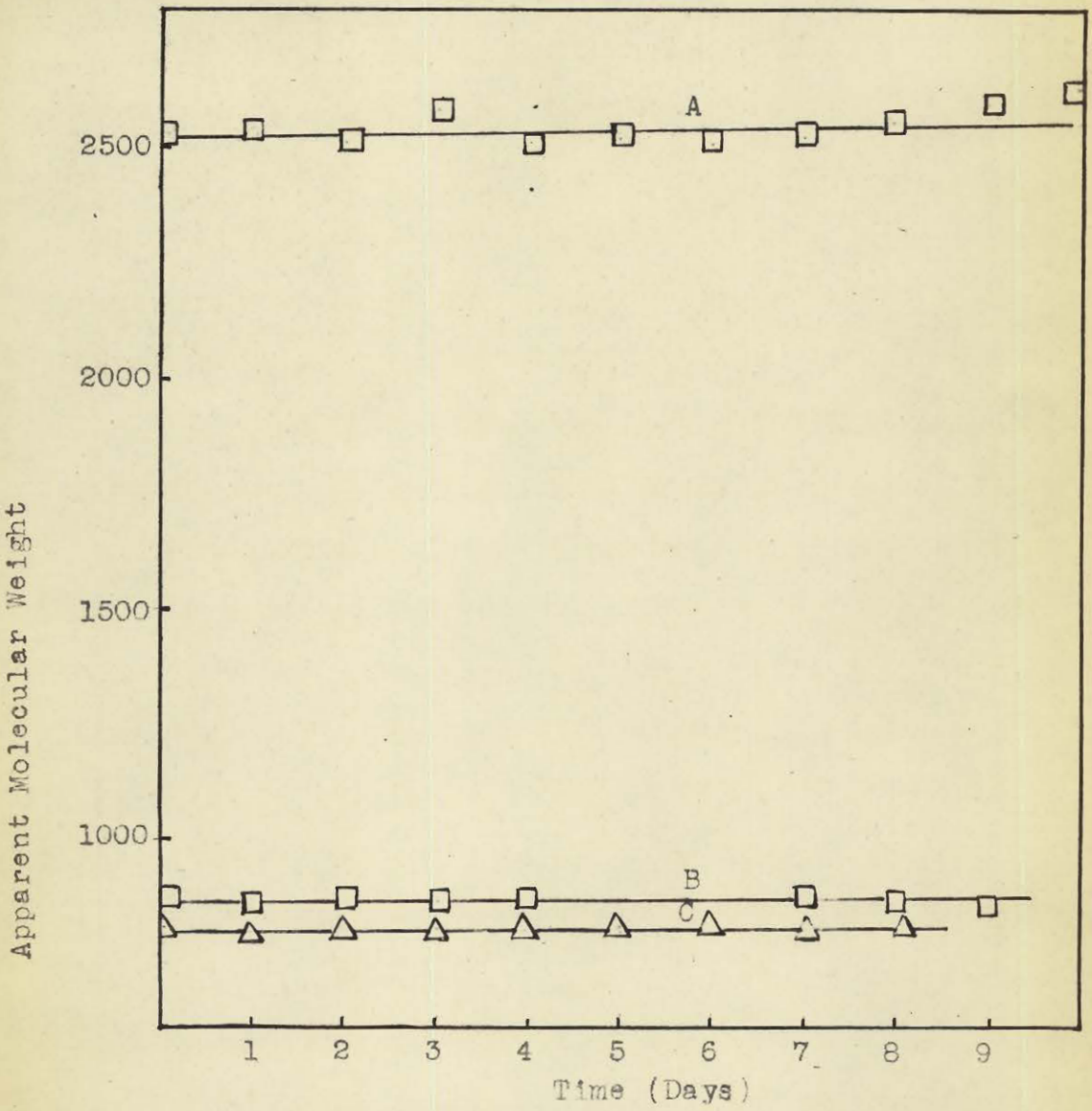


Fig.5. The Apparent Molecular Weights of Oxylignin B by the Signer Method.

Plot A Oxylignin B, 4.81 mg., in 1.010 cc. of Methanol and 1.28 mg.of Sucrose Octaacetate in 1.000 cc. of Methanol at Room Temperature.

Plot B Oxylignin B, 10.0 mg., in 1.050 cc of Methanol and 8.29 mg. of Sucrose Octaacetate in 1.130 cc of Methanol at Room Temperature.

Plot C Oxylignin B, 1.37 mg., in 0.840 cc.of Methanol and 1.99 mg. of Sucrose Octaacetate in 1.435 cc of Methanol at Room Temperature.

between 980 and 895. This method depended upon the attainment through isothermal distillation of equal vapor pressures by a solution of oxylignin B and a control solution containing a substance of known molecular weight. Figure 5 shows the result of repeating this work; no change occurred when the molecular weight as indicated by the concentrations in the apparatus, was set at 800 or 2500. Since it was suspected that the solutions of oxylignin B-8 employed were too dilute, an approximately 10% solution was set against the control solution for a molecular weight of 880, but there was still no change after five weeks. The results had another abnormal aspect in that a decrease of molecular weight would be expected from a slow esterification of oxylignin B by the solvent, but no decrease was observed. Signer's method was abandoned at this juncture. The freezing point method of determining molecular weights was not attempted because of the low values it was known to give for lignins and their derivatives (75) (48).

Oxylignin B had been methylated with diazomethane by Levitin (18), but it was decided to re-investigate the products from this reaction. The oxylignin, in dioxane solution, was methylated four times with diazomethane and isolated by evaporation of the solvent. Residual solvent was removed by solvent exchange with petroleum ether for sixteen hours followed by maintenance in a high vacuum for eight hours, the two processes being carried out over a period of three days. This treatment was not enough for

18.4% of methoxyl groups were present, while after an additional extraction with petroleum ether for four days, the methoxyl content decreased to 16.6%. A fifth methylation yielded a product also containing 16.6% of methoxyl. The methylation produced a slight increase in the amount of ash and a decrease in the chlorine content from 8.9% to 7.9% (corrected for ash and increase in methoxyl). After the fifth methylation, the oxylignin lost most of its solubilities in organic liquids as well as in sodium hydroxide and sodium bicarbonate solutions.

TABLE VIII

The Analysis of Diazomethane Methylated Oxylignin B-8.

Total Number of Methylations	Yield[a] (%)	Methoxyl[b] (%)		Chlorine[a] (%)		Ash (%)	
4	89.7	18.0	18.7	-		-	
Rewashing	-	16.5	16.7	-		-	
5	98.0	16.6	16.7	7.7	8.0	3.6	3.6

(a) Corrected for increase in methoxyl and ash.
(b) Not corrected for chlorine or ash.

For the calculation of the base molecular weight of this derivative and of other methylated derivatives, the formula proposed by Hibbert and Brauns (76) was used, where D represented the number of methoxyl groups and H the molecular weight of the methoxyl free unit. If a molecular weight of (1193 - 28)=1165

$$\%OCH_3 = \frac{31.02 \times D \times 100}{H \quad 14D}$$

was assumed for this unit, the number of methoxyl groups introduced into the methylated oxylignin B-8 containing 17.2% methoxyl (on an ash free basis) was 6.98. Thus about 5 new methoxyl groups had been introduced into oxylignin B-8 by diazomethane. These new groups were attached either to carboxylic acid or phenolic hydroxyl groups.

An attempt was then made to esterify only the carboxylic acid groups of oxylignin B-8 with methanol and to force the reaction to completion by means of "Drierite". The mixture was heated under reflux to hasten the reaction, the results of which are shown in Table IX.

TABLE IX

The Analysis of Oxylignin B-8 Methylated with Methanol

Methylation	Time of Methylation (hours)	Yield[a] (%)	Methoxyl[b] (%)		Chlorine[a] (%)		Ash (%)	
First	16	89.4	14.3	14.5	-		-	
Second	16	84.5	15.8	15.9	-		-	
Third	16	85.6	15.5	15.6	9.0	9.2	1.1	1.2

(a) Corrected for increase in methoxyl and ash.
(b) Not corrected for ash.

The slight decrease in methoxyl content brought about by the third methylation was disregarded as being too small to be of significance, but this methylation partially destroyed the organic solubilities of the derivative. A determination of its molecular weight was therefore impossible. If the same base molecular weight of (1193 - 28) 1165 was

assumed, together with an ash free methoxyl content of 16.1%, then approximately 4.5 additional methoxyl groups were introduced and were probably a true reflection of the number of carboxylic acid groups present. Since the total number of phenolic and carboxylic acid groups revealed by diazomethane methylation was 5, there were no more than about 0.5 moles of phenolic hydroxyl. This estimate was probably a maximum one, because complete esterification might well not have been achieved. A loss of carbon dioxide by the oxylignin when it was heated under reflux might have caused the observed small decrease in methoxyl content.

Another esterification of oxylignin B-10 employed 2% methanolic hydrogen chloride under mild conditions. After standing for 40 hours at room temperature, the solution was heated under reflux for 3 hours and an aliquot was then treated with silver carbonate to remove the hydrochloric acid, filtered and evaporated to dryness giving Fraction 1, Table X. Fraction 2 was removed after the solution had stood for an additional 40 hours and after an additional 1 hour period of heating under reflux. The following day, the residual solution was heated under reflux for 3 hours and decanted from a residue, giving Fraction 3. The dark brown residue was purified and called Fraction 4.

TABLE X

The Analysis of Oxylignin B-10 Methylated with Methanolic Hydrogen Chloride

Fraction Number	Yield (Grams)(a)	Methoxyl (%)	Ash (%)	Molecular Weight (b)
1	0.684	12.2	-	-
2	0.754	14.2 14.2	-	-
3	2.300	15.7 15.8	0.3	1250 1248
4	0.680	15.6	-	-

(a) The low yield, only 4.418 gm. recovered out of 5.00 gm., was probably due to the fourfold increase in the manipulative errors.
(b) Molecular weights by the method of Menzies and Wright (74).

The first three fractions from this methylation were completely soluble in methanol. Two molecular weight determinations by the method of Menzies and Wright (74) were made on the third fraction using methanol as solvent, the average result being 1249. Since the esterification introduced 4.3 methoxyl groups, the theoretical molecular weight of the oxylignin was increased to 1193 (4.3 x 14)= 1253, in excellent agreement with the experimental value.

The behaviour of the hydroxyl groups in oxylignin B-8 toward acetylation was studied next. Sodium acetate and acetic anhydride proved to be ineffective either at room temperature or at 50°C. The Schotten Baumann technique (77) did not introduce any acetyl groups, but a mixture of acetyl chloride and acetic anhydride

dissolved a portion of the oxylignin and acetylated it. The insoluble residue, which had not changed in appearance, was not acetylated, but could be acetylated if treated again with the reagents. A mixture of ether and petroleum ether (1:10) was found suitable for removing the last traces of acetic acid from the acetylated oxylignin which swelled but did not dissolve in a higher concentration of ether. The most satisfactory acetate was obtained by the use of acetic anhydride and sulphuric acid at room temperature. Since the acetate proved to be completely insoluble in water it could be washed free of the sulphuric acid which might otherwise have tended to char the oxylignin when it was dried. The results of the latter experiments are given in Table XI.

TABLE XI

The Analysis of Oxylignin B-8 Acetylated with Acetic Anhydride and Sulphuric Acid.

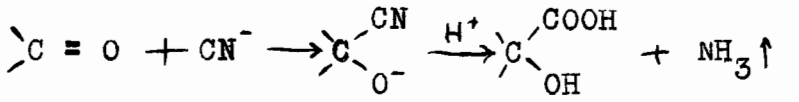
Duration of Acetylation (days)	Yield[a] (%)	Chlorine (%)	Acetyl (%)	Molecular Weight[b]
3	78	-	16.5 17.0	-
	-	-	16.5 17.0[c]	-
5	70	-	19.0 19.5	-
13	100	6.8 6.9	19.9 20.4	1245 1790 1399

(a) Corrected for acetyl content
(b) Molecular Weights by the method of Menzies and Wright (74).
(c) After re-extraction with ether and petroleum ether (1:10).

The acetylated oxylignin contained about 20.2% of acetyl groups, corresponding to the acquisition of 7.0 such groups by a substance of base molecular weight 1193. The acetate was a light yellow, fluffy powder, quite different in appearance from oxylignin B and its methylated derivatives. Fortunately, acetylated oxylignin B was completely soluble in acetone and gave molecular weights by the ebullioscopic method of Menzies and Wright (74) which did not indicate any abnormal behaviour. The average of the three experimental values was 1458, in good agreement with the calculated value of 1487. The molecular weights obtained for methylated and acetylated oxylignin B were close to the upper limit of the ebullioscopic method, and the least trace of moisture or residual solvent would have exerted a

great influence on the values observed.

The method chosen to measure the carbonyl content of oxylignin B depended upon the cyanohydrin reaction (72).



In the first experiment, the cyanide ion remaining unused was estimated by titration with the ammonaical silver ion (78), but a greater number of carbonyl groups were present than the number of available oxygen atoms in the molecule. In the second method, the cyanohydrin solution was made strongly alkaline, and the cyanohydrin groups were hydrolysed to a carboxylic acid salt and ammonia. The ammonia could be estimated easily by distillation into boric acid and titration of the distillate with acid (79). Since oxylignin B reacted with sodium hydroxide, an alkaline buffer was prepared which would hold the pH at 10.5 for 24 hours; after this interval the consumption of alkali by the oxylignin was negligible. The addition of the cyanide to the freshly prepared buffered solution of oxylignin B soon caused the colour to become that of wine, and the maximum production of ammonia corresponded to 1 carbonyl group in 1212 grams of oxylignin B. This reaction, of course, gave no information as to whether the carbonyl group was an aldehyde or ketone.

Levitin (18) found that low pressure hydrogenation caused oxylignin B to absorb one mole of hydrogen per 1100 grams and he considered that a double

bond was present. In an attempt to check this conclusion, the addition of bromine was studied, although it was realized that bromine would also react with any keto-enol system and might substitute reactive hydrogen atoms in aromatic neuclei or certain aliphatic units. On the other hand, the reaction with chlorine dioxide had probably destroyed all the readily substituted phenolic nuclei in oxylignin B (18). The bromine for the reaction was generated from potassium bromate and potassium bromide in acidic solution, and the bromine consumed was calculated from the amounts present in a blank and in the reaction vessel at different times. Figure 6 shows that the bromination was not rapid and that about 5.5 bromine atoms were eventually consumed by 1200 grams of the oxylignin. The measurement was not accurate because the dark colour of the suspended oxylignin rendered the determination of the end point by iodometric means very difficult. This result provided no decisive evidence for the presence or absence of double bonds.

According to the review by Jones (80) the ultra-violet absorption spectra of lignin and model compounds contained two characteristic absorption bands in the wave length regions 200 to 230 mμ and 275 to 285 mμ. The former was customary for aromatic compounds, while the latter could be attributed to benzene nuclei substituted with oxygen. A further absorption in the region 300 to 350 mμ was probably the masked maxima of

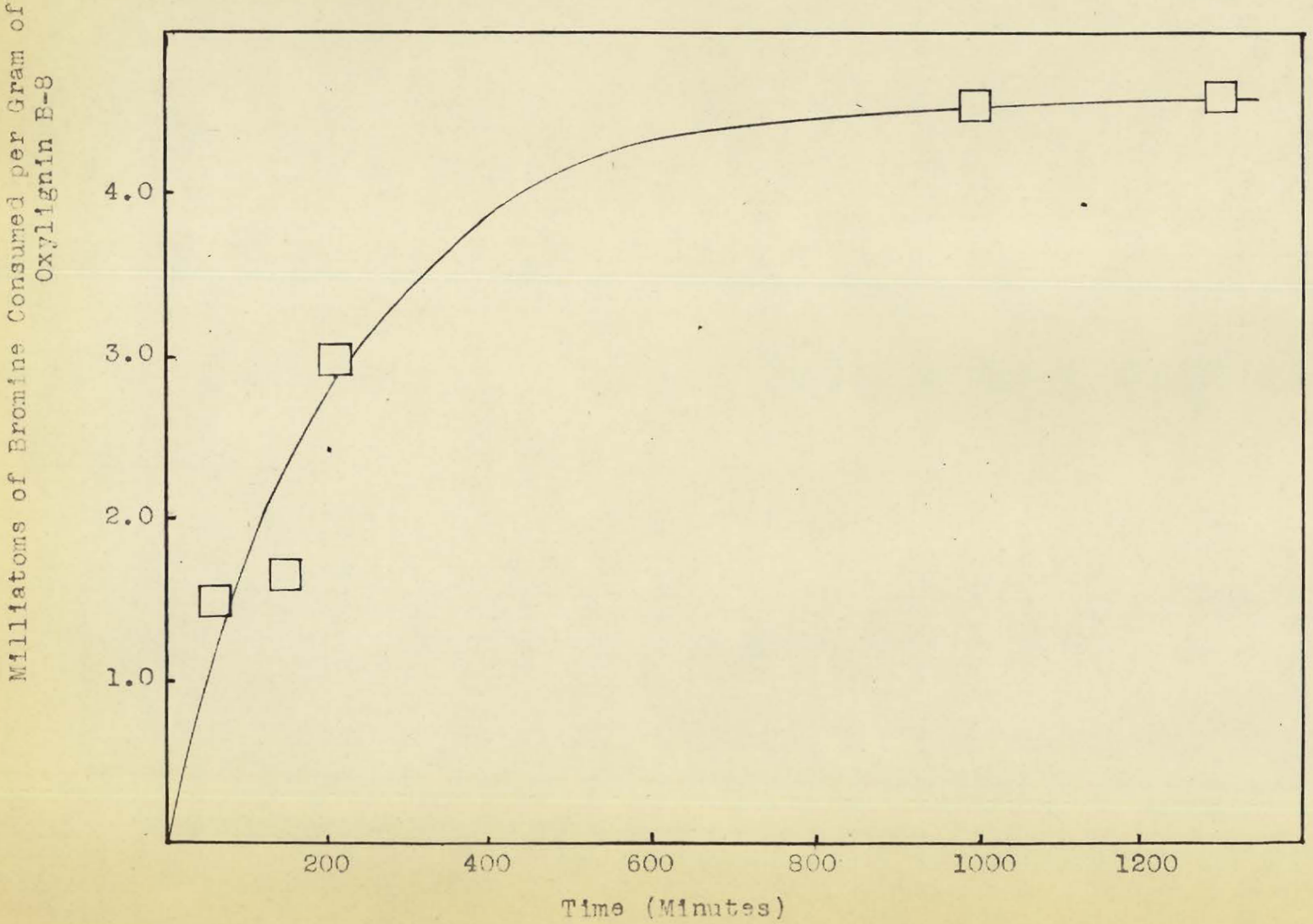


Fig.6. The Reaction of Oxylignin B-8 with Bromine. (see Table XX).

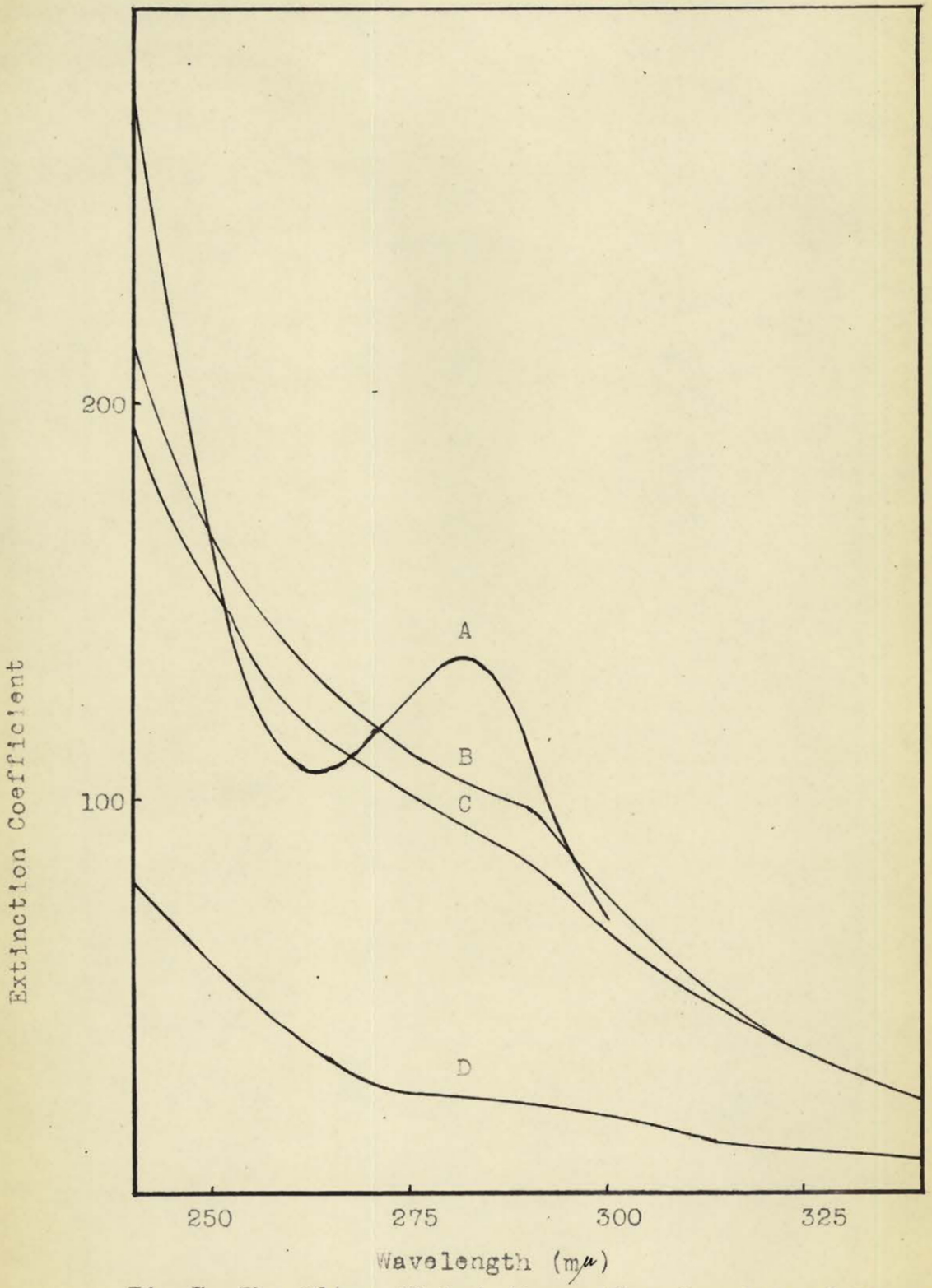


Fig.7. The Ultra-Violet Absorption Spectra of Degraded Lignins.
Plot A Calcium Lignosulphonate, 10.0×10^{-3}%, in NaOH.
Plot B Oxylignin B-8, 4.4×10^{-3}%, in Methanol.
Plot C Oxylignin F, 5.6×10^{-3}%, in Methanol.
Plot D Oxylignin C-8, 11.5×10^{-3}%, in Methanol.

other chromophoric groups. Jones (80) suggested that if the hydroxyl groups and ether linkages in lignin were replaced by hydrogen or alkyl groups the maxima of these other chromophoric groups might be clearly exposed. In Figure 7, the ultra-violet absorption spectrum of oxylignin B-8 has been compared with that of a lignosulphonic acid since periodate lignin was insoluble and did not lend itself to the determination. The data for the absorption spectra of the lignosulphonic acid was kindly supplied by Dr. G. Allen of the Pulp and Paper Institute of Canada. Instead of a maximum at 282 mμ, a point of inflection seemed to be present at 291 mμ and the shape of the curve suggested that the number of phenolic nuclei in oxylignin B was very small, while the maxima which appeared below 240 mμ indicated the presence of other types of aromatic nuclei. The same remark seemed true in even greater degree of the more highly oxidized oxylignin C fraction.

If phenolic groups were absent, then oxidation of oxylignin B with nitrobenzene and alkali should yield no vanillin, in agreement with Levitin's (18) observation. This oxidation was repeated using the new micro technique of Stone and Blundell (81). No aldehyde could be detected among the oxidation products by chromatography on paper, and the ultra-violet investigation of the chromatographed products showed that only 0.09% of vanillin was present. This result was at the lower limit of the sensitivity of the Beckman Spectrophotometer used and was not

necessarily in conflict with the entire absence of vanillin.

The foregoing results could be approximated by the following molecular formula for oxylignin B, if the doubtful assumption was made that the substance was homogeneous.

$$(OCH_3)_2 \left[C_{41} H_{33} O_8 \begin{matrix} (COOH)_5 \\ (OH)_7 \\ (C{=}O) \end{matrix} \right] Cl_3$$

Not more than 0.5 moles of the hydroxyl groups, if indeed any at all, were phenolic. This formula, however, disregards the low neutralization equivalent of 120 found after oxylignin B was kept in an excess of alkali. A later section of this thesis describes attempts to gain more information about the C_{41} nucleus, which was known to be non-phenolic or nearly so, but to be aromatic at least in part.

Examination of Oxylignin C.

The acid-soluble portion of the oxidation of periodate lignin with chlorine dioxide was called oxylignin C by Levitin (18) who had been able to isolate the fraction only as a tar contaminated with butanol and with an apparent methoxyl content of 14.8%. Oxylignin C was isolated in the present investigation as an almost colourless powder by adding the butanol extract to petroleum ether. Some change in the oxylignin had occurred, as it was no longer soluble in water and its varying methoxyl content,

9.4% to 10.4% suggested esterification with butanol. Trial showed that pure n-butanol contained 40.7% of apparent methoxyl groups when analysed by the standard Vieboch procedure (82), and traces of hydrochloric acid extracted together with oxylignin C by the butanol would certainly catalyse an esterification. The water soluble portion of the oxidation was therefore isolated by evaporation of the solvent, first at 2 cm. pressure and finally at 1 mm. Under suitable circumstances a froth was formed which could be crushed to a fine yellow powder, the acetone-soluble portion of which was also light yellow in colour and contained only 1.2% of methoxyl groups but 17.1% of chlorine. The yield was 29% by weight of the periodate lignin. Since the isolation required the use of a high vacuum over considerable periods of time, it was possible that a relatively large quantity of organic matter was lost. Some evidence for this conjecture was given by the purple colour imparted to the potassium hydroxide employed as desiccant. An extraction of the crude oxidation liquors with benzene also gave a few milligrams of a purple tar which was not investigated further.

The acetone-insoluble fraction from the extraction with acetone mentioned further above amounted to 10% of the periodate lignin and contained 30% of ash and 0.76% of methoxyl groups. A portion was examined by the chromatographic technique of Barker and Kennedy (83), using methanol-ammonia as solvent, and a spot of acidic

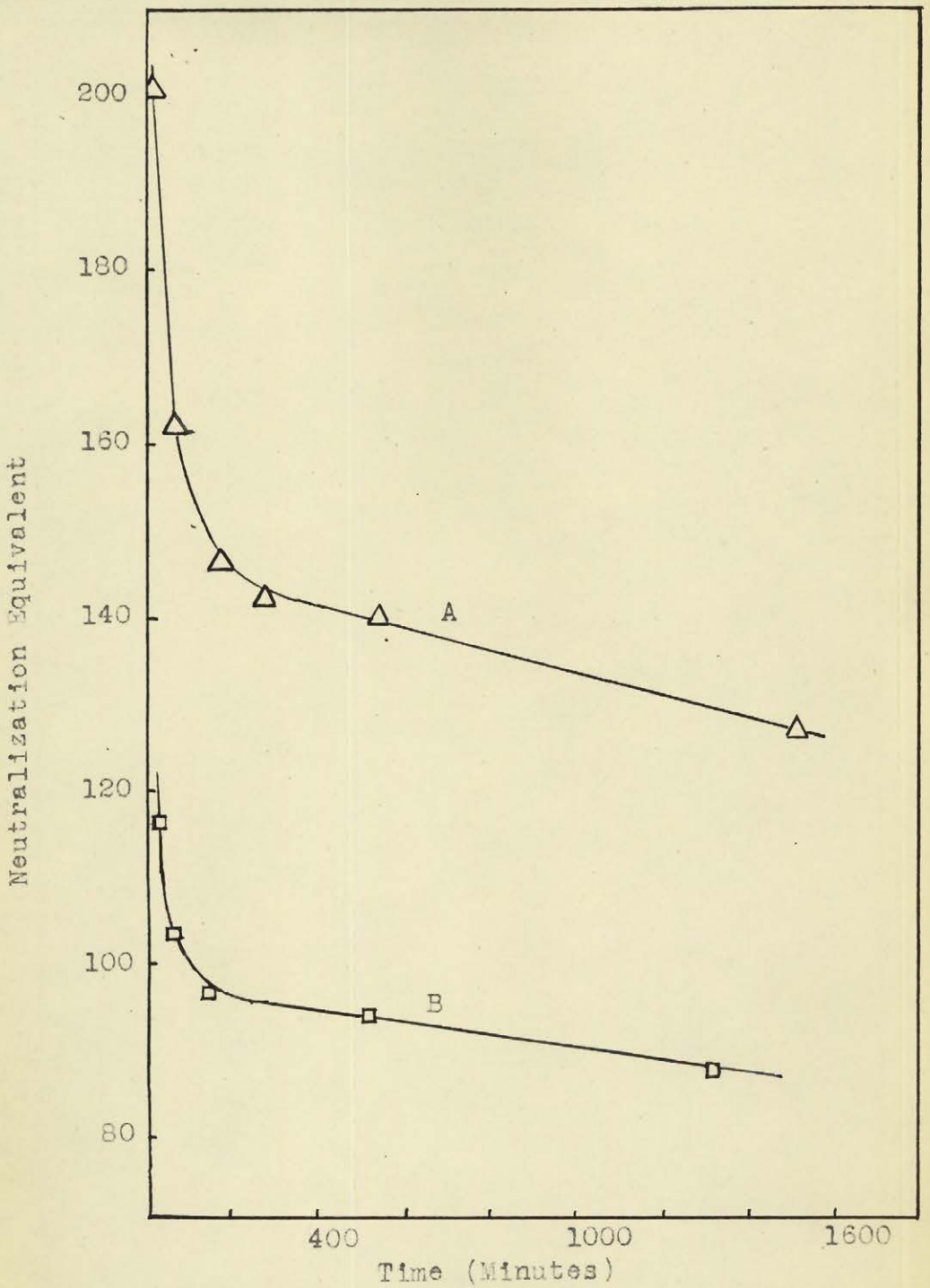


Fig.8. The Reaction of Oxylignin C with 0.0865 N Sodium Hydroxide at Room Temperature.

Plot A Oxylignin C-7,91mg., in 25 cc. of NaOH.

Plot B Oxylignin C-8,106mg., in 25 cc of NaOH.

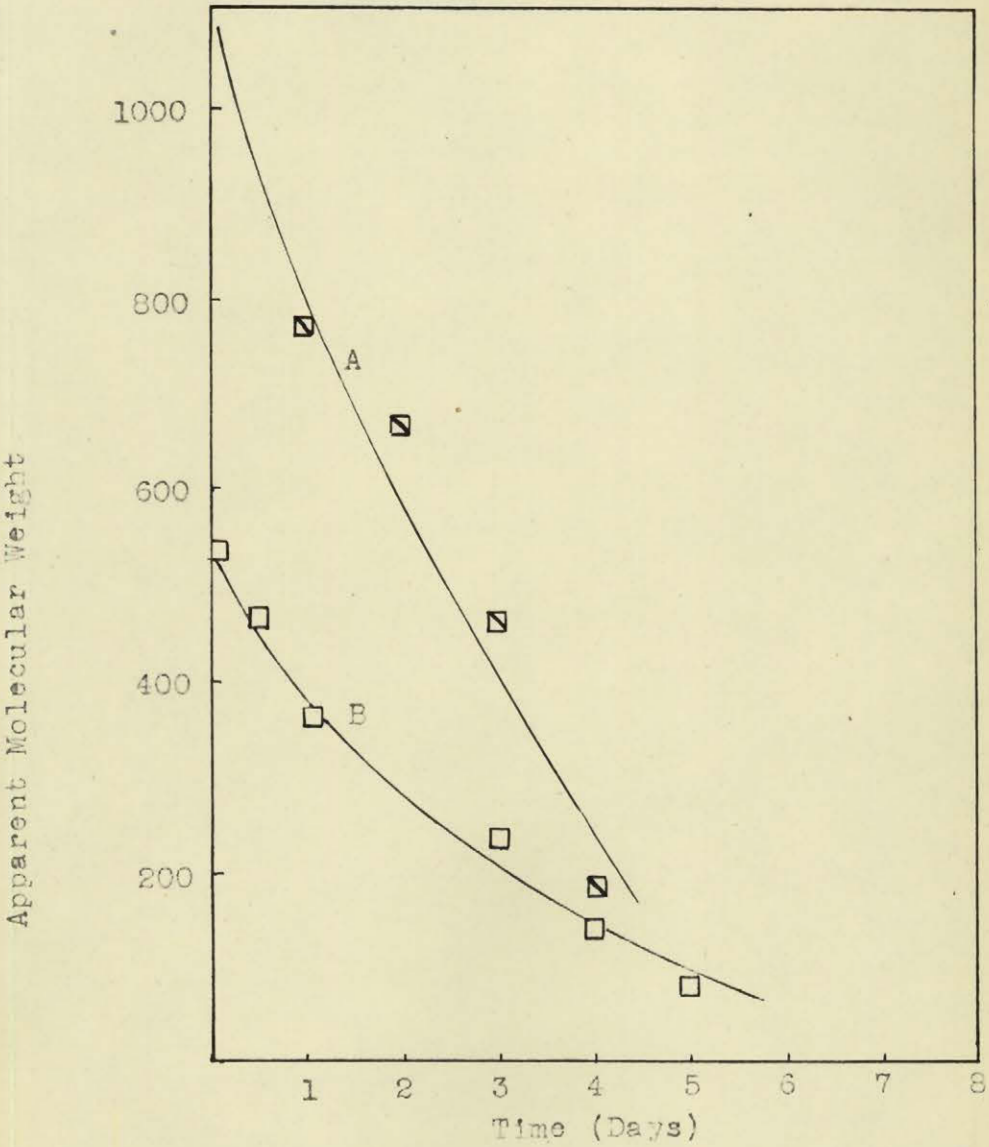


Fig.9. The Apparent Molecular Weight of Oxylignin C by the Signer Method.

Plot A Methylated Oxylignin C-8, 1.82 mg., in 1.550 cc.of methanol and 0.926 mg. of Sucrose Octaacetate in 1.305 cc.of Methanol at Room Temperature.

Plot B Oxylignin C-8, 1.88mg.,in 1.055 cc. of Methanol and 1.00 mg. of 2,3-Dimethyl Methyl Glucoside in 1.315 cc.of Methanol at Room Temperature.

material was detected with an Rf value of 0.90. This acetone-insoluble fraction yielded a methanol-soluble fraction which was only partly soluble in sodium hydroxide but which was hydroscopic and readily soluble in dilute hydrochloric acid. The investigation of this mixture was discontinued at this point.

Oxylignin C, considered to be the acetone-soluble fraction mentioned above, was soluble in water, bases, acids, acetone and alcohols, but was insoluble in benzene, camphor, chloroform and ether. Oxylignin C reduced Fehling's solution and periodic acid. The high degree of oxidation of this fraction, (Table IV), was reflected by its low carbon and hydrogen content, even after allowance was made for its high chlorine content. By assuming oxylignin C-8 to be homogeneous, which was extremely unlikely, an empirical formula could be calculated which corresponded to the analyses in Table IV.

$$C_{46.35}\ H_{52.61}\ Cl_{6.79}\ O_{33.94}\ (OCH_3)_{0.5}$$

No systematic fractionations were carried out, but another reaction to be described later resulted in a fraction with a higher methoxyl content. It was suspected therefore that oxylignin C was a mixture of compounds some of which were free or nearly free of methoxyl groups.

Since all the water-soluble products isolated had a methoxyl content of less than 1.2%, and since oxylignin C extracted with butanol had an apparent methoxyl content of 9.4% to 10.4%, contamination with butanol must have

occurred during the isolation by that method. The difference in neutralization equivalents, shown in Figure 8, between oxylignin C-7 (isolated with butanol) and oxylignin C-8 (isolated directly), could be explained not only by esterification but also if the two oxylignins happened to represent fractions with different neutralization equivalents. The yield of C-7 was only one half the yield of C-8.

The results of methylating oxylignin C-8 with diazomethane are given in Table XII. The chlorine content decreased from 17.1% to 11.3% as a result of this reaction, and the product from the ninth methylation had lost its solubilities in organic liquids.

TABLE XII

The Analysis of Oxylignin C-8 Methylated with Diazomethane.

Number of Methylations	Yield[a] (%)	Methoxyl[b] (%)		Chlorine[a] (%)		Ash (%)	
6	91	12.5	12.6	-		-	
8	69[c]	13.8	13.8[d]	11.2	11.4	2.4	2.4
9	89	14.3	14.0	-		-	

(a) Corrected for increase in methoxyl and ash.
(b) Not corrected for ash or change in chlorine content.
(c) Low yield due to mechanical losses.
(d) Methoxyl content corrected for ash and chlorine 15.7%

TABLE XIII

The Analysis of Oxylignin C-8

Esterified with Methanol and "Drierite"

Time of Methylation (Hours)	Yield[a]	Methoxyl[b] (%)		Chlorine[a] (%)		Ash (%)	
3	89	13.0	13.0	-		-	
5	94	13.8	13.8[c]	18.5	18.7	6.0	6.6
25	89	13.8	13.9	-		-	

(a) Corrected for increase in methoxyl and ash.
(b) Not corrected for ash.
(c) Corrected for ash and increase in chlorine 17.6%.

Oxylignin C-8 was also methylated to constant methoxyl content using methanol and "Drierite" in the same manner as described for oxylignin B. The chlorine content increased slightly to about 18.6% when corrected for ash and the additional methoxyl and therefore no chlorine was displaced. Diazomethane was found to introduce about 15.7% of methoxyl on an ash and chlorine free basis into oxylignin C-8 while methanol introduced 17.6% on a similar basis. Although the accuracy of these corrected values was not great, their similarity suggested that diazomethane had methylated carboxylic acid groups exclusively, and that oxylignin C contained no free phenolic groups.

An attempt was made to determine the molecular weight of oxylignin C-8 by the Signer method (69) using

methanol as solvent, but the values decreased from 550 to 95, (Figure 9). The value for the product esterified with methanol also decreased from 800 to 190. Labile chlorine atoms, which were later shown to be present in the oxylignin, might have interfered with these estimations and brought about their failure.

The quantitative recovery of an acetylated oxylignin B suggested that the composition of an acetylated oxylignin C might give an accurate measure of the hydroxyl groups present, even though the oxylignin was probably a mixture. Acetyl chloride at room temperature introduced 7.8% acetyl groups, but introduced only 4.3% at 48°C. Since the yield in both cases was only 50%, other methods were investigated. Sulphuric acid and acetic anhydride gave a light yellow powder in 29% yield containing 15.5% of acetyl groups. All the acetates of oxylignin C were water soluble and the quantitative recovery of acetylated products was impossible. The only conclusion drawn was that hydroxyl groups were present in a portion of oxylignin C at least.

Since the chlorine atoms in oxylignin C seemed to vary in lability, their nature was explored in greater detail. When oxylignin C-8 (Cl 17.1%) was dissolved in dilute sodium hydroxide, only 5.1% of chlorine remained on the oxylignin after 40 hours (Figure 10). From this experiment, the chlorine seemed to be of two types, 5.1% resembling aromatic chlorides in stability, and the

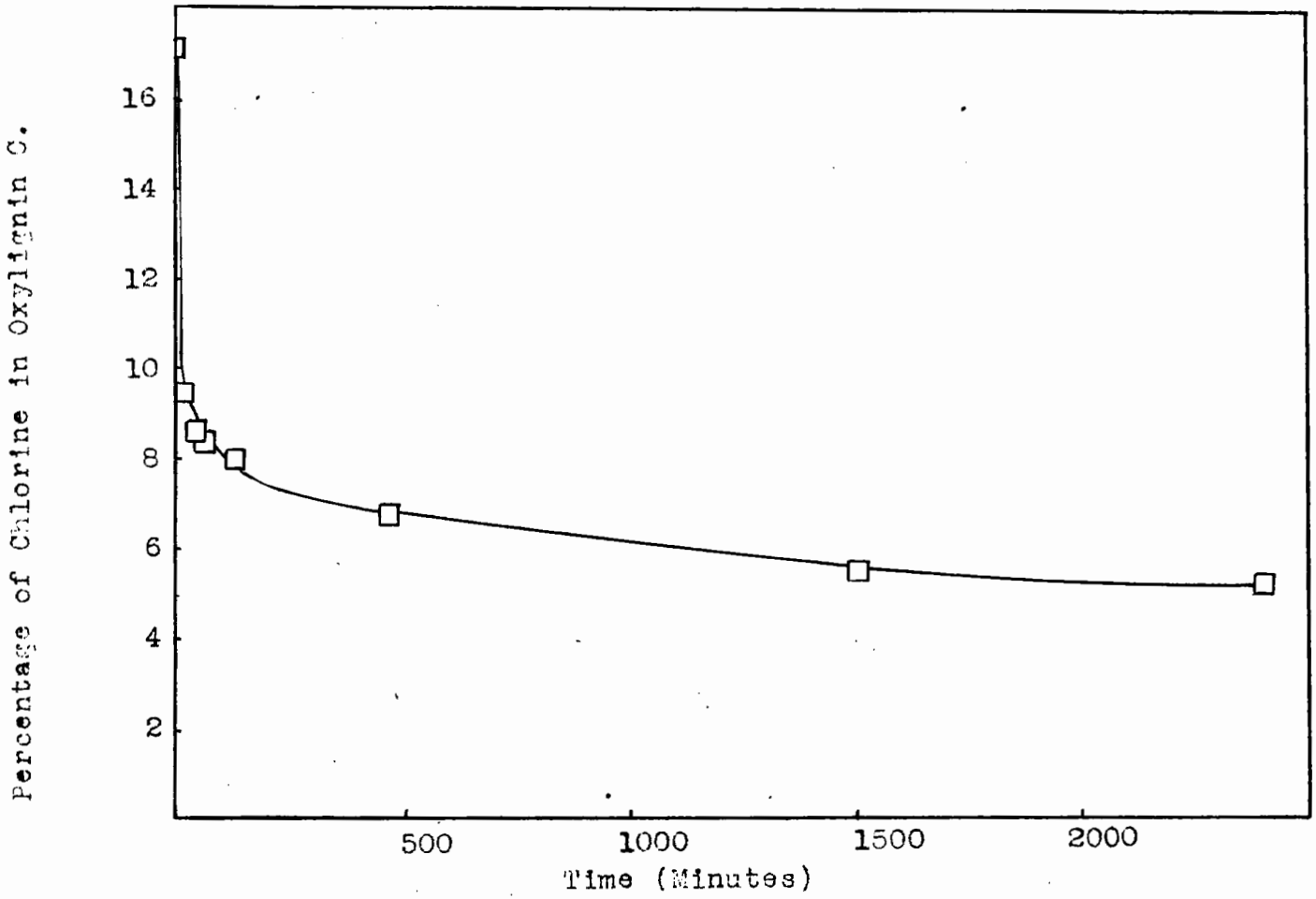


Fig.10. The Loss of Chlorine by o.1287 gm. of Oxylignin C-8 (17.1% Cl) Dissolved in 20 cc. of 1 N Sodium Hydroxide at Room Temperature.

remaining 12% of a more labile nature, resembling aliphatic chlorides. Since 2.3% chlorine was removed by silver nitrate when oxylignin C-8 was dissolved in water, it was concluded that a portion of the aliphatic chlorine resembled a tertiary chloride in its lability.

Attempts were next made to isolate a dechlorinated oxylignin C having satisfactory physical characteristics. In 24 hours, sodium hydroxide left only 6.5% of chlorine on a sample of oxylignin C-8, but the caustic soda converted the oxylignin into an unmanageable black tar. Sodium bicarbonate gave a similar tar, while "Amberlite I R 4-B" was basic enough to darken the oxylignin without removing much chlorine. An attempt was made to remove the halogen with nickel-aluminum alloy and sodium hydroxide (84), but the damaging effect of the alkali, together with the difficulty of removing the aluminum ion, rendered the method useless. Zinc and hydrochloric acid reduced oxylignin C to a light yellow powder in 40% yield containing 3.6% of methoxyl and only 7% of chlorine. The product appeared suitable for further study, but the low yield and the tedious method of removing the zinc ion with hydrogen sulphide were serious drawbacks to the process. At this point efforts to remove labile chlorine without promoting what appeared to be deep-seated changes in oxylignin C were abandoned.

Oxidations of oxylignin C with nitrobenzene using the technique of Stone and Blundell (81) failed to

show the presence of vanillin either by a paper chromatographic method or by examination of the chromatographed products with a Beckman D U Spectrophotometer. The ultraviolet absorption spectrum of oxylignin C-8, (Figure 7), showed a complete lack of any maximum at 285 m . These results were not inconsistant with the view that oxylignin C represented a lignin fraction containing little or no phenolic nuclei although other aromatic nuclei might be present. The labile nature of oxylignin C, and the difficulty of isolating it in a powder form suitable for quantitative experiments, made it less attractive for detailed study than oxylignin B, which was readily isolated in almost twice the amount. The remainder of the work was accordingly devoted to the degradation of oxylignin B.

Degradation of Oxylignin B

Potassium permanganate has proven to be a useful instrument in the hands of many investigators for the preparation of low molecular weight compounds from lignin. Oxylignin B-8, as Figure 11, Plot A shows, consumed 0.0145 moles of potassium permanganate per gram. Chromatographic investigation of the ether extract of such an oxidation did not reveal the presence of any low molecular weight products, but several amorphous products were isolated from another oxidation which were not investigated because of their unsatisfactory physical state. The experiments, although materially a failure, did introduce the major problem to be faced; namely the

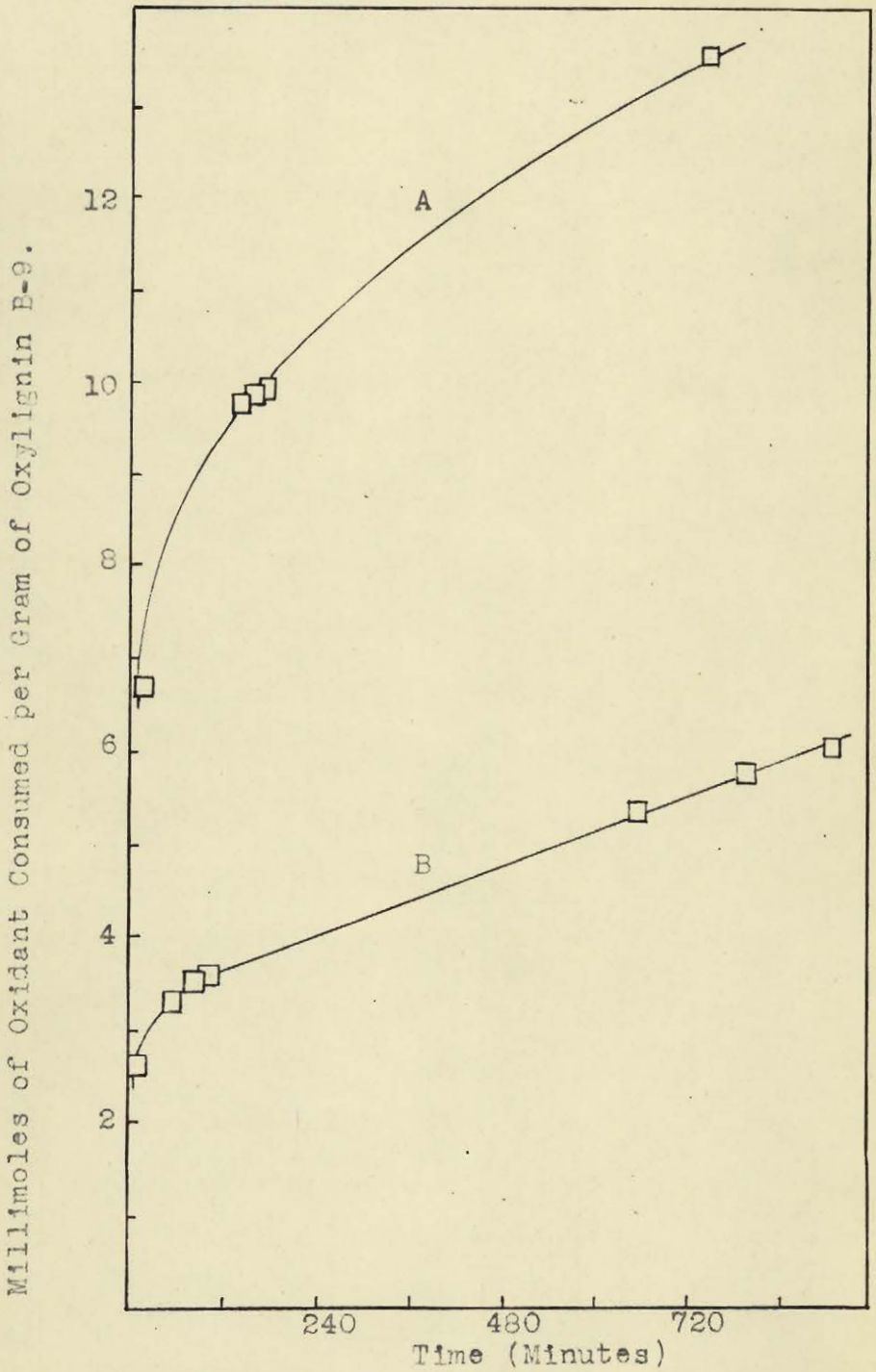


Fig.11. The Degradation of Oxylignin B with Potassium Permanganate and Sodium Periodate at Room Temp.
Plot A Oxylignin B-9, 0.105 gm., in 110 cc. of 0.0186 M Potassium Permanganate at pH 8.5.
Plot B Oxylignin B-9, in 68 cc. of 0.0353 M Sodium Periodate at pH 4.0.

isolation of rather high molecular weight compounds which were acidic, labile, non-crystalline, and not able to be removed from aqueous solution by any solvent but butanol, which would probably esterify them.

Since oxylignin B had been shown to contain 7 hydroxyl groups in 1193 grams, some of these groups might be on adjacent carbon atoms and thus permit the molecule to be cleaved into fragments of lower molecular weight by means of sodium metaperiodate. Tests with periodic acid according to the method of Shriner and Fuson (85) indicated that a reaction occurred and oxylignin B was later found to consume 0.0033 moles of oxidant per gram of oxylignin B or 3.95 moles by 1193 grams (Figure 11, Plot B). This value was obtained by extrapolating a slower reaction to zero time and in succeeding experiments the oxidation was stopped at this stage by means of ethylene glycol. An acid-insoluble portion called oxylignin F was isolated, whose methoxyl content varied between 4.9% and 5.5% for several preparations while the chlorine content was 9.5%. Tests (86) showed that no iodine was present. Attempts to determine the molecular weight of this derivative were unsuccessful; oxylignin F, (Figure 12), exhibited the same abnormal behaviour during the Signer determination (69) as oxylignin B. The ebullioscopic method of Menzies and Wright (74) was equally unsuccessful, such large variations in the boiling point of the solvent occurred when oxylignin F was added that no estimate could be made.

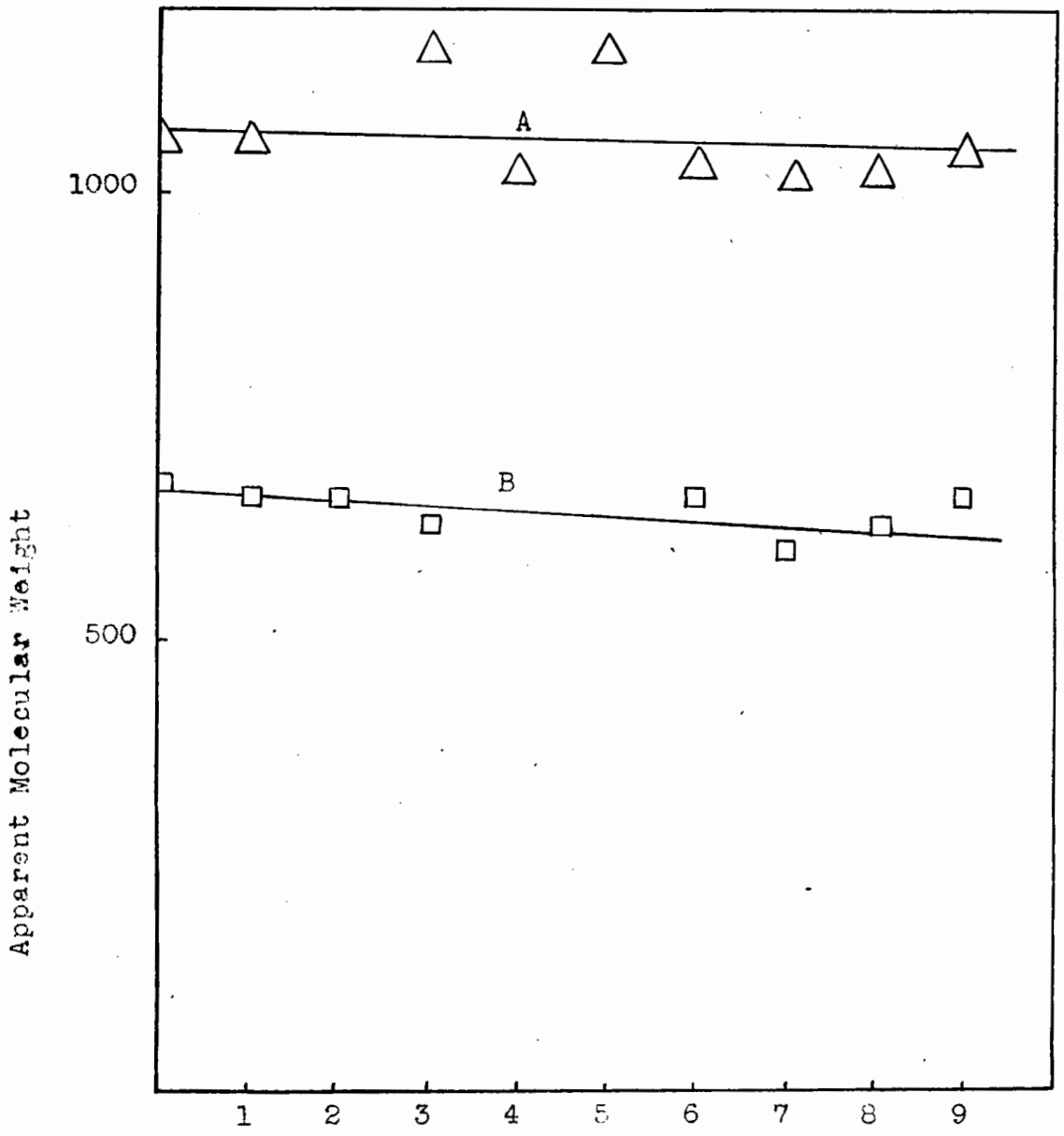


Fig.12. The Apparent Molecular Weights of Oxylignin F by the Signer Method.

Plot A Oxylignin F, 1.16 mg., in 1.000 cc. Methanol and 1.07 mg. Sucrose Octaacetate in 1.435 cc. of Methanol at 28.7°C.

Plot B Oxylignin F, 1.30 mg., in 1.140 cc. of Methanol and 1.27 mg. Sucrose Octaacetate in 1.100 cc. of Methanol at Room Temperature.

Methylation of oxylignin F with methanol and "Drierite" gave a derivative with 11.5% of methoxyl groups, but the low molecular weight of 105 indicated that stability had not been achieved in the determination. The ultra-violet spectrum, as shown in Figure 7, was almost identical with that of oxylignin B, and it was concluded that no deep seated changes had occurred in oxylignin B when it was oxidized with sodium metaperiodate.

The aqueous oxidation liquor contained no sodium metaperiodate, although sodium iodate and perhaps some sodium iodide were present. Preliminary tests showed that the iodate and periodate ions could be reduced to iodide ion by hydrogen sulphide. Since the extraction of the oxidation liquor with ether gave poor yields of organic material, hydrogen sulphide was used to convert all the iodate ion to iodide ion to prevent further possible oxidation of the organic residue. The first attempt to separate the organic material was by dialysis but the organic material also dialysed indicating it had a low molecular weight. The next attempt to isolate the organic material was to replace the iodide ion with chloride ion using silver chloride followed by evaporation of the liquors to dryness. Only a small amount of organic material contaminated with sulphur was isolated. Further investigation of the water-soluble product was not carried out as its physical state was unsuitable for analysis.

Sodium hypochlorite was next chosen for the

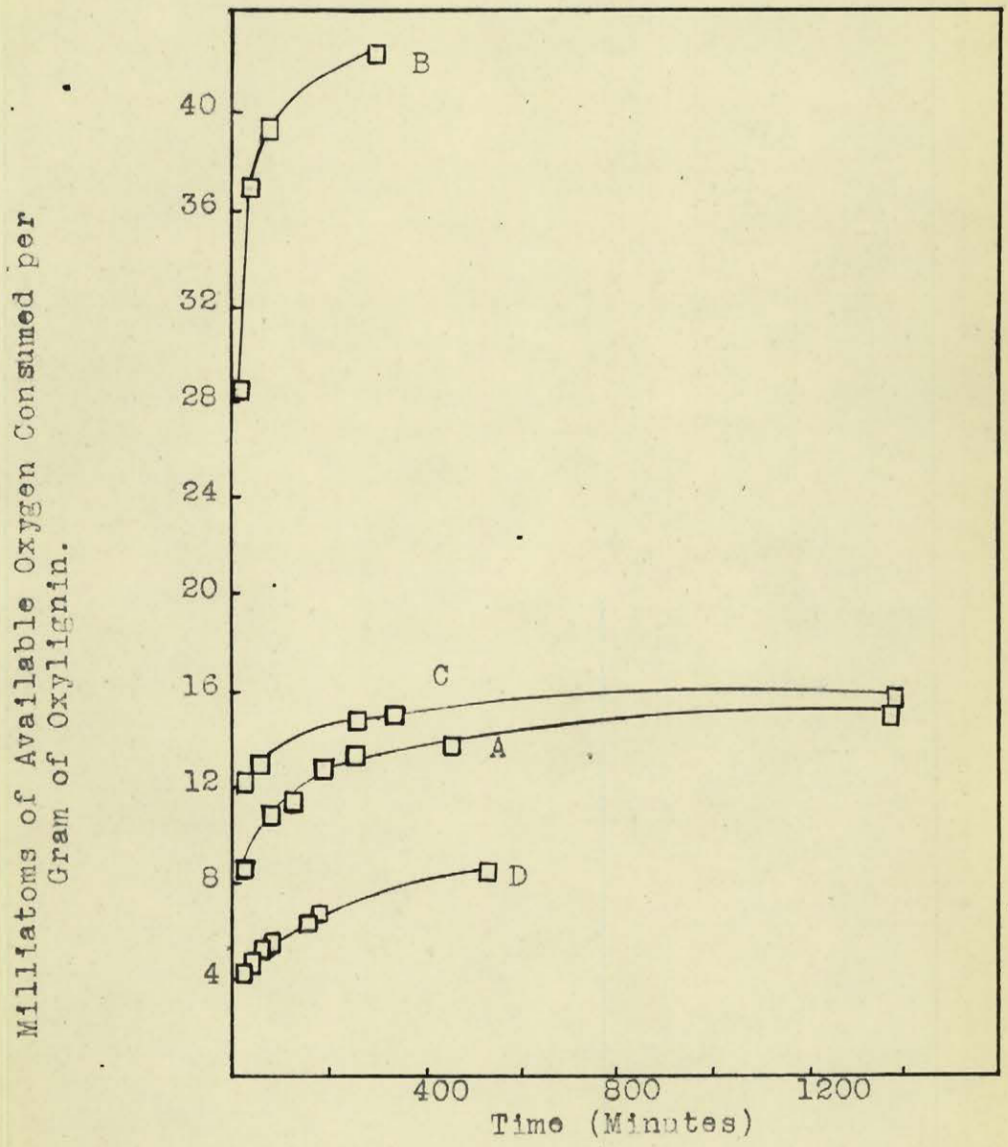


Fig.13. The Reaction of Spruce Periodate Oxylignins with Sodium Hypochlorite.

Plot A Oxylignin B-9, 0.549 gm., with 50 cc. of 0.592 N Sodium Hypochlorite at pH 11 and at Room Temperature.

Plot B Oxylignin B-9, 1 gm., with 100 cc.of 0.900 N Sodium Hypochlorite at pH 7.2 to 7.8 and at Room Temperature.

Plot C Oxylignin H-2, 0.203 gm., with 20 cc.of 0.974 N Sodium Hypochlorite at pH 10.8 and at Room Temperature.

Plot D Oxylignin B-9, 2 gm., with 100 cc of 0.648 N Sodium Hypochlorite at pH 11 and at 5°C.

degradation of oxylignin B because it was successfully used by Whittal (47) and Brounstein (48) to degrade periodate lignin. Figure 13, Plot A shows that 14.6 milliatoms of oxygen were consumed by 1 gram of oxylignin B-9 when it was oxidized by 0.592 N sodium hypochlorite at pH11 and room temperature. After adding acetone to reduce the residual hypochlorite, the solution was acidified to pH 1 and evaporated to a thick tar, the acetone extract of which was converted to a sticky brown powder in 40% yield still contaminated with residual acetone.

A second oxidation was carried out under similar circumstances, and the consumption of oxidant was found to be identical to that given above. Acidification of the alkaline solution to pH 1 after the removal of the excess oxidant with acetone, precipitated an insoluble oxylignin G in 6% yield, and extraction of the aqueous liquors with ether gave a small amount of oil (15%) which would not distill under reduced pressure. Paper chromatographic investigation of the oil by the method of Barker and Kennedy (83) using ethanol-ammonia as solvent, showed the probable presence of acetic acid. This particular solvent system was capable of separating simple aliphatic monocarboxylic acids, but Barker and Kennedy (83) found that methanol-ammonia was the best solvent to separate more complex aliphatic acids. When this solvent system was tried on the ether soluble fraction of a later oxidation carried out at pH11, two spots were found which

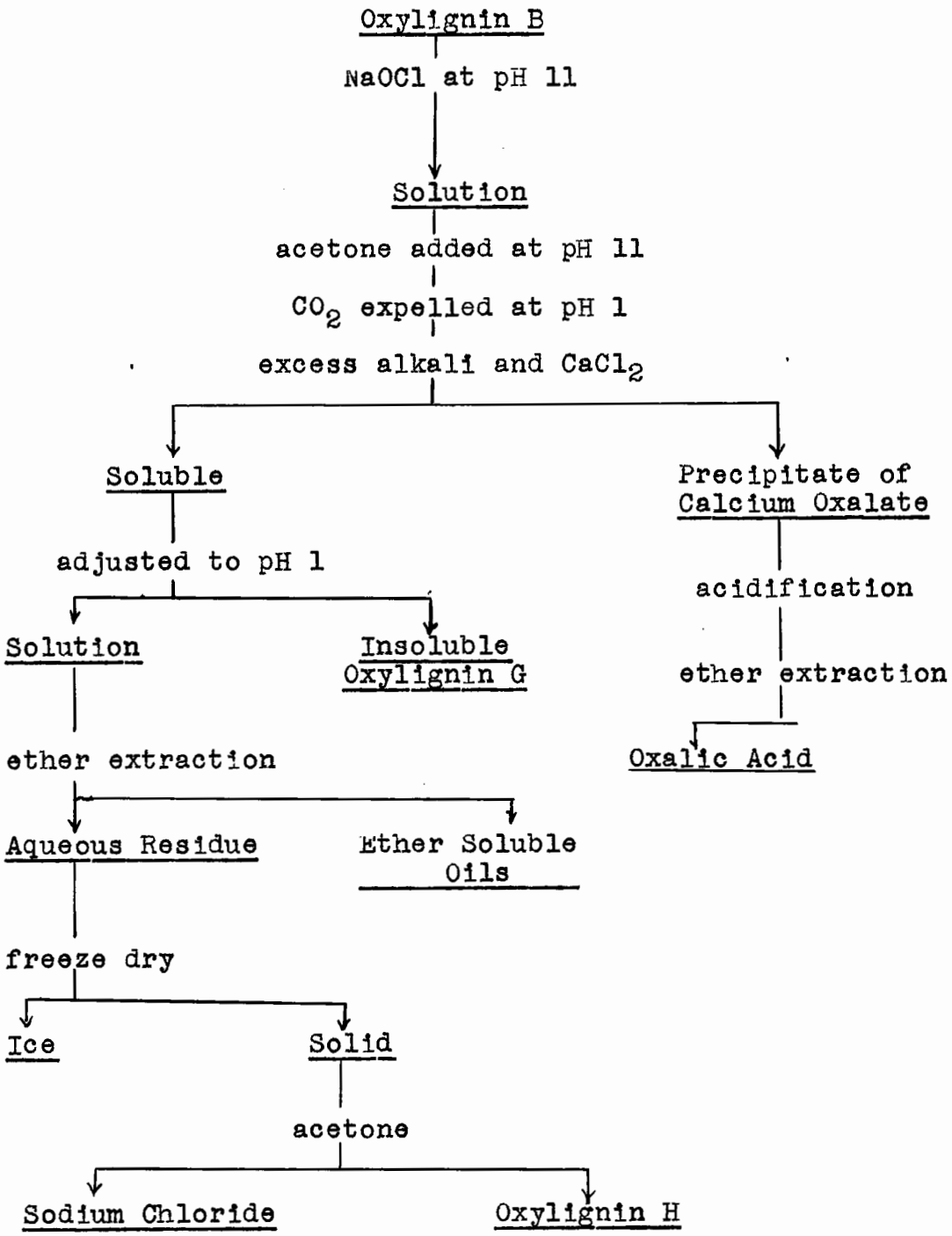


Figure 14

Flow Sheet for the Oxidation of Oxylignin B with Sodium Hypochlorite

corresponded closely to control spots of acetic and oxalic acids.

The acidified oxidation liquors from the second oxidation were freeze-dried according to the technique given by Cabot (19). Acetone extracted all the organic material from the mass of sodium chloride crystals and isolation of the extract gave a hydroscopic, lemon-yellow solid called oxylignin H. A trace of organic crystals was detected in the acetone-petroleum ether liquor from which oxylignin H had been precipitated, and extraction of oxylignin H itself gave some crystalline material imbedded in a red oil. When recrystallized these crystals proved to be impure oxalic acid. About 5.5% of oxalic acid was isolated from the alkaline oxidation liquors of the third oxidation as the calcium salt and its identity was definitely established. Molecular weight determinations of oxylignin H by the method of Menzies and Wright (74), using acetone as solvent, gave values of 509, 546, and 577. Oxylignin H was soluble in acetone, alcohols, water, acidic aqueous solutions, sodium hydroxide and sodium bicarbonate with the evolution of carbon dioxide. The substance was insoluble in benzene, chloroform, ether and petroleum ether. Oxylignin G had the same solubilities as oxylignin H except it was insoluble in water and aqueous acidic solutions.

From this preliminary examination it appeared that sodium hypochlorite had oxidized oxylignin B to

oxalic acid, an ether soluble oil, and two amorphous solids called oxylignin G and H. Acetic acid was also detected in the ether extract but it could have been formed from a reaction between acetone and the residual oxidant. Figure 14 schematically shows the method employed for the isolation of the different fractions from these oxidations. In addition to the products mentioned, a small amount of oil was isolated from the petroleum ether-acetone solution from which oxylignin H was isolated. This particular fraction was very large for the large scale oxidations to be mentioned later. Freeze drying was necessary to isolate oxylignin H as a powder, since evaporation of the oxidation liquor at room temperature gave a resinous product which was very difficult to purify.

As it was discovered that oxylignin H was itself readily oxidized by sodium hypochlorite at pH 10.8, (Figure 13, Plot C) the study of oxylignin B was resumed in an attempt to isolate a product stable to the conditions used. An oxidation carried out at pH 7, instead of pH 11, required 42.4 milliatoms of oxygen per gram of oxylignin B in 5 hours. About 15.1% of oxalic acid was isolated as the calcium salt, and extraction of the acidified liquors with ether gave 27.5% of an oil from which about 1% of a volatile acid, probably acetic acid, was steam distilled. A paper chromatogram (83) confirmed the presence of acetic and oxalic acids and also showed the presence of a new acidic spot having an Rf value of 0.77. Although this

spot could be detected when methanol-ammonia was used as a solvent, the reverse was true when ethanol-ammonia was used, indicating that the spot might correspond to a more complex substance. A trace of white amorphous solid was isolated from the ether soluble oil, but later oxidations failed to produce more of this fraction. The aqueous oxidation liquor was freeze dried but no organic matter could be detected in the mass of sodium chloride crystals. In another experiment when all the oxidant had been consumed by the oxylignin, chromatographic investigation failed to show the presence of acetic acid, although oxalic acid and the unknown acid could be detected. It was concluded that all the acetic acid detected previously had resulted from the action of sodium hypochlorite on acetone, and that none had arisen from the oxylignin.

A mild oxidation of oxylignin B-9 with 0.648 N sodium hypochlorite at pH 11 and 5°C. instead of near 20°C. gave a greatly increased yield of oxylignin G, which might therefore be the less degraded of the two new oxylignins. A comparison of the products from the three types of oxidation is given in Table XIV.

TABLE XIV

A Comparison of the Products from the Oxidation of Oxylignin B-9 with Sodium Hypochlorite under Different Conditions.

Normality of Oxidant	Temperature (°C.)	pH	Oxalic Acid (%)	Ether Soluble Oil (%)	Oxylignin G (%)	Oxylignin H (%)
0.648	5	11	7.7	0.1	15.5	30.0
0.874	(a)	11	5.5	15.0[b]	3.0	29.0
0.900	(a)	7	15.1	27.5[c]	0	0

(a) Room temperature.
(b) From the second oxidation.
(c) An ether soluble solid, 1.4%, was also isolated.

This table shows that the two oxidations carried out at pH 11 gave the best yields of products, but examination of the products themselves indicated that oxylignin H isolated from the oxidation carried out at room temperature had the best solubilities in water and acetone. Large scale oxidations were then carried out to obtain a superior oxylignin H, and the details are summarized in Table XV.

TABLE XV

The Oxidation of Oxylignin B-10 with Sodium Hypochlorite at pH 11 and at Room Temperature.

Grams of Oxylignin B-10	Normality of Oxidant	Volume of Oxidant	Millimoles of Oxidant Consumed(a)	Oxylignin G (%)	Oxylignin H (%)	Oil (%)
17.0	2.89	185	16	20.0	15.5	
20.0	3.01	200	16	20.7	19.0	10.5(b)
16.4	2.47	200	14	19.3	13.2	

(a) Per gram of oxylignin B-10.
(b) This oil was isolated from the purification of oxylignin H and represents the combined yield of all three oxidations, and is in addition to the ether soluble oil isolated previously.

The concentration of the oxidant was increased in an attempt to decrease the amount of solution to be freeze dried, and this change seemed to manifest itself in a slightly different ratio of degradation products. Oxalic acid and the ether-soluble fraction were isolated from these oxidations but were not investigated. Oxylignin G was isolated in the normal manner, but oxylignin H was isolated as an oil from the acetone extract of the freeze dried liquor. Exhaustive extractions of the oily oxylignin H fraction with ether made it possible to recover oxylignin H as a powder after the residual oil had been kept *in vacuo* for several hours. Attempts made to froth the combined ether extracts were unsuccessful. The powdery oxylignin H was purified by petroleum ether extractions followed by drying under vacuum and the

analysis of this oxylignin and oxylignin G are given in Table XVI. The first analysis of oxylignin H indicated that it had a methoxyl content of 9%, but further purification decreased the methoxyl content to 5.3%.

TABLE XVI

Analysis of Oxylignins G and H

	Oxylignin G		Oxylignin H	
Methoxyl (%)	6.2	6.3	8.8	9.2
			5.2	5.3(a)
Chlorine (%)	11.7	12.1	9.0	
Ash (%)	2.8	1.9	4.6	4.7
Molecular Weight(b)	222		283	300

(a) These values were obtained after careful purification.
(b) By the method of Menzies and Wright (74).

TABLE XVII

Fractionation of Oxylignin G and Oxylignin H.

Oxylignin G

Fraction	Yield (%)	Methoxyl (%)(a)	Molecular Weight(b)	Colour
1	2.0	3.0	insol.	cream
2	37.0	5.7	900	red-brown
3	40.0	6.9	370	yellow
4	19.7	8.0	250	light-orange
		Number average	548	

Oxylignin H

1	8.2	2.5	insol.	cream
2	24.1	4.4	236	yellow
3	42.5	4.0	170	yellow
4	24.0	7.3	234	light orange
		Number average	203	

(a) Single determinations only.
(b) The average of several determinations by the method of Menzies and Wright (74).

Both oxylignins G and H were fractionally precipitated from their acetone solutions by means of successive additions of petroleum ether. The results (Table XVII) showed that both oxylignins were inhomogeneous with respect to methoxyl content and molecular weight. The fractions isolated from oxylignin G, however, had much higher molecular weights than the oxylignin itself. No drifting of the boiling point of the solutions was observed during the determinations, but the hydroscopic nature of the oxylignins, particulary of oxylignin H, and possible contamination with solvent, made the determinations technically uncertain. All that could be concluded was that both oxylignins G and H had average molecular weights much less than the value of about 1200 found for oxylignin B. Oxylignin H appeared to be more homogeneous than G with respect to methoxyl content, as approximately 66.6% contained between 4.0% and 4.4% of methoxyl.

Oxylignin G and oxylignin H were separately methylated with diazomethane and also esterified with methanol and "Drierite" with the results given in Table XVIII.

TABLE XVIII

The Methylation of Oxylignin G and Oxylignin H.

	Methylated (CH_2N_2)		Esterified (CH_3OH)	
	% OCH_3	Molecular Weight(a)	% OCH_3	Molecular Weight(a)
Oxylignin G	11.5	162	11.6	315
Oxylignin H	10.8	425	12.8	330

(a) By the method of Menzies and Wright (74).

Although the lack of material prevented these methylations and esterifications from being exhaustive, the concordance of the two methoxyl contents for oxylignin G in the two preparations suggested that no phenolic groups were present and that all carboxyl groups were completely methylated; on the other hand, the esterification of oxylignin H might have been incomplete. The observed molecular weights of these derivatives emphasized once more the unreliability of this determination in the lignin field, even when the strongly polar carboxylic acid groups had been removed by esterification. It seemed likely, however, that oxidation of oxylignin B (molecular weight about 1200) by alkaline hypochlorite had produced products G and H in substantial yields and of markedly reduced molecular weights. These oxidations had therefore proceeded with little or no condensation or resinification of oxylignin B.

When titrated directly with sodium hydroxide, oxylignin G had a neutralization equivalent of 203

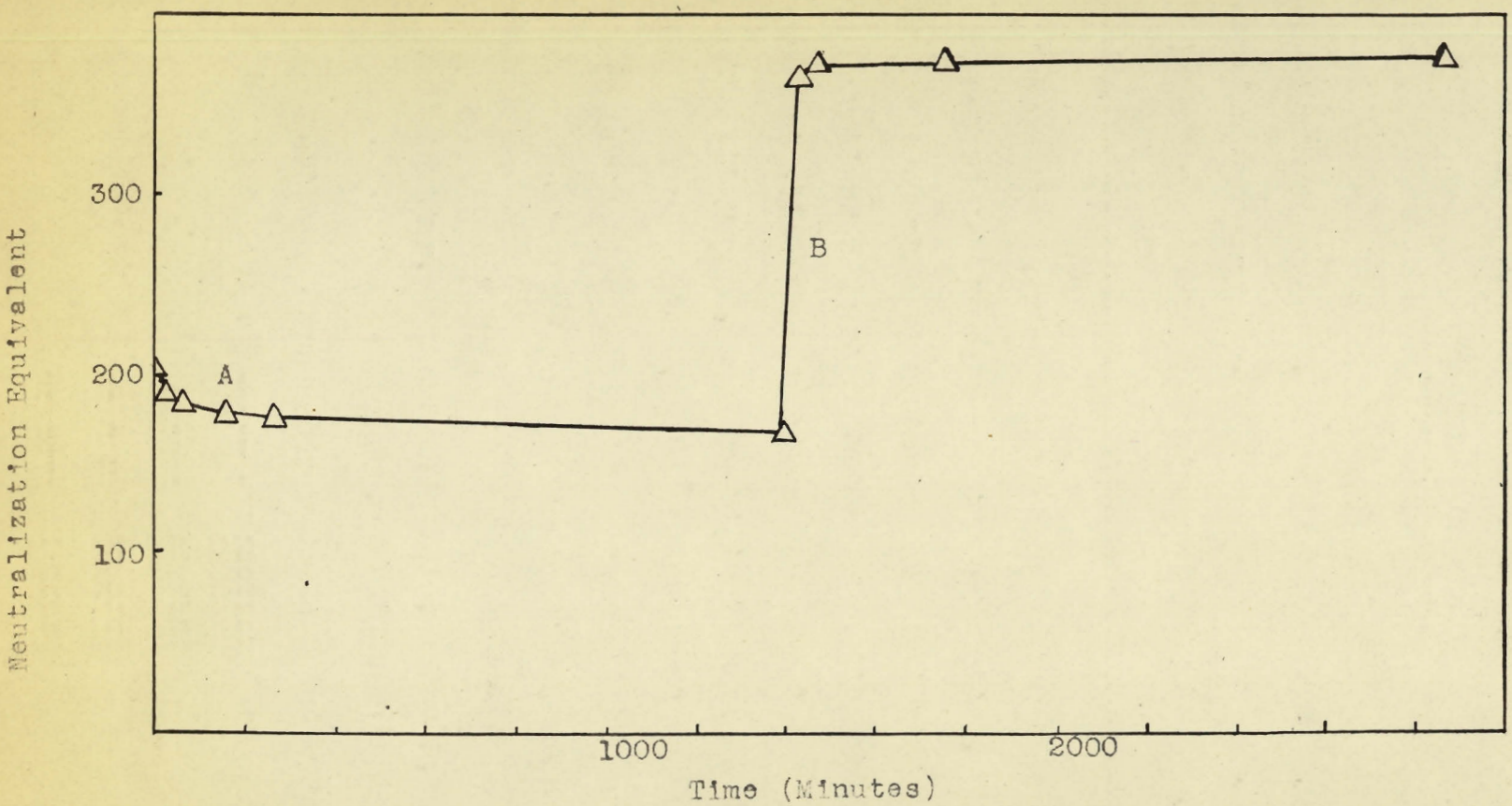


Fig.15. The Change of the Neutralization Equivalent of Oxylignin G.

Plot A Oxylignin G, 0.244 gm., in 100 cc. of 0.0490 N Sodium Hydroxide.

Plot B Fifty cc. Aliquot of A (after 1390 minutes)added to 50 cc. of 0.118 N Hydrochloric Acid.

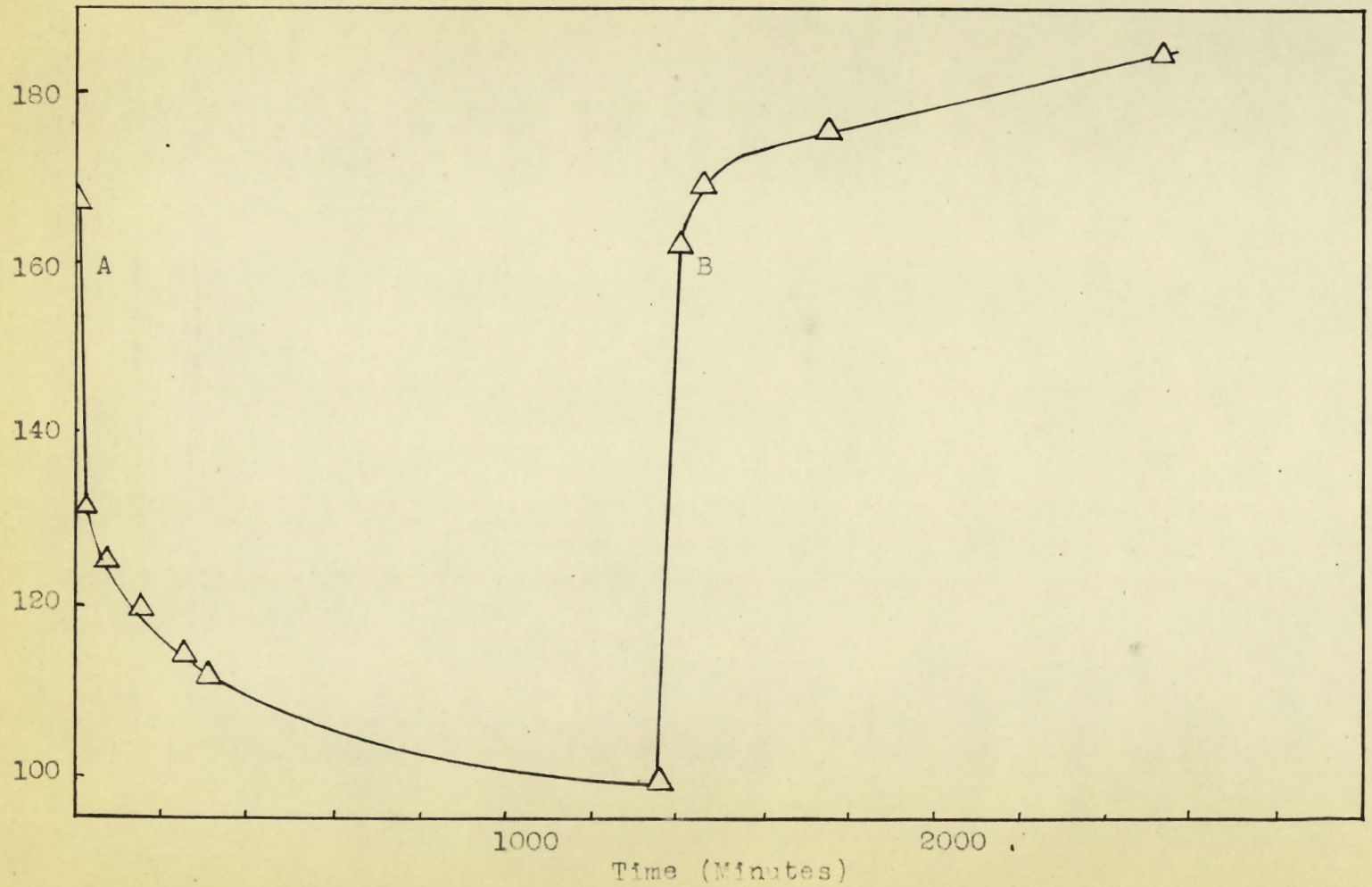


Fig.16. The Change of Neutralization Equivalent of Oxylignin H.

Plot A Oxylignin H, 0.264 gm., in 100 cc.of 0.0490 N Sodium Hydroxide.

Plot B Fifty cc. Aliquot of A (after 1360 minutes) added to 50 cc. of 0.118 N Hydrochloric Acid.

(Figure 15), but when kept in excess alkali this value decreased to 167. Acidification with excess hydrochloric acid caused this value to increase slowly to 375. Figure 16 shows that the neutralization equivalent of oxylignin H underwent a similar change from 167 to 100 and back to 185. Both degradation products, therefore, retained the lactone-like behaviour characteristic of oxylignin B. If present, some of these lactones were stable enough to resist cleavage and methylation with diazomethane or esterification with methanol, because the resulting increases in methoxyl content (Table XVIII) corresponded to equivalent weights of about 600 and 550 to 400 for oxylignins G and H respectively. Although mixtures, a detailed study of these oxylignins would probably reveal much of the structure of lignin itself. Time and material, however, did not suffice for this task to be undertaken in the present research.

EXPERIMENTAL SECTION

Standard Analytical Methods

Ash

Samples of 10 to 15 mg. were ashed as described by Niederl and Niederl (87) on page 62 using a semi-micro apparatus.

Methoxyl

The methoxyl contents of preparations were determined by the method of Vieboch and co-workers (82), (88) as modified by Clark (89) and Penniston and Hibbert (90). The percentage methoxyl was calculated from the following expression:

$$\%OCH_3 = \frac{V \times N \times 31.02 \times 100}{W \times 1000 \times 6}$$

where V was the volume of sodium thiosulphate required, N the normality of the sodium thiosulphate, and W the weight of the sample.

Lignin Content

The standard method as outlined in T.A.P.P.I. Standards (91) using 72% sulphuric acid, was employed.

Chlorine

Samples, 10 to 15 mg. were subjected to a peroxide fusion in a semi-micro Parr bomb according to the procedure described by the Parr Instrument Company (92), the chlorine produced being isolated and weighed as silver

chloride in semi-micro Gooch Crucibles. Determinations of labile chlorine were carried out according to the method given later in the Experimental Section.

Carbon and Hydrogen

Samples of 10 to 15 mg. were burnt in a semi-micro combustion train according to the procedure outlined by Niederl and Niederl (93) on pages 101 to 139.

Acetyl Analysis

The acetyl content was measured by the method of Clark (94) in which samples weighing 10 to 15 mg. were saponified with alcoholic potassium hydroxide, acidified with a sulphuric acid-magnesium sulphate solution, steam distilled, and the distillate containing acetic acid titrated with barium hydroxide using phenol red as indicator. The percentage of acetyl was calculated from the following expression:

$$\%Ac = \frac{V \times N \times 4300}{W \times 1000}$$

where V was the volume of the barium hydroxide, N its normality and W the weight of the sample. Owing to the tendency of the acetylated oxylignin to froth during the analysis, a special apparatus was designed with the help of Dr. W. J. Brickman. The still in this apparatus consisted of a flask with a curved side arm 1.5 cm. in diameter.

Molecular Weight Determinations

The Signer method (69) depending upon the isothermal distillation of solvent under reduced pressure and at room temperature was used to determine molecular weights. The apparatus, described by Clark (95), consisted of two bulbs with graduated extensions, joined by a horseshoe-shaped tube. Arms, extending at right angles to the graduated extensions and in the same plane as the horseshoe tube, served for the introduction of solvent and sample. Solutions of the oxylignin in known amount and in a suitable solvent were introduced through one arm of the apparatus into one bulb and solutions of sucrose octaacetate or other standard were introduced through the other arm into the other bulb. The volumes of the solutions were measured by tipping the solutions into the graduated extensions. The two solutions were cooled in dry ice and acetone slush and one of the arms was sealed off. The apparatus was evacuated to 1 mm. pressure and the other arm was sealed using an oxygen torch.

The method of Menzies and Wright (74) was also employed for the determination of molecular weights. Methanol and acetone were the solvents employed, the change in boiling point being measured with the aid of a cathetometer. The molecular weight was calculated from the formula

$$M = \frac{Wu \times Ms \times Ts}{Ws \times Tu}$$

where Wu was the weight of the unknown, Ms the molecular weight of the standard, Ts the temperature change of the

standard, Tu the temperature change of the unknown, and Ws the weight of the standard.

The Detection of Aliphatic Acids

Sheets of Whatman No. 1 Paper 40 cm. by 11 cm., were employed in the Barker and Kennedy (83) chromatographic method for the separation of aliphatic acids. Ammonia solutions of 0.01 to 0.06 cc. and containing 0.5 to 2.0 micromoles of the acid mixtures, were spotted at 2 to 3 cm. distance along a base line 2.5 cm. from the end of the sheet. A solution of 100 cc. of ethanol or methanol and 1 cc. of concentrated ammonia was used as solvent over a period of 6 to 8 hours for the ascending method of separation. The sheets were dried at 100°C for five minutes and sprayed with an indicator formed from 100 cc. of water containing 50 mg. of bromophenol blue and 200 mg. of citric acid.

Neutralization Equivalents

Suitable amounts of the oxylignins were dissolved in an appropriate amount of water and titrated with caustic using a Beckman pH Meter. If the oxylignin was not soluble in water, it was dissolved in the least amount of acetone and this solution was diluted with water. Some samples were back titrated with acid after the initial titration with sodium hydroxide. Other samples were dissolved in sodium hydroxide and the change of neutralization equivalent with time was followed by

titrating suitable aliquots with acid.

Ultra-Violet Absorption Spectra

The ultra-violet absorption spectra of several oxylignins were determined in the range 240 m to 350 m using a Beckman D. U. Spectrophotometer according to the procedure published in Bulletins 91 G and 89 C (96) by the Beckman Instrument Company. The concentration of the oxylignins in ethanol were 0.0044% for oxylignin B, 0.0115% for oxylignin C and 0.0056% for oxylignin F. The extinction coefficient, $E_{1\,cm.}^{1\%}$, was calculated from the following expression:

$$E_{1\ cm}^{1\%} = \frac{\log \frac{T\%}{100}}{c\ d}$$

where C represents the percentage concentration of the oxylignin, d the thickness of the absorption cell in centimeters and T% the percentage transmission. The results were plotted in Figure 7 together with data from a 0.010% solution of a lignosulphonic acid in dilute sodium hydroxide kindly provided by Dr. G. Allan of the Pulp and Paper Institute of Canada.

The Preparation of Periodate Lignin

Trisodium paraperiodate was prepared according to the method of Lange and Paris (97) by dissolving 75 gm. of sodium iodide in 3 liters of water with vigorous stirring. The solution was heated to 40°C. and 400 gm. of sodium hydroxide was added. The temperature rose to

80°C and was maintained there while 120 cc. of bromine was carefully added from a dropping funnel, the end of which was immersed 10 cm. into the reaction mixture. After the bromine had been added, the mixture was heated for 30 minutes and allowed to stand overnight. The voluminous white precipitate of the paraperiodate was collected in a sintered glass funnel and carefully washed with a little ice water.

The solution used for oxidizing the wood meal was prepared according to the directions of Ritchie and Purves (11) by adding sufficient trisodium paraperiodate to water to give a 5% solution. The pH was then adjusted to 5 and the mixture was stirred overnight. Decantation separated the solution from any undissolved material and the concentration of the clear liquor was adjusted to about 4.5% by dilution. The analysis followed the procedure of Fleury and Lange (98) whereby 5 cc. aliquots were diluted to 30 cc. with distilled water and made alkaline to phenolphthalein with caustic soda. Sodium bicarbonate was added to adjust the pH to about 8.5; an excess of potassium iodide was added together with a known excess of arsenious acid, and after 10 minutes the solution was back-titrated with decinormal iodine. The chemistry involved was as follows:

$$Na_3H_2IO_6 + 2KI + H_2O \rightarrow 2KOH + 2NaOH + NAIO_3 + I_2$$

$$H_2O + I_2 + H_3As\ O_3 \rightarrow H_3AsO_4 + 2HI$$

The spent oxidizing liquors, containing slightly diluted sodium iodate and some paraperiodate, were worked up to recover the paraperiodate according to the method of Ritchie and Purves (11). When the solution was made alkaline, unreacted trisodium paraperiodate was precipitated and the mixture was treated with chlorine at 100°C. to oxidize the iodate to periodate. After recovery and washing, the salt was in a form suitable for re-use.

The preparation of periodate lignin, together with the recovery and preparation of reagents, was carried out with the co-operation of Dr. W. J. Brickman. Chips of Black Spruce wood, approximately 70 years of age, were ground in a Wiley Mill to particles of approximately 40 mesh. The wood meal was extracted in a Soxhlet extractor for 48 hours with ethanol-benzene (1:1) to remove fats and waxes and other extraneous material. After drying in the air for several days, the wood meal was extracted for an additional 24 hours with ethanol and dried in the air.

The periodate lignin was prepared by the method of Ritchie and Purves (11) as modified by Brounstein (48) although certain features such as the washing technique had to be abandoned because of the finer nature of the wood meal. The wood meal was stirred with an approximately 4.5% solution of oxidant at 20°C. and a pH of 4.1 for 24 hours, and the residual oxidant was removed by suction through a Buchner funnel having a 200 mesh

copper screen as filter. The spent oxidizing liquors were collected in an aspirator bottle in series with the system. The oxidized wood meal was washed with water and after a thorough soaking, the water was removed in the same manner as the oxidizing liquors. Usually two changes of water were carried out every 24 hours, although the first washings were sometimes carried out more frequently. When the emerging wash water failed to liberate any iodine from a potassium iodide-acetic acid solution the washing was discontinued. The residual wood meal was extracted with 12 liters of boiling distilled water at a pH of about 6. After the removal of the wash liquors the hydrolysed material was ready for the next oxidation. The material isolated after the 7th oxidation of the first batch was found to contain 10.7% of methoxyl, 4.3% of ash, 10.8% of moisture, 85% of Klason lignin, and negligible holocellulose, as determined by the method of Kurth and Ritter (99) employing ethanolamine. Since 20% of this material remained insoluble after a standard cook in calcium bisulphite it was thought that the holocellulose determination was faulty and the batch was set aside to be combined with the final oxidation of a second batch. The conditions of the oxidations and the analysis of the products are given in Tables II and XIX.

TABLE XIX

The Oxidation of Black Spruce Wood Meal with Trisodium Paraperiodate

Oxidation	Sodium Paraperiodate (cc.)	%	pH
Series I[a]			
1	23500	4.39	4.10
2	14750	3.90	4.10
3	8300	4.45	4.10
4	8100	4.50	4.10
5	8500	4.50	4.10
6	9870	4.50	4.10
7	8000	4.68	4.10
8	combined with Series II, oxidation 7.		
Series II[b]			
1	24600	4.35	4.15
2	17500	4.50	4.15
3	20000	4.50	4.15
4	18000	3.50	4.15
5	10000	4.78	4.15
6	8000	4.02	4.15
7	Combined with Series I 12500	4.50	4.10

(a) Weight of wood 2366 gm.

(b) Weight of wood 2113 gm.

The Preparation of Chlorine Dioxide

The method of Schacherl (73) as used by Husband (56) and Logan (57) was employed for the preparation of chlorine dioxide. The apparatus consisted of a 500 cc. reaction flask followed by a trap to prevent any spray from being carried over, a trap containing 5 cc. of a saturated sodium chlorite solution to convert any chlorine to chlorine dioxide, a third trap to prevent any spray from being carried over from the second trap, and finally a bubbling tube which was inserted into a 500 cc. flat bottomed flask. The reagents employed were 25 gm. of potassium chlorate, 20 gm. of oxalic acid dihydrate and 80 cc. of a sulphuric acid solution prepared by dissolving 100 cc. of concentrated sulphuric acid in 225 cc. of water. The absorption flask contained 200 cc. of distilled water cooled at $0^{o}C.$ by means of crushed ice.

The chlorine dioxide was prepared in batches as large scale preparations were reported to get out of control and cause serious explosions. In a typical run, the reaction flask was heated to $65^{o}C.$ by means of hot water and after half an hour the reaction began. Aliquots of 1 cc. were removed periodically and titrated until the concentration of chlorine dioxide became 0.4 to 0.5 moles per liter. These aliquots were analysed by diluting to 25 cc. and removing a 10 cc. sub-aliquot to a solution composed of an excess of potassium iodide in 5 cc. of 10% sulphuric acid and 10 cc. of water. The iodine

liberated was titrated with standard sodium thiosulphate using starch as an indicator. Dilutions were so arranged that the concentration in moles per liter was given by the following expression with the usual constants eliminated:

$$M = V \times N$$

When a suitable concentration of chlorine dioxide had been achieved the solution was stored in a stoppered flask in the dark under refrigeration and a fresh preparation was begun. The combined chlorine dioxide in a sufficient number of these solutions was determined, was diluted to the proper concentration, and used immediately. The reactions involved in the preparation were as follows:

$$2KClO_3 + (COOH)_2 + 2H_2SO_4 \rightarrow 2ClO_2 + 2CO_2 + H_2O + 2KHSO_4$$

$$Cl_2 + 2NaClO_2 \rightarrow 2NaCl + 2ClO_2$$

Oxidations 1 to 6

The first 6 oxidations were of an exploratory nature and may be exemplified by the third oxidation in which eight grams of periodate lignin was suspended in 768 cc. of 0.260 molar chlorine dioxide solution in a liter flask fitted with a mercury seal and connected to a gas trap. After stirring for 18 hours at room temperature, the resulting orange solid was collected in a sintered glass funnel and washed with a little water. The residue was allowed to remain under suction until most of the moisture adhering to it had been removed. After the wash liquors had been combined with the original mother liquor, the remaining chlorine dioxide was blown out with nitrogen.

The slightly moist residue was placed in a 250 cc. Erlenmeyer flask and allowed to stand overnight in 100 cc. of methanol. During this interval, the oxylignin swelled considerably and in a few hours went into solution. The resulting red liquor was centrifuged to remove any suspended oxylignin A and a white residue of unknown nature. The clear solution was decanted from the centrifuge cup into sufficient petroleum ether to cause the formation of a very viscous red oil. If too much petroleum ether was used, the oxylignin became powdery and very difficult to remove from the walls of the containing vessel. The red oil, on the other hand, could be scooped out with a spatula and placed in a weighed sintered glass funnel containing petroleum ether. The oil usually powdered in a few minutes, but if not, it could be powdered by rubbing it on the sides of the funnel with a spatula. The petroleum ether was removed by suction and the sample was suspended in fresh petroleum ether until it assumed a light yellow colour. Storage in a vacuum removed the last traces of petroleum ether, and was continued until the weight of the oxylignin became constant. This fraction, insoluble in the oxidizing liquors but soluble in methanol, was called oxylignin B by Levitin (18).

The residual aqueous mother liquors were extracted 3 times with 200 cc. volumes of butanol, and the combined extracts were concentrated to 100 cc. at 35°C. and 2 cm. pressure, using a stream of nitrogen gas to

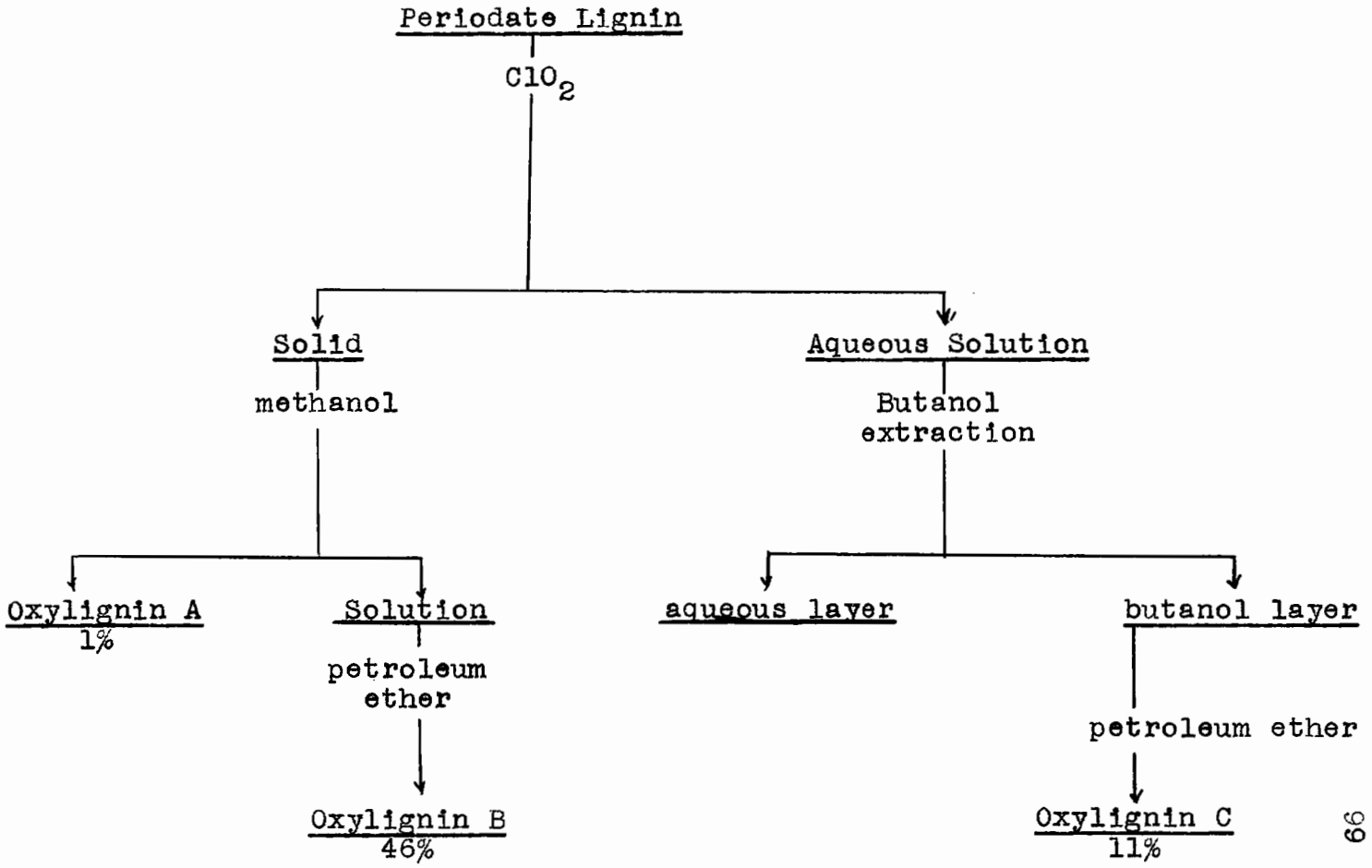
prevent bumping. Addition of the concentrated solution to 300 cc. of petroleum ether gave a suspension which was allowed to separate overnight. The insoluble material was collected in a sintered glass funnel, was treated with petroleum ether and stored in a vacuum until a constant weight was obtained. When the aqueous residue from the extraction was allowed to stand at room temperature for a sufficiently long time, the water evaporated leaving a red oil which could be separated into three fractions, one soluble in acetone, another acetone insoluble but soluble in methanol, and a white residue containing mostly ash. As the last traces of solvent could not be removed from these fractions, no attempt was made to analyse them, and new techniques for isolation were investigated. Other fractions isolated are shown in Figure 17.

Oxidation 7

A sample of periodate lignin was oxidized in the manner previously described, the conditions being given in Table III. The crude oxylignin was collected in a sintered glass funnel and was divided into two portions after it had been sucked dry. One portion was dissolved in methanol and the oxylignin B isolated according to the procedure given above. The other portion was taken up in acetone (60 cc.) and after centrifuging to remove a small amount of oxylignin A, was poured into 200 cc. of petroleum ether. The red oil produced by this treatment was converted into an orange powder by repeated washings

Figure 17

The Isolation of Products from Chlorine Dioxide Oxidation 3



with petroleum ether. The powder was collected in a sintered glass funnel and the two oxylignin fractions (the first designated as oxylignin B-7[a] and the second as oxylignin B-7[b] in Table IV) were given identical exhaustive washings with petroleum ether and were stored in a vacuum until their weights became constant.

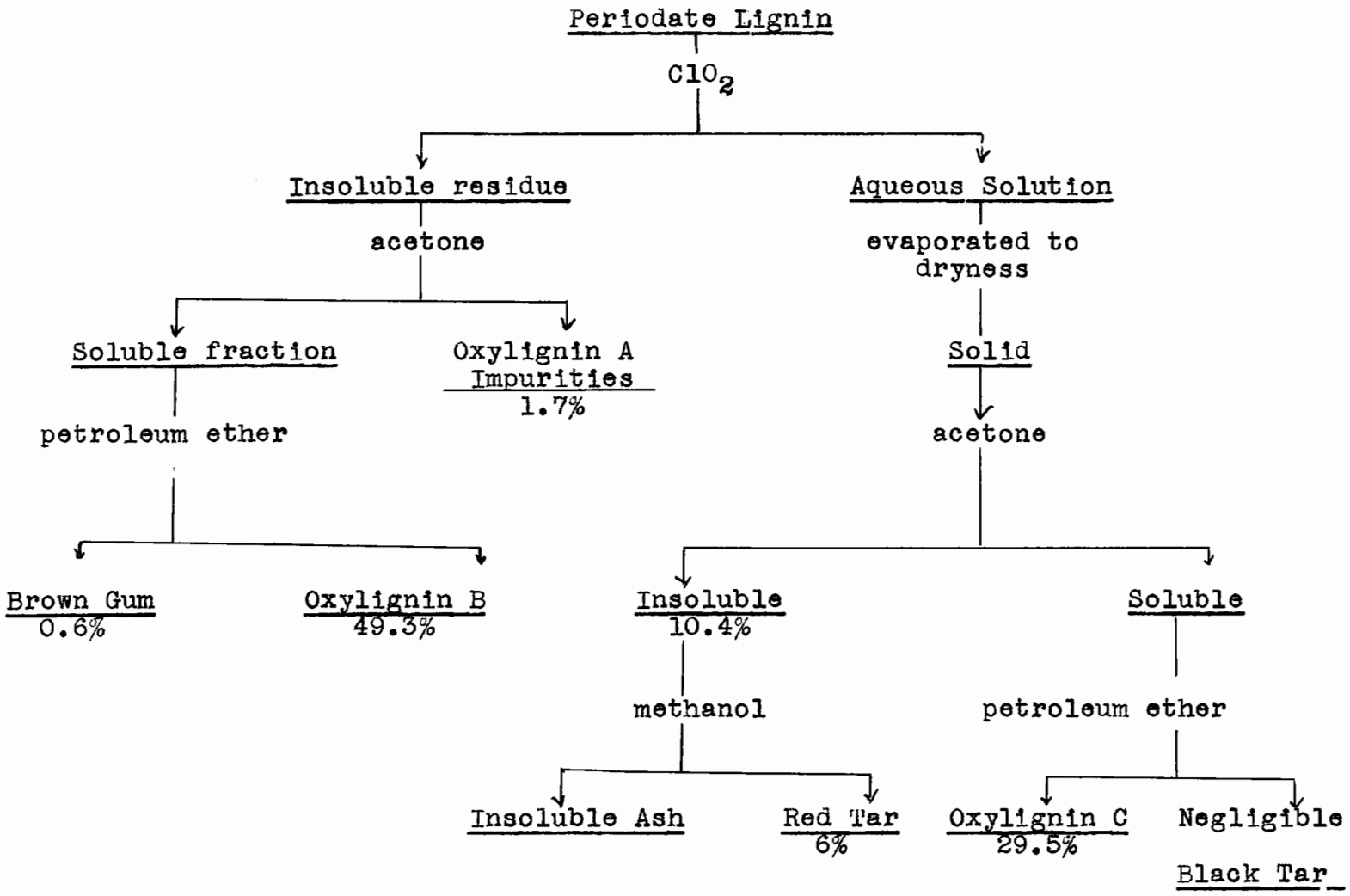
An oxylignin C fraction was isolated by extracting the yellow aqueous oxidation liquor with butanol in the manner described previously.

Oxidation 8

The batchwise preparation of chlorine dioxide made it necessary to carry out a large scale preparation of oxylignin B in four batches under the conditions described in Table III. On completion of the oxidation, the B fraction was separated as described previously and dissolved in acetone. Each acetone extract was treated separately, but the powders when formed were combined and mixed before being analysed. It was found very difficult to convert the red solution, obtained by pouring the acetone solution, 200 cc., into an excess of petroleum ether, 600 cc., into a powder since this oil retained some residual acetone very strongly. After repeated washings with petroleum ether, the oxylignin was obtained as a sticky red solid. Further treatments with petroleum ether alternated with storage in vacuum, produced finally a light orange powder free of the odour of acetone. Other sub-fractions were obtained from the oxidation, their

FIGURE 18

Isolation of Products from Chlorine Dioxide Oxidations 8 and 9.



sources and yields are shown in Figure 18.

The combined residual aqueous mother liquor from the four oxidations (4200 cc.) was placed in a 6 liter flask, and nitrogen gas was blown through for 7 hours to remove the residual chlorine dioxide. The yellow solution, under a pressure of 2 cm., was heated to 40°C. to assist evaporation. When the volume had been reduced to 500 cc., the liquid was evaporated over potassium hydroxide at reduced pressure to an orange syrup. Care was taken at this stage to stop the dessication before a viscous tar was formed, since the next step of the isolation required a certain minimum amount of solvent for its success. Dilution of the tar with water was all that was required to remedy a too viscous condition. The syrup, 75 cc., was exposed suddenly to a pressure of 0.5 to 1.0 mm. and after a few minutes it frothed up and filled the 500 cc. beaker in which it was contained. In a short time the froth hardened to a solid foam. The foam was crushed to a light yellow powder and kept under high vacuum until it had a constant weight and did not smell of hydrogen chloride. The dry powder was extracted with 70 cc. volumes of acetone until no further colour was removed. The combined acetone extracts were then evaporated, first at a pressure of 2 cm., then under high vacuum until the frothing occurred. Washing with petroleum ether was necessary to cause the foam to harden. The oxylignin was kept <u>in vacuo</u> until the weight of the sample was constant. Table IV contains

the analysis of this fraction.

Oxidation 9

Three batches of periodate lignin were oxidized by chlorine dioxide under the standard conditions at room temperature. The material of each batch which had not dissolved was placed in a centrifuge cup and treated with 110 cc. of acetone. The extract was centrifuged as before and decanted from the insoluble residue into an evaporating dish and allowed to stand exposed to the atmosphere overnight. The red-brown, sticky mass was covered with three times its volume of petroleum ether and whipped with a spatula until the liquid had thoroughly penetrated the sticky mass. The excess petroleum ether was decanted from the semi-solid which was then spread out on a glass plate and allowed to dry at room temperature in a ventilated cupboard. The damp mass was crushed periodically during the drying process to prevent the formation of large hard lumps. A fine yellow powder resulted which was scraped from the glass plate into a weighed sintered glass funnel and was treated with petroleum ether and vacuum until the weight became constant.

The yellow oxidation liquor from this oxidation was evaporated as before to recover oxylignin C. An oxylignin fraction similar in appearance and analysis to that isolated from the previous oxidation was isolated. A 200 cc. aliquot of the original oxidation liquor had previously been removed and titrated with a standard

silver nitrate solution using the Mohr procedure (100) for the estimation of chloride ion. Calculation showed that 3.54 grams of silver carbonate was necessary to remove the chloride ion from solution, but only 3.44 grams was added in order that a small amount of hydrochloric acid would be left in solution to neutralize the ash present. After standing overnight, the silver chloride was removed and the oxylignin C isolated by the procedure mentioned above.

Oxidation 10

This oxidation followed the procedure given for oxidation 9, except that 188 grams of periodate lignin was oxidized in 4 batches as indicated in Table III. No attempt was made to isolate the oxylignin C fraction.

Fractionation of Oxylignin B

A sample of oxylignin B-8 weighing 1.001 gm. was dissolved in 10 cc. of 5% sodium bicarbonate, and a further 10 cc. of distilled water was added to facilitate handling. After standing at room temperature for one hour, the red-brown solution was acidified with strong hydrochloric acid to pH 0.9. A light brown suspension was centrifuged to the bottom of the centrifuge cup and the supernatent light-yellow, aqueous layer was decanted. The residue was scraped out of the cup with a spatula and placed in a weighed sintered glass funnel. The last traces of hydrochloric acid were removed by washing the residue with 20 cc. of water and the wash water was combined with the mother liquor. The precipitated oxylignin B was sucked dry in a Büchner funnel, taken up in acetone, filtered, precipitated with petroleum ether and finally dried under high vacuum. Table VII contains the analysis of this and of the succeeding fractions. The combined mother liquors were evaporated to dryness at 2.5 cm. pressure and 35°C. and the mixture of sodium chloride and organic material was leached with 30 cc. of acetone. The brown extract was filtered to remove any suspended salt and evaporated to dryness. Purification of the dark brown oily residue was by washing with petroleum ether and drying until a constant weight was obtained, although the powder still retained a slight odour of acetone.

The acid precipitated oxylignin B fraction was redissolved in 7 cc. of 5% sodium bicarbonate and 5 cc. of water was added. The sample was treated exactly as described above, but some mechanical losses occurred when the acetone extract splashed under vacuum. The acid soluble fraction of the second fractionation was also isolated.

The acid-insoluble residue of the second fractionation was dissolved in 7 cc. of 5% sodium bicarbonate and fractionated in the manner described. The acid-insoluble fraction was isolated as before, but the acid-soluble fraction, isolated by evaporation of the aqueous liquor, was found to be insoluble in acetone.

Oxylignin B and Diazomethane

A sample of oxylignin B-8, 5.10 gm., was dissolved in 30 cc. of anhydrous methanol giving an orange-brown solution. When diluted with 60 cc. of pure dioxane, a quantity of the oxylignin was rendered insoluble, but it was not removed. The mixture was distilled at 35°C. under 2 cm. pressure until the refractive index of the distillate was equal to that of pure dioxane. The distillation was interupted periodically to replace the dioxane lost by distillation. The final volume was 75 cc. The mixture, now free of methanol, was methylated with diazomethane in the following manner. A solution of potassium hydroxide, 9 gm. in 10 cc. of water, and 30 cc. of ether were placed in the generating flask of the

methylation apparatus described by Cabot (19). The flask was cooled to 0°C. with crushed ice, and 5 gm. of methylnitroso urea was added to the alkali. The generating flask was connected to the methylation apparatus and the diazomethane, driven over by a stream of nitrogen, was carried through the apparatus and bubbled into the dioxane suspension of the oxylignin. The evolution of diazomethane from the methylnitroso urea was controlled by cooling the generating flask with cold water.

The methylation was carried out 4 times over a period of 2 days and the dioxane was removed at 30°C. and 5 cm. pressure in a distillation apparatus. A stream of nitrogen gas was passed through the suspension to prevent bumping. When the volume had decreased to 20 cc., the remainder of the solvent was removed by evaporation at 1 mm. pressure in a dessicator. The resulting brittle, orange solid was crushed with a spatula to a fine yellow powder which was soaked in petroleum ether for 18 hours. The bulk of the petroleum ether was removed by decantation, and the sample was kept in a high vacuum until the weight became constant. After 4 days the sample was analysed, subjected to further purification for 1 week and analysed again (Table VIII).

The methylated oxylignin B was suspended in 90 cc. of dioxane for 24 hours and finally vacuum was applied for 6 hours. When the volume had decreased to 70 cc., the swollen mass was methylated once with

diazomethane. An orange powder was isolated in the manner described above. Diazomethane methylated oxylignin B was found to be insoluble in water, acetone, camphor, ether, methanol, sodium hydroxide and sodium bicarbonate solutions. Because of its insolubility, no molecular weight determinations could be carried out.

Oxylignin C and Diazomethane

A sample of oxylignin C-8, 2.353 gm., was dissolved in 100 cc. of methanol and 100 cc. of pure dioxane was added. The methanol was removed in the manner described previously and the sample was methylated 6 times over a period of 3 days with diazomethane. The excess dioxane was removed at 2.5 cm. pressure until the volume was 25 cc. and the evaporation was continued at 1 mm. pressure until the volume had decreased to 10 cc. Thirty cc. of petroleum ether was added causing the oxylignin to precipitate as a sticky powder. The colourless petroleum ether layer contained no organic residue. After storage in vacuum for 1 week, the oxylignin was analysed.

The methylated oxylignin was taken up in dioxane, methylated twice and analysed. A final methylation was again carried out on this methylated product and the analyses are shown in Table XII.

Oxylignin B and Methanol

Oxylignin B-8, 0.665 gm., was dissolved as completely as possible in 100 cc. of anhydrous methanol,

only a very small amount failed to go into solution. One half gram of "Drierite" was added and the solution was heated under gentle reflux for 16 hours. The solution was filtered, evaporated at a pressure of 2.5 cm. to a thick red oil and was thoroughly shaken with 50 cc. of petroleum ether. Evaporation of the residue under high vacuum for 24 hours gave a brittle brown solid which was easily crushed to a dark yellow powder. After washing with petroleum ether and drying under high vacuum until constant weight was obtained, the powder was analysed. The methylation was carried out twice more, the products being isolated by the procedure given above. The analyses are given in Table IX. The methylated lignins became increasingly insoluble in methanol and after the third methylation, the oxylignin became almost completely insoluble in methanol.

Oxylignin C and Methanol

A 0.595 gm. sample of oxylignin C-8 was dissolved in 27 cc. of anhydrous methanol and 0.5 gm. of "Drierite" was added. The red solution was heated under reflux for 3 hours and was filtered. It was evaporated to a red oil under reduced pressure and allowed to stand overnight in contact with 50 cc. of petroleum ether. The clear petroleum ether layer, which contained no dissolved organic matter, was decanted and the oxylignin was frothed by subjecting it to a pressure of 1 mm. for several days. The sticky foam was hardened by repeated washings with petroleum ether

and was crushed to a yellow powder which was dried for one week *in vacuo*. The methylated oxylignin was methylated twice more, the times of methylation being 2 and 20 hours and it was analysed after each methylation (Table XIII).

Oxylignin B and Methanolic Hydrogen Chloride

Five grams of oxylignin B-10 was suspended in 100 cc. of 2% methanolic hydrogen chloride for 40 hours at room temperature. Nearly all the oxylignin dissolved and after 3 hours heating under reflux, a 20 cc. aliquot was removed and treated with silver carbonate to remove the hydrogen chloride. The aliquot was filtered and evaporated to dryness *in vacuo*. This solid, called Fraction 1, was carefully purified in vacuum before analysis (Table X). The residual methanol solution was allowed to stand for an additional 40 hours and a second fraction was removed after a 1 hour reflux. The following day, the residual 60 cc. was heated under reflux for 3 hours and the soluble portion (Fraction 3) was decanted from the dark brown insoluble residue called Fraction 4 in Table X.

Acetylation of Oxylignin B

(a) Small scale preliminary experiments showed that oxylignin B-8 would not dissolve or swell in a sodium acetate-acetic anhydride acetylation mixture at room temperature. Another sample of the oxylignin, 0.325 gm., was suspended in a mixture of 0.122 gm. of sodium acetate

and 5 cc. of acetic anhydride and was heated at 50°C. for 24 hours. The oxylignin partially dissolved but an unmanageable black tar was formed when the dark solution was poured into distilled water. No further investigations were carried out on this product.

(b) One half gram of oxylignin B-8 was added to a solution composed of 1 cc. of acetyl chloride and 5 cc. of acetic anhydride at room temperature in a 50 cc. Erlenmeyer flask. After 46 hours the solution darkened considerably but much of the oxylignin remained insoluble. The supernatent liquid was decanted into 10 cc. of water in a weighed Erlenmeyer flask. A light coloured precipitate was formed and was isolated by decantation. Vacuum treatment over potassium hydroxide for 1 week completed the isolation procedure. Found: weight, 0.0992 gm., acetyl, 9.3%. The oxylignin B insoluble in the acetylation mixture was extracted with distilled water and dried in vacuo over potassium hydroxide. Found: weight, 0.402 gm., acetyl, negligible. The unacetylated oxylignin fraction (0.380 gm.) was heated with 4 cc. of acetic anhydride and 4 cc. of acetyl chloride on a steam bath at 50°C. for 9 hours and at room temperature for 2 days. Most of the oxylignin dissolved while the insoluble portion became a dark red swollen mass. The mixture was added to 15 cc. of water and shaken well to assist the penetration of the water into the oxylignin. The mixture was filtered and the residue was dried, first at 2 cm.

pressure over potassium hydroxide and finally at 1 mm. pressure to remove traces of acetic acid. The yellow powder was then soaked in a solution of ether and petroleum ether (1:10) overnight and dried in vacuo for 1 week. Found: weight, 0.217 gm., acetyl 11.1% and 10.6% after washing with the ether-petroleum ether solution for an additional 2 days.

(c) An attempt was made to carry out a Schotten-Baumann reaction (77) on oxylignin B-8, but analysis showed that no acetyl groups were introduced.

(d) A 0.2463 gm. sample of oxylignin B-8 was mixed with 10 cc. of acetic anhydride and one drop of sulphuric acid. The oxylignin reacted slowly to form a dark solid, and after 3 days the solid dissolved completely to give a dark red solution. The solution was carefully poured into 50 cc. of distilled water and allowed to stand for several hours. A solid was collected in a weighed sintered glass funnel and washed with 20 cc. of water. After washing with ether and petroleum ether (1:10) and storing in a vacuum for 1 week, the oxylignin was isolated as a light-yellow, fluffy solid. After analysis it was washed for two more days and analysed again (Table XI).

(e) A 0.147 gm. sample of the acetylated oxylignin was reacetylated with 2 cc. of acetic anhydride and 1 drop of sulphuric acid. After 2 days, the acetylated oxylignin was isolated in the manner described and was analysed (Table XI).

(f) Another sample of oxylignin B-8, 1.01 gm., was acetylated with 10 cc. of acetic anhydride and 2 drops of concentrated sulphuric acid. After 13 days, only a portion of the oxylignin had dissolved and that which remained was a dark swollen mass. The mixture was poured carefully into 75 cc. of distilled water to precipitate the acetylated product and was allowed to stand for 3 hours. The oxylignin was transferred quantitatively to a weighed sintered glass funnel. The yellow fluffy solid was washed repeatedly with distilled water to remove the last trace of sulphuric acid after which it was purified in the manner described above. The acetylated oxylignin was soluble in acetone, but was completely insoluble in ether, water and chloroform. The analysis of this product is given in Table XI.

Acetylation of Oxylignin C

(a) Two grams of oxylignin C-8 was treated with 20 cc. of acetyl chloride in a small Erlenmeyer flask at room temperature for 3 days. The oxylignin dissolved giving a dark red solution which was poured into 50 cc. of water. A tan precipitate appeared, but the yellow colour of the acetic acid solution indicated that a considerable amount of oxylignin remained in solution. The crude solid was dried in the usual manner, giving a light brown powder. Found: weight 1.1 gm.; acetyl, 7.8%; chlorine, 13.9%.

(b) A 0.7525 gm. sample of oxylignin C-8 was treated with 8 cc. of acetyl chloride at 48°C. for 6 days.

Only 4.3% of acetyl was introduced by this more drastic treatment.

(c) Two hundred milligrams of oxylignin C-8 were kept at room temperature for 2 days with 0.0552 gm. of sodium acetate and 2 cc. of acetic anhydride. The solution turned dark and a black gummy residue remained insoluble. No additional precipitate appeared when the mixture was diluted with 10 cc. of water. The dark residue proved to be unmanageable and the experiment was abandoned.

(d) A 0.2285 gm. sample of oxylignin C-8 was dissolved in 1 cc. of acetic anhydride and 1 drop of sulphuric acid. After standing at room temperature for 7 days, the solution was poured into 5 cc. of water. The resulting cream-coloured precipitate was isolated and dried. Found: weight, 0.0685 gm.; acetyl, 15.5% and 15.7%. This material was soluble in acetone and water but was only partially soluble in chloroform and ether.

Oxylignin B and Potassium Cyanide

A buffer solution was prepared consisting of equal volumes of N sodium hydroxide and saturated sodium borate. A preliminary test showed that 20 cc. of this buffer would dissolve 1 gm. of oxylignin B-10 and keep the pH of the solution at 10.5 for 24 hours. Another gram of the oxylignin was then dissolved in 20 cc. of the buffer, 200 cc. of 0.092 M potassium cyanide was immediately added and the reaction vessel stoppered. A blank was prepared at the same time. The first 25 cc.

aliquot was removed after 2 hours and was added to 10 cc. of 50% sodium hydroxide. A blank was treated similarly and both solutions were steam distilled. The apparatuses were arranged so that the ammonia evolved had to bubble through the receiver flasks, each of which contained 50 cc. of saturated boric acid solution to absorb the ammonia. When 50 cc. of the distillates had passed over, the solutions were titrated with standard hydrochloric acid using bromocresol green as indicator according to the procedure given by Clark (89). Considerable care had to be exercised lest the samples froth over into the receivers. After subtracting the blank, it was found that 3.9×10^{-4}, 7.9×10^{-4} and 8.2×10^{-4} moles of ammonia had been evolved per gram of oxylignin B after 2, 24 and 48 hours respectively. Of interest was the change in colour of the basic oxylignin solution from red-brown to a rich wine colour shortly after the potassium cyanide had been added.

Oxylignin B and Bromine

An attempt was made to detect double bonds using the classical method involving the addition of bromine as described by Robert and Traynard (44). The reactions of shorter length were carried out in open flasks, the longer reactions in stoppered flasks. The samples of oxylignin B-8 were dissolved in 5 cc. of 0.1 N sodium hydroxide and were immediately precipitated with 10 cc. of 4 N sulphuric acid. Five cc. of 3.3 M potassium bromide was added to this mixture followed immediately by 10 cc. of

0.1203 M potassium bromate. A blank was prepared at the same time and the solutions were cooled in an ice bath in a dark cupboard. After a suitable length of time, the samples were removed and 10 cc. of 10% sulphuric acid was added to each. The mixtures were then treated with an excess of potassium iodide and titrated with 0.0297 N sodium thiosulphate.

TABLE XX

The Reaction of Bromine with Oxylignin B

Time (minutes)	Grams of Oxylignin B	Bromine Reacted (In cc. of 0.0297 N Sodium Thiosulphate)			Milliatoms of Bromine Consumed per Gram of B.
		Blank	Actual Titration	Difference	
60	0.07574	39.07	35.28	3.79	1.49
142	0.08438	37.17	32.58	4.59	1.62
205	0.08320	39.92	31.67	8.25	2.94
985	0.07412	40.35	29.15	11.20	4.49
1320	0.08593	40.10	26.82	13.28	4.59

Oxylignin B and Nitrobenzene

(a) Oxylignin B-8, 0.02531 gm., was heated with 0.06 cc. of nitrobenzene and 1 cc. of sodium hydroxide in a 2 cc. stainless steel bomb at 160°C. for 2.5 hours, according to the procedure of Stone and Blundell (81) as modified by Towers (102). The bomb was cooled, opened and centrifuged to remove any suspended material and any unreacted nitrobenzene. An aliquot of the clear solution, 0.2 cc., was spotted in 10 spots of 0.02 cc. on 2 sheets of Whatman No. 1 filter paper 40 cm. long and 11 cm. wide.

The spots were acidified with acetic acid vapor and allowed to dry for 10 minutes before they were chromatographed using a butyl ether-water mixture. After 8 hours the sheets were removed and dried. The sheets were sprayed with a 2,4-dinitrophenyl hydrazine solution, but no positive indication of an aldehyde could be detected.

(b) A second oxidation using 0.01471 gm. of oxylignin B-8 was carried out under identical conditions. The sample was chromatographed together with a sample of lignosulphonic acid kindly provided by Dr. W. J. Brickman which had been oxidized under identical circumstances. The strips corresponding to the lignosulphonic acid spot and to one of the oxylignin B spots were sprayed with the indicator referred to above; a postive test for vanillin was given by the lignosulphonic acid but none by oxylignin B. The area of the remaining channels corresponding to the location of vanillin was cut out and extracted for 2 hours with 25 cc. of ethanol to which 4 cc. of 0.2% potassium hydroxide had been added. The extract was diluted to 50 cc. with ethanol and examined with a Beckman D. U. Spectrophotometer in the region 320 mμ to 400 mμ. A very slight decrease in the percentage transmission was discovered at 352 mμ, the region in which vanillin absorbs energy. A calculation of the amount of vanillin from calibration curves prepared for the Spectrophotometer by Dr. W. J. Brickman showed only 0.09% vanillin resulted from the oxidation of oxylignin B-8

with nitrobenzene.

Oxylignin C and Nitrobenzene

(a) Oxylignin C, 0.03059 gm., was treated in an identical manner with nitrobenzene; no positive indication of vanillin was found by chromatographic means.

(b) A second sample of the oxylignin, 0.01650 gm., was oxidized with alkaline nitrobenzene and examined with the Beckman Spectrophotometer in the region 320 mμ to 400 mμ. No vanillin whatsoever could be detected.

The Nature of the Chlorine Atoms on Oxylignin C

(a) In a preliminary experiment, 0.0548 gm. of oxylignin C-8 was dissolved in 10 cc. of distilled water and was acidified with nitric acid to pH 1. An excess of 5% silver nitrate was added and the white precipitate was digested in the dark for half an hour at 60°C. The precipitate was collected in a weighed Gooch crucible, dried and weighed. Found: 2.3% of chlorine by weight of the oxylignin was removed by this treatment.

(b) A 0.1287 gm. sample of oxylignin C-8 was dissolved in 20 cc. of N sodium hydroxide and 2 cc. aliquots were removed periodically, acidified with nitric acid and treated with 5% silver nitrate. The silver chloride was isolated by the procedure mentioned above. The results of this experiment are plotted in Figure 10.

(c) A 1.383 gm. sample of oxylignin C-8 was dissolved in 30 cc. of 0.5N. sodium hydroxide and allowed to stand

at room temperature for 40 hours. After acidification to pH 1 with hydrochloric acid, the excess solvent was removed by evaporation at 2 cm. pressure. The thick dark solution was then subjected to high vacuum in an unsuccessful attempt to make it froth. The residue was extracted with acetone to remove the organic material. Evaporation of the extract gave a thick black tar which could not be powdered nor could the residual solvent be removed from it. After standing for 2 months in petroleum ether, the product was converted by vacuum into a sticky dark brown solid still smelling of acetone. Found: weight, 0.993 gm.; chlorine 6.4%, 6.5%.

In another attempt to convert the product into a powder, a sample of the above material, 0.578 gm., was dissolved in methanol, 0.2 gm. of "Drierite" was added, and the mixture was heated under gentle reflux for 6 hours. After standing at room temperature for a further 18 hours, the mixture was filtered and evaporated to a shiny black, glass-like material which could not be powdered. Treatment of oxylignin C with sodium bicarbonate gave a similar unmanageable material.

(d) In another attempt to remove chlorine atoms, 0.331 gm. of the oxylignin was dissolved in 50 cc. of distilled water and the solution was passed through a column of "Amberlite I R 4-B" 45 cm. long and 1 cm. in diameter which had previously been thoroughly washed with distilled water. The yellow effluent was then evaporated

to dryness at 2 cm. pressure over potassium hydroxide and the last traces of moisture were removed with vacuum over phosphorous pentoxide. Found: weight, 0.197 gm.; chlorine, 13.4% and 13.6%.

(e) Another sample of oxylignin C, 0.307 gm., was dissolved in 50 cc. of sodium hydroxide and 1.5 gm. of nickel-aluminum alloy was added slowly over a 15 minute period at such a rate that the reaction of the alloy with the alkali was not too vigorous, following the method of Papa, Schwenk, and Whitman (84). The solution, containing suspended nickel, was heated for 1 hour at 70°C., during which interval the nickel settled to the bottom of the reaction vessel. The supernatant liquid was decanted and acidified with hydrochloric acid using Congo-red as indicator. The organic material was not extracted by ether, and three 20 cc. volumes of butanol had to be employed. Evaporation of the combined extracts to 10 cc. at 35°C. and 2 cm. pressure, and storage at 1 mm. pressure for several days finally gave a red tar smelling of butanol. Treatment with petroleum ether ultimately gave a sticky black powder in 25% yield which still had a butanol odour. Further attempts at purification were unsuccessful. The aqueous residue from this experiment was evaporated to dryness giving a black tar which could not be leached from the contaminating inorganic crystals with acetone, ether, dioxane or butanol.

(f) In another experiment, 0.7114 gm. of the

oxylignin was dissolved in 35 cc. of N hydrochloric acid and 0.80 cm. of zinc powder was added over a period of 4 hours, during which time the temperature of the reaction was maintained at 50°C. The solution was neutralized to pH 2.5, and hydrogen sulphide was bubbled into it for 1 hour. The white zinc sulphide was centrifuged to the bottom of the reaction vessel and the pH was again adjusted to 2.5. Hydrogen sulphide was again added and the process was repeated until no further zinc sulphide was deposited. The aqueous layer was decanted and evaporated to dryness after acidifying to pH 1. Unsuccessful attempts were made to extract the organic matter carried down by the zinc sulphide. The residue from the evaporation of the aqueous solution was leached with 20 cc. of acetone, filtered to remove a small amount of ash and evaporated to a red oil. High vacuum converted the oil to a yellow powder in 42% yield. As a considerable amount of ash was still present, the powder was taken up in acetone and filtered. A high vacuum treatment again gave the yellow powder. Found: weight, 0.268 gm.; OCH_3 3.6% and 3.6%; chlorine, 6.7% and 7.2%; ash, negligible. The material was soluble in acids, bases and organic liquids such as acetone and alcohols but was insoluble in ether.

(g) A 1.06 gm. sample of the oxylignin was dissolved in 100 cc of distilled water and placed in the hydrogenation chamber of an Adams hydrogenator. Palladium black, 0.11 gm., was added and after flushing the chamber 3 times with

hydrogen, the sample was treated with that gas at 46.5 p.s.i. After shaking overnight, the pressure decreased to 44.0 p.s.i. The aqueous solution was filtered and evaporated to a dark brown powder. Found: weight, 0.756 gm; chlorine, 16.7%.

Oxylignin B and Sodium Hydroxide

(a) This experiment was carried out according to the method given by Levitin (18). One gram of oxylignin B-6 was dissolved in 8 cc. of 1.02 N sodium hydroxide and allowed to stand at room temperature for 24 hours. The solution was acidified with strong hydrochloric acid to pH 0.8 and the fine suspension was removed by filtration. It was washed with 10 cc. of distilled water and dried in a vacuum over phosphorous pentoxide. The dark brown powder, called oxylignin D-1, was isolated in 46% yield. Found: methoxyl, 4.93% and 5.01%; ash, negligible.

The aqueous mother liquor was extracted 3 times with 25 cc. of butanol, the extract was concentrated to one-third of its original volume, filtered to remove a little inorganic material and poured into 3 times its volume of petroleum ether. A dark brown, oily solid called oxylignin E-1, was isolated in 10% yield. Found: methoxyl, 7.65%; ash, 2.9%.

(b) Levitin's procedure was altered in a second oxidation in which 1.043 gm. of oxylignin B-8 was dissolved in 8 cc. of 1.06 N sodium hydroxide and allowed to stand at room temperature for 24 hours. The "D"

fraction was isolated as above in 51% yield. Found: methoxyl, 4.5% and 4.5%; chlorine, 8.2% and 8.3%; ash, negligible.

The aqueous liquor was evaporated at 2 cm. pressure over potassium hydroxide to a brown organic tar contaminated with inorganic crystals. Extraction with acetone gave a red solution which was filtered and evaporated to a red gum. Storage in a high vacuum, alternated with petroleum ether extractions, gave a brittle brown tar which was crushed to a light brown powder called oxylignin E-2. Found: weight, 0.290 gm.; methoxyl, 4.1% and 4.3%; chlorine, 9.1% and 9.1%; ash, 1.4% and 1.5%. A small amount of organic matter remained insoluble after the acetone extraction but this fraction was not investigated further.

Degradation of Oxylignin B

Oxylignin B and Potassium Permanganate

(a) A 0.1050 gm. sample of oxylignin B-9 was dissolved in 10 cc. of water saturated with sodium bicarbonate and 100 cc. of 0.1020 N potassium permanganate was added with stirring. At suitable intervals 5 cc. aliquots were removed and analysed by the method of Bray and Miller (103) in which 5 cc. aliquots were acidified with 10 cc. of 10% sulphuric acid, treated with an excess of potassium iodide and titrated with 0.0303 N sodium thiosulphate.

TABLE XXI

The Consumption of Potassium Permanganate by Oxylignin B-9[a]

Time (minutes)	Oxidant Consumed (in cc. of 0.0303 N Sodium Thiosulphate[b][c]	pH	Milliatoms of Potassium Permanganate Consumed per Gram Oxylignin.
0	0	8.25	0
22	5.80	8.15	6.67
238	8.20	8.05	9.74
243	8.26	8.05	9.81
290	8.30	----	9.85
1258[d]	11.16	8.80	13.46

(a) The oxylignin, 0.1050 gm., was dissolved in 10 cc. of of sodium bicarbonate buffer and oxidized with 100 cc. of 0.1020 N potassium permanganate.
(b) Five cc. aliquots were removed.
(c) As a difference from a blank of 15.25 cc.
(d) All the oxidant was consumed in an additional 24 hours.

(b) In another experiment, 0.2132 gm. of oxylignin B-9 was oxidized under similar circumstances until 8.12

millimoles of oxidant per gram of oxylignin had been consumed. The solution was acidified, sodium bisulphite was added until the excess oxidant was destroyed, and it was extracted with ether for 6 hours. The ether extract was evaporated to a few milligrams of an oil which contained no detectable aliphatic acids when examined chromatographically (83). The aqueous solution was evaporated on the steam bath to a small amount of red tar contaminated with inorganic salts.

(c) One gram of oxylignin B was dissolved in an excess of 10% sodium hydroxide, a solution of 1.9 gm. of potassium permanganate in 200 cc. of water was added and the mixture was allowed to stand at room temperature for 2 hours. When the solution was acidified, an appreciable quantity of gas was evolved and sodium bisulphite was added to destroy the excess oxidant. About 14% of the oxylignin was precipitated by the addition of acid. The yellow aqueous filtrate was extracted with ether overnight. This extract yielded 6.2% of a dark brown gum containing no aliphatic acids detectable by chromatographic means (83). The residual oxidation liquor was evaporated to one-third its volume under reduced pressure and was extracted with three 50 cc. volumes of butanol. Evaporation of the butanol extract gave a brown tar which could not be powdered nor could the residual solvent be removed from it.

Oxylignin B and Sodium Metaperiodate

(a) In order to verify the ability of hydrogen sulphide to reduce potassium iodate, the gas was bubbled into 10 cc. of 0.101 N potassium iodate for one hour. No reaction occurred for several minutes, then the solution became coloured by iodine and a milky precipitate of sulphur was deposited. The gas was added until the iodine colour disappeared and the saturated solution was allowed to stand for several days until all the sulphur had deposited. After the excess hydrogen sulphide had been removed by boiling, the solution was acidified with 5 cc. of 10% sulphuric acid and potassium iodide was added. No iodine was liberated nor was any blue colouration produced when starch indicator was added to the mixture.

(b) In another experiment, hydrogen sulphide was added to 10 cc. of 0.048 M sodium metaperiodate. Once again no reaction occurred for some time, but quite suddenly a very strong colour of iodine appeared followed by a heavy deposit of sulphur. Much of the iodine in the solution was carried down by the sulphur and was difficult to reduce. After standing for several days the iodine left the sulphur layer and entered the solution where it was readily reduced by hydrogen sulphide. No free iodine could be detected when potassium iodide and starch were added to the boiled, acidified solution.

(c) A 0.207 gm. sample of oxylignin B-9 was dissolved in 13 cc. of N sodium hydroxide and was immediately

acidified with 5.04 cc. of hydrochloric acid to pH 4.0. Fifty cc. of 0.048 M sodium metaperiodate was added. Two cc. aliquots were removed at suitable intervals and analysed for residual oxidant according to the procedure given by Kholtoff (104). The aliquots were buffered with sodium bicarbonate to pH 8.5, potassium iodide was added and the liberated iodine was titrated with 0.025 M sodium arsenite.

TABLE XXII

The Consumption of Sodium Metaperiodate by Oxylignin B-9 [a]

Time (minutes)	Oxidant Consumed (in cc. of 0.025 M Sodium Arsenite [b][c])	Millimoles of Sodium Metaperiodate Consumed per Gram Oxylignin
22	0.64	2.63
97	0.81	3.34
150	0.96	3.53
174	0.85	3.50
1100	1.30	5.32
1325	1.41	5.80
1521	1.46	5.99

(a) The oxylignin, 0.207 gm., was brought to a swollen state with 18.04 cc. of solvents described in experiment (c) and oxidized with 50 cc. of 0.048 M sodium metaperiodate.
(b) Two cc. aliquots were removed.
(c) As a difference from a blank of 14.71 cc.

(d) One gram of oxylignin B-9 was dissolved in 20 cc. of N sodium hydroxide and concentrated hydrochloric acid was added until the pH of the solution was 3.2. To this solution was added 80 cc. of 0.048 M sodium metaperiodate,

and after 4.5 hours tests showed that most of the oxidant had been consumed. A few drops of ethylene glycol were added and the solution was acidified to pH 1 with hydrochloric acid. A heavy deposit was removed by centrifuging the mixture, and the solid was freed of excess moisture by suction in a sintered glass funnel. The damp solid was taken up in the least amount of acetone and poured into a large excess of petroleum ether. Evaporation of the residual solvent from the precipitated oil gave an orange powder called oxylignin F-1. Found: weight, 0.54 gm.; methoxyl, 7.3%; chlorine, 12.0%.

The aqueous oxidation liquor was extracted with ether for three 24 hour periods. The first extraction gave 8% of a black tar, the second extraction 8% of a viscous red tar, and the last a little ash.

(e) A third oxidation was carried out using 2.03 gm. of oxylignin B-9 dissolved in 30 cc. of 3% sodium bicarbonate and enough hydrochloric acid to adjust the pH to 4.9. One hundred and fifty cc. of 0.048 M sodium metaperiodate were added and after 4 hours the oxidation was stopped with ethylene glycol. The solution was acidified, the precipitate centrifuged and then dried in a sintered glass funnel for 4 hours with suction. This solid was dissolved in acetone, filtered and poured into petroleum ether. Exhaustive washing with petroleum ether freed the precipitate from traces of iodine and drying was with vacuum until the weight became constant. The

analysis of this fraction is given in Table XXIII.

Hydrogen sulphide was bubbled into the aqueous residue for 4 hours, and after standing for 24 hours the sulphur deposit was removed. The foul smelling solution was evaporated to 75 cc. in an unsuccessful attempt to remove this odour and a further crop of sulphur was removed. The concentrate was placed in a dialysing sack surrounded by 2 liters of distilled water. After 24 hours, iodine could be detected on both sides of the membrane and a fresh supply of distilled water was added. After an additional 24 hours very little halogen could be detected within the membrane and the solution was evaporated to a few milligrams of organic and inorganic salts.

(f) A fourth oxidation was carried out under identical conditions. The oxylignin F fraction was isolated as before and its analysis is given in Table XXIII. The aqueous oxidation liquor was treated with hydrogen sulphide and after the removal of the insoluble sulphur, was evaporated to one-half the original volume and filtered again. Two gm. of silver chloride were added and the mixture was stored in the dark for 20 hours. The yellow inorganic deposit was removed and the solution evaporated to dryness <u>in vacuo</u>. The organic material was leached with acetone. Evaporation of the extract gave a dark powder in 10% yield.

TABLE XXIII

The Analysis of Oxylignin F

	Third Oxidation		Fourth Oxidation		
Yield (%)	65.8		65.1		
Methoxyl (%)	5.5	5.4	4.9	4.9	(a)
Chlorine (%)	-	-	9.3	9.7	(a)
Ash (%)	7.2	7.2	1.8	2.1	

(a) No trace of iodine present (86).

(g) In another experiment, hydrogen sulphide was added to the oxidizing solution at pH 4.9 before the removal of oxylignin F. After removal of the sulphur, acidification of the solution produced only 6% of a dark brown powder.

(h) A 1 gram sample of oxylignin F from the fourth oxidation was methylated by refluxing with methanol overnight according to the procedure given earlier for the methylation of oxylignin B with methanol and "Drierite". Since the product gave unsatisfactory molecular weight determinations, no detailed analysis was carried out. Found: weight, 0.770 gm.; methoxyl, 11.4% and 11.6%; ash, 1.4% and 1.5%.

Oxylignin B and Sodium Hypochlorite

The hypochlorite solutions used in these experiments were prepared by bubbling chlorine gas into a solution of sodium hydroxide, preferably cooled, containing twice as many moles of caustic as the desired number of moles of sodium hypochlorite. Various reagents,

most commonly sodium carbonate, were added to buffer the solutions to the required pH. The nature of the buffer will be described for each experiment.

Since oxylignin B was not suspected of having a large number of phenolic groups, the simplest method of analysis was employed (48). Aliquots of 2 cc. were dissolved in 10 cc. of 10% sulphuric acid, an excess of potassium iodide was added and the resulting iodine titrated with sodium thiosulphate. The total number of equivalents of hypochlorite was given by the following expression.

$$\text{Equivalents} = \frac{\text{Vol. (of thiosulphate)} \times \text{N. (of thiosulphate)}}{2000}$$

(a) A 0.549 gm. sample of oxylignin B-9 was dissolved in 50 cc. of a solution containing 0.592 equivalents per liter of sodium hypochlorite and buffered to pH 11 with 1 gm. of sodium carbonate. At suitable intervals 2 cc. aliquots were removed and analysed for residual sodium hypochlorite, the results of which are shown in Table XXIV and plotted in Figure 13, Plot A. At the conclusion of the experiment, the residual hypochlorite was destroyed with acetone and the solution, after acidification to pH 1, was evaporated to dryness at reduced pressure over potassium hydroxide. The residue was leached with acetone, the extract was filtered and evaporated to a thick red tar which could not be powdered. A little acetone was added to dissolve the tar, and it was then subjected to a pressure of 1 mm. for several days in

an unsuccessful attempt to make it froth. After standing for two weeks in petroleum ether, vacuum treatment converted it to a sticky brown powder in 71% yield.

TABLE XXIV
(See Fig. 13 Plot A)

The Consumption of Sodium Hypochlorite by Oxylignin B-9 at pH 11 and at Room Temperature. (a)

Time (minutes)	Oxidant Consumed (in cc. of 0.0268 N Sodium Thiosulphate(b)(c)	Milliatoms of Available Oxygen Consumed per Gram Oxylignin
16	14.46	8.7
68	17.32	10.6
117	18.58	11.3
185	20.49	12.8
247	22.02	13.4
445	22.32	13.6
1370	23.97	14.6

(a) The oxylignin, 0.549 gm., was oxidized with 50 cc. of 0.592 N sodium hypochlorite.
(b) Two cc. aliquots were employed.
(c) As a difference from a blank of 44.17 cc.

(b) A second oxidation was attempted using 4 gm. of the oxylignin dissolved in 250 cc. of 0.92 sodium hypochlorite buffered to pH 11 with sodium carbonate. The consumption of oxidant was followed and was identical to that given above. After destruction of the residual oxidant with acetone, the solution was acidified to pH 0.8 with hydrochloric acid and a fine suspension was deposited by centrifugation. The residue was leached with acetone, the extract was decanted from some inorganic material and was evaporated to a dark brown powder in 6% yield.

The aqueous liquor was extracted with ether

for 24 hours, and the yellow extract was reduced from 300 cc. to 60 cc. by distillation. After drying with "Drierite", the ether extract was evaporated to a pleasant smelling oil in 15% yield. A preliminary test showed that the bulk of this material would not distill under reduced pressure, although a small amount of liquid boiling at 84°C. (1 atm.) was isolated. Chromatographic examination of the crude oil by the method of Barker and Kennedy (83) using ethanol-ammonia as solvent, showed the presence of an acid with an Rf value of 0.27. Test spots of isobutyric acid and acetic acid gave values of 0.57 and 0.33 respectively.

The residual aqueous oxidation liquor was evaporated to dryness using a modification of the freeze dry technique described by Cabot (19), the details of which will be given later. The mixture of organic and inorganic material was leached with five 50 cc. volumes of acetone, the extracts were combined and evaporated to 50 cc. and poured into 300 cc. of petroleum ether. The precipitated red oil was caused to froth by high vacuum treatment and was crushed to a yellow, hydroscopic powder under petroleum ether. The acetone and petroleum ether mother liquor was taken to dryness and a red tar contaminated with a little crystalline material was isolated. The yellow froth was extracted with 100 cc. of ether but during the filtration of the extract, the powder was converted to a red gum. Its original condition

was restored by means of a brief vacuum treatment over phosphorous pentoxide. The etherial solution was evaporated to a mass of needle-like crystals imbedded in a red oil. Although the crystals dissolved in a little water and some of the oil was converted to an amorphous mass, tests showed that the crystalline material could not be extracted in a pure state. Ethanol, methanol, petroleum ether, acetone, ethyl acetate, benzene and ether were likewise unsatisfactory as extractants. The oil was taken up in 1 cc. of acetone and 6 cc. of petroleum ether was added, causing most of the red oil to separate. Evaporation of the supernatent liquid gave some crystals contaminated with a little oil. The original red oil was extracted in this manner until evaporation of the solvent did not cause the separation of any crystalline material. The crude crystals were crystallized from ether but the product was still contaminated with a minute amount of oil. Found: weight, 0.0505 gm.; melting point, 98.1° to 99.7° C.; mixed melting point with oxalic acid dihydrate, 100° to 101° C.

A 0.2034 gm. sample of the yellow powder, called oxylignin **H-2**, was dissolved in 20 cc. of 0.974 N sodium hypochlorite and buffered to pH 10.8 with sodium carbonate. The consumption of oxidant was followed by analysing 2 cc. aliquots for residual sodium hypochlorite, the results of which are given in Table XXV and plotted in Figure 13, Plot C.

TABLE XXV

The Consumption of Sodium Hypochlorite by Oxylignin H-2 at Room Temperature and pH 10.8[a]

Time (minutes)	Oxidant Consumed (in cc. of 0.0338 N Sodium Thiosulphate[b][c])	Milliatoms of Available Oxygen Consumed per Gram Oxylignin
17	14.72	12.2
42	15.52	12.8
254	17.45	14.5
334	17.97	14.9
1376	18.82	15.6

(a) The oxylignin, 0.2034 gm., was oxidized with 20 cc. of 0.974 N sodium Hypochlorite.
(b) Two cc. aliquots were employed.
(c) As a difference from a blank of 57.62 cc.

(c) A third oxidation, carried out to isolate products free of oxalic acid, employed 4.00 gm. of oxylignin B-9 in 250 cc. of 0.874 N sodium hypochlorite, buffered as before to pH 11. The oxidation was stopped when 11.8 milliatoms of available oxygen per gram of oxylignin were consumed. Acidification of the solution destroyed the excess carbonate and rendered 3% of the oxylignin insoluble. The yellow solution was made alkaline again and 0.5 gm. of calcium chloride was added with stirring. A faintly yellow precipitate appeared which was isolated and dried at 105°C. This residue, weighing 0.478 gm., was acidified with strong hydrochloric acid and extracted with five 15 cc. volumes of ether. Evaporation gave 0.218 gm. of crude crystals which were recrystallized from water. The product was still slightly contaminated with oil. Found: melting point, 99° to 100°C.; mixed m.p. with oxalic acid dihydrate,

98.5 to 99.5°C.; neutralization equivalent, 63.7; calculated for $H_2C_2O_4.2H_2O$, 63.0.

The residual aqueous oxidation liquors were acidified and extracted for 48 hours with pure ether. The yellow extract was dried over "Drierite" and evaporated to a red gum. Attempts to distill this gum under reduced pressure were unsuccessful, although a trace of sublimate thought to be oxalic acid appeared when the temperature exceeded 150°C. The aqueous solution was freeze dried and the organic material isolated as before in 29% yield.

(d) A further degradation of 1.00 gm. of oxylignin B-9 was carried out using 100 cc. of 0.900 N sodium hypochlorite buffered to pH 7.2 with sodium carbonate. At the conclusion of the experiment, the pH had risen to 7.8. The results are given in Table XXVI and in Figure 13, Plot B.

TABLE XXVI

The Consumption of Sodium Hypochlorite by Oxylignin B-9 at Room Temperature and at pH 7.2 to 7.8[a]

Time (minutes)	Oxidant Consumed[b][c] (in cc. of 0.0338 N Sodium Thiosulphate)	Milliatoms of Available Oxygen Consumed per Gram Oxylignin.
12	33.80	28.5
32	43.43	36.7
77	46.37	39.2
300	50.19	42.4

(a) One gram of oxylignin oxidized by 100 cc. of 0.900 N sodium hypochlorite.
(b) Two cc. aliquots were employed.
(c) As a difference from a blank of 53.28 cc.

The oxidation was stopped with acetone after increasing the pH to 11, and approximately 0.4 gm. of calcium chloride was added. The white precipitate was dissolved in hydrochloric acid to destroy the calcium carbonate and the solution was made alkaline to precipitate calcium oxalate. After digesting on the steam bath, the residue was isolated, dried and weighed. Found: 0.215 gm. calcium oxalate which corresponded to 0.151 gm. oxalic acid.

The aqueous oxidation liquor was acidified to pH 1 and extracted with ether for 48 hours. The etherial solution was dried with "Drierite" and allowed to evaporate overnight to 1.59 gm. of a crude oil and 0.0245 gm. of an amorphous solid. A portion of the oil which had been freed from volatile matter by high vacuum treatment corresponded to 27.5% of the oxylignin. Steam distillation of the oil gave an apparent acetyl content of 1% based on the weight of the oxylignin B oxidized. Chromatographic examination of the oil by the method of Barker and Kennedy (83) using ethanol-ammonia as solvent showed only one spot with an Rf value of 0.33, while the literature values (83) for acetic and formic acids were 0.32 and 0.33. A control spot of acetic acid gave an Rf value of 0.33. A second chromatogram was run using methanol-ammonia as solvent and three spots were detected having Rf values of 0.07, 0.59 and 0.77. Control spots of oxalic and acetic acids gave Rf values of 0.07 and 0.61.

The insoluble, amorphous solid refered to above was extracted with hot ethyl acetate; the soluble portion was removed and evaporated to dryness. It was taken up again in ethyl acetate, filtered and evaporated to a white amorphous residue in 1.4% yield. This material was soluble in hot ethyl acetate, acetone, ether, and ethyl alcohol and gave an amorphous deposit when the solutions were cooled. The residual aqueous liquors from the oxidation were freeze dried but no organic material could be isolated from the mass of sodium chloride.

(e) An unsuccessful attempt was made to isolate a further quantity of the amorphous solid by oxidizing 4 grams of oxylignin B. Chromatographic investigation showed the presence of the same three acids detected previously when methanol-ammonia was used as solvent.

(f) In another experiment at pH 7.3 using sodium hydroxide as buffer, all the oxidant was consumed in 24 hours. Acidification of this solution resulted in the liberation of a gas, and chromatographing the ether extract revealed the presence of oxalic acid, the unknown acid but no acetic acid.

(g) A mild oxidation of 2 gm. of oxylignin B-9 was carried out using 100 cc. of 0.648 N sodium hypochlorite buffered to pH 11 with 5 gm. of sodium carbonate. The oxidizing solution was cooled to 5°C. with crushed ice and the oxylignin was added slowly with stirring so that the temperature did not exceed 9°C. The solution was kept at

5°C. for the rest of the experiment, the results of which are shown in Table XXVII and Figure 13, Plot D.

TABLE XXVII

The Reaction of Sodium Hypochlorite with Oxylignin B-9 at 5°C. and pH 11.(a)

Time (minutes)	Oxidant Consumed(b)(c) (in cc. of 0.338 N Sodium Thiosulphate)	Milliatoms of Available Oxygen Consumed per Gram Oxylignin.
15	9.92	4.2
27	10.84	4.6
60	12.84	5.4
82	13.14	5.5
150	14.60	6.2
180	15.74	6.6
525	19.24	8.1

(a) Two gm. of oxylignin oxidized by 100 cc. of 0.648 N sodium hypochlorite.
(b) Two cc. aliquots were employed.
(c) As a difference from a blank of 38.34 cc.

The reaction was stopped with acetone and the solution was carefully acidified with concentrated hydrochloric acid. At pH 1 a precipitate separated which was isolated by centrifugation and extraction with acetone. The extract was added to petroleum ether, and the resulting brown precipitate, after drying, amounted to 16.5% of the oxylignin. The aqueous solution was made alkaline and calcium chloride was added. The crude calcium oxalate was isolated, dissolved in dilute hydrochloric acid and extracted with ether. Recrystallization of the ether extract from water gave 7.7% oxalic acid. The alkaline oxidation liquor was re-acidified and extracted with ether for 24 hours to remove 0.2 gm. of a thick red oil. The aqueous

liquor was freeze dried, and the residue gave an acetone extract in 30% yield. Found: methoxyl, 8.2%; ash, 6.8%; molecular weight by the ebullioscopic method of Menzies and Wright (74), 400.

(h) A large scale oxidation was attempted but because of the delays introduced by the use of the freeze dry technique, the preparation had to be carried out in 3 conveniently sized batches. The concentration of reagents and other data are collected in Table XV. The oxidized solutions were treated as before to remove oxalic acid but this fraction was not investigated further. The alkaline oxidation liquor was then acidified and the oxylignin which separated was removed by centrifugation. Acetone was employed to remove the wet brown gum from the cups and the red solution was poured into twice its volume of petroleum ether. After several hours, the orange oil which precipitated was separated from the supernatent petroleum ether and acetone layer. Two hours under high vacuum sufficed to convert the oil to a brown powder. The acid insoluble fractions from all 3 oxidations were combined and called oxylignin G.

The residual aqueous solution from the oxidation was extracted for 24 hours with ether and the extracts were stored for further study. During the extraction, a brown gum was deposited on the walls of the extractor. This gum was taken up in acetone, centrifuged, poured into petroleum ether, powdered and added to the

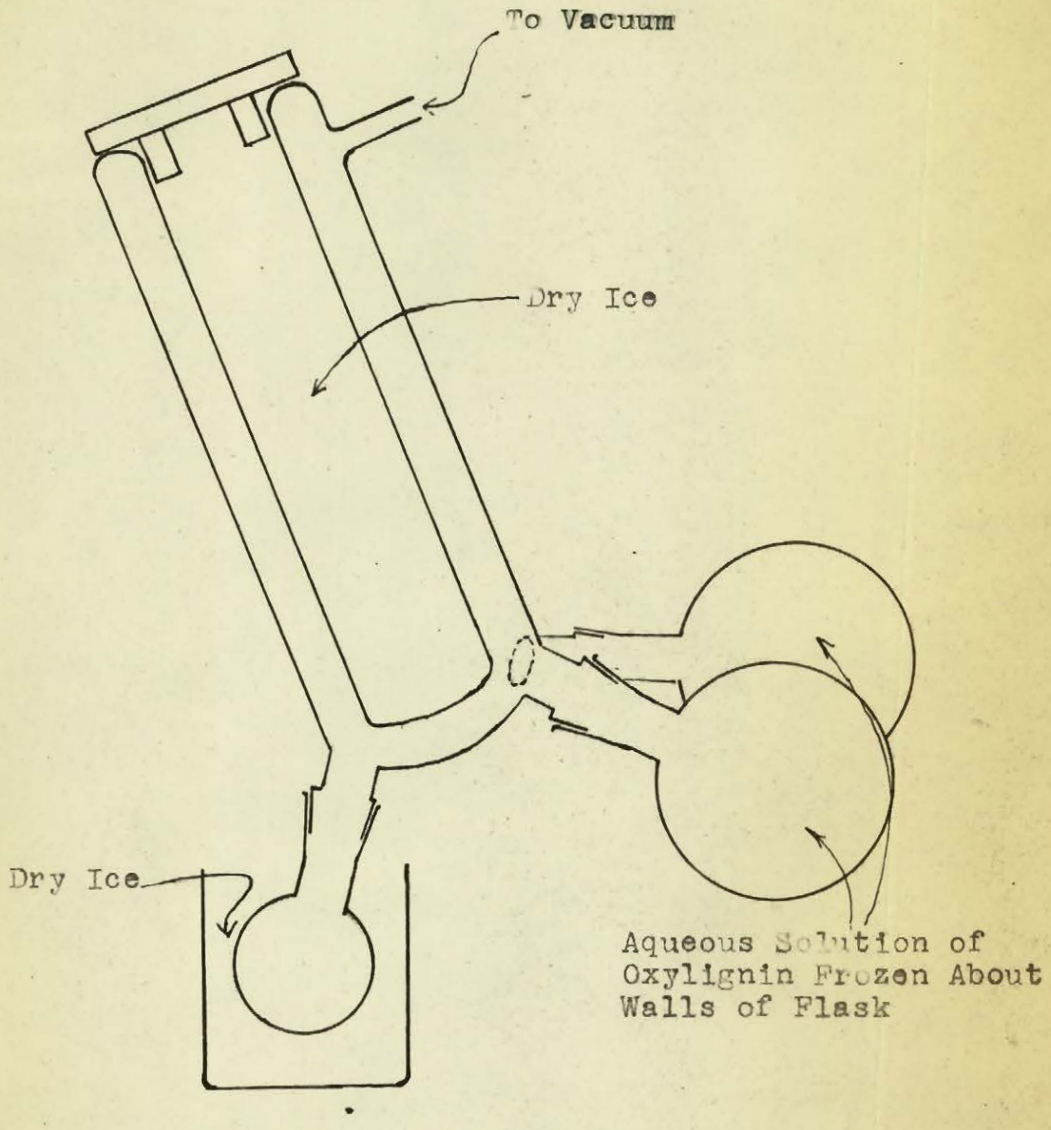


Figure 19.

The Freeze Dry Apparatus Employed for the Isolation of Oxylignin H.

oxylignin G fraction. The clear yellow aqueous residue from one of the oxidations (350 cc.) was freeze dried in the apparatus depicted in Figure 19. One of the 3 outlets was set lower than the other two and was cooled with dry ice so it might act as a trap to collect excess water vapor. The condenser was charged with dry ice and two 1 liter flasks, each containing 175 cc. of the residual oxidation liquor frozen about their walls, were attached to the other two outlets. A vacuum of 1 mm. was carefully applied and the ice was evaporated from the non volatile material. The residue was extracted with four 70 cc. volumes of acetone, the dark orange liquid was centrifuged and decanted into 400 cc. of petroleum ether. After standing overnight, the clear acetone petroleum ether layer was decanted and the residual orange oil was extracted with equal volumes of ether until the extract was colourless. The oil was converted to a yellow froth by exposure to vacuum. The yellow powders from the 3 oxidations were combined and purified by means of petroleum ether washings. After drying for 1 week in vacuo, the product was analysed. It was then taken up in acetone, precipitated and carefully dried. The analyses of this fraction and the others from this oxidation are given in Table XVI.

The Fractionation of Oxylignins G and H

One gram of the oxylignins were each dissolved in 10 cc. of acetone containing 1 drop of water. After

standing overnight, they were filtered to give Fractions 1. To each was added 3 cc. of petroleum ether and after standing overnight, the acetone-petroleum ether solutions were decanted from the precipitated oils. The oils were frothed under high vacuum, washed carefully and dried to give Fraction 2. The residual solutions were treated with an additional 4 cc. of petroleum ether, the insoluble fractions collected after standing overnight were called Fractions 3 and the soluble fractions were called Fractions 4. Fractions 3 were purified and dried in the manner described, while Fractions 4 were evaporated to dryness, crushed under petroleum ether and dried by exhaustive vacuum treatments. The yields and analysis of the fractions obtained from oxylignin G and oxylignin H are given in Table XVII.

Methylation of Oxylignins G and H

(a) A 0.993 gm. sample of oxylignin G was methylated with two 5 gram charges of methylnitroso urea on succeeding days according to the procedure given for the methylation of oxylignin C. A thick oil was produced when the solution was evaporated, and high vacuum treatment converted it into a powder. After careful washing and drying under high vacuum, a brown powder was isolated in 83% yield and the analysis of this product, together with those of the following methylated products are given in Table XVII.

(b) A 0.978 gm. sample of oxylignin H was methylated

under circumstances identical to those given in (a), and a light yellow powder was isolated in 80% yield.

(c) Oxylignin G, 0.500 gm., was heated under reflux with 50 cc. of anhydrous methanol and 0.5 gm. of "Drierite" for 24 hours. The product, after working up by the procedure given above, was isolated in 96% yield.

(d) Oxylignin H, 0.503 gm., was methylated under identical circumstances to those described in (c) and gave a light yellow powder in 83% yield.

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. The oxidation of isolated spruce periodate lignin ($OCH_3$10.3%) with unbuffered 0.26 M aqueous chlorine dioxide for 18 hours at room temperature and pH 4 to 1, was carried out on ten occasions substantially as described by Levitin (18). In the later experiments, however, acetone was used instead of methanol to extract the oxylignin B fraction from the water-insoluble mixture of oxylignin A and B, and oxylignin C was isolated by direct evaporation of the aqueous mother liquor from the oxidation. These changes made it clear that Levitin's oxylignin B ($OCH_3$5.5 to 7.0%) had been partly esterified with methanol during the isolation, and that his oxylignin C (apparent OCH_3 14.4%) was contaminated with the butanol used to extract C from the aqueous liquor. The proper methoxyl contents were 5% and 1.2% respectively.

2. Methylation of oxylignin B, a cream coloured powder, with diazomethane, and esterification with methanol, produced similar products with nearly the same methoxyl content (17%) and Levitin's (18) inference that oxylignin B contained few, if any, phenolic groups was confirmed. The acetylation of oxylignin B with acetyl chloride was very incomplete. Acetic anhydride and sodium acetate produced a black gum, but with sulphuric acid as catalyst an acetate was recovered in nearly quantitative yield as a yellow powder. The presence or latent presence of aliphatic hydroxyl groups was thus demonstrated for the

first time. When oxylignin B was condensed with aqueous sodium cyanide at pH 10.5, and the liquor later heated with caustic soda, the evolution of ammonia corresponded to the presence of one gram molecular weight of carbonyl group in 1.2 kilogram of oxylignin B. Levitin's result was one such group in 2.8 kilograms. On the other hand, the one double bond discovered by Levitin could not be detected by the addition of bromine to oxylignin B.

3. When dissolved in alkali and reprecipitated by acidification next day to pH 1, about half of oxylignin B was recovered as oxylignin D. The soluble portion was oxylignin E. Neither the chlorine nor the methoxyl contents of D and E, however, differed markedly from those of oxylignin B, and no evidence was found to support Levitin's view that alkali cleaved B into two fragments each of lower molecular weight. Oxylignin B when titrated back and forth with alkali and acid, behaved as a lactone or a keto-enol system.

4. Although B was probably a mixture, the fact was not decisively established. By assuming that the substance was homogeneous, the mass of analytical data underlying the conclusions in (2) and (3) agreed with the empirical formula:

$$Cl_3\left[\begin{matrix}(OCH_3)_2\end{matrix}\right.\left.\begin{matrix}C_{41}H_{33}O_8\end{matrix}\right]\left.\begin{matrix}(COOH)_5\\(OH)_7\\C{=}O\end{matrix}\right.$$

in which five additional acidic functions exposable by

caustic soda might be present. The chlorine was probably aromatic in nature, but evidence for phenolic nuclei could not be detected in the ultra-violet spectrum. As Levitin showed, oxidation with alkaline nitro-benzene yielded no vanillin. Levitin's different formula and his divergent results could be explained by assuming that his oxylignin B was isolated as a partial methyl ester.

5. The cautious oxidation of oxylignin B with aqueous potassium permanganate under various conditions, and also with sodium metaperiodate, yielded intractable products that were not examined in detail. Alkaline hypochlorite, the most promising oxidant found, yielded from 7 to 15% of oxalic acid, about 20% of an oxylignin G that was insoluble in aqueous acid, 16% of a water soluble oxylignin H and 11% of an ether soluble oil. Esterification with methanol and diazomethane showed that the products G and H contained fewer carboxyl groups than oxylignin B. Both G and H contained fractions of varying methoxyl content and probably of varying molecular weight.

6. Much effort was expended in attempts to establish the molecular weights of the above oxylignins, their acetates and methyl esters using methanol or acetone as solvents with Signer's isothermal distillation or Menzies and Wright's ebullioscopic techniques. Although the variable results showed that the methods were in general untrustworthy, those of the acetate of oxylignin B (1200 to 1500) and its methyl ester (1300) were reproducible

and agreed with the molecular weight (1193) given in (4) for oxylignin B itself. Those for various fractions of oxylignins G and H (160 to 900) and for their methyl esters (160 to 330) suggested that they represented substantially degraded products of oxylignin B.

7. Oxylignin C, which Levitin isolated as a red-brown gum contaminated with butanol, was recovered in purer condition as a light yellow, water soluble powder with the empirical formula:

$$C_{46.35}\ H_{52.61}\ Cl_{6.79}\ O_{33.94}\ (OCH_3)_{0.5}$$

but no molecular weights could be obtained to prove or disprove the suspicion that it was just as complex as oxylignin B. Of the chlorine present (17%), about 2% resembled the chlorine in tertiary butyl chloride in its reactivity, and a further 8% was readily removed by dilute alkali. The remaining chlorine, (about 7%) stable both to alkali and to reduction with zinc and hydrochloric acid or hydrogen and palladium, appeared to be aromatic in nature. No phenolic or vanillin units were discovered, but esterification with methanol and methylation with diazomethane suggested the presence of 7 to 8 carboxylic acid groups in the molecule. The instability of oxylignin C, together with its solubility in water, hampered its investigation in other directions.

BIBLIOGRAPHY

1. Brauns, F. E., "The Chemistry of Lignin", pp. 49-132 , Academic Press Inc., New York (1952).

2. Brauns, F. E., J. Am. Chem. Soc., 61, 2120 (1939).

3. Hess, C. L., T.A.P.P.I., 35, 312 (1952).

4. Brauns, F. E., Paper Trade J., 111, No. 4, 35 (1940).

5. Ploetz, T., Ber., 73B, 57, 61, 74, 790 (1940).

6. Schubert, W. J., and Nord, F. F., J. Am. Chem. Soc., 72, 3835 (1950).

7. Harris, E. E., Ind. Eng. Chem., 32, 1049 (1940).

8. Klason, P., Cellulosechem., 4, 81 (1923); 12, 36 (1931).

9. Willstatter, R., and Zeichmeister, L., Ber., 46, 2401 (1913).

10. Freudenberg, K., Zocher, H., and Durr, W., Ber., 62, 1814 (1929).

11. Ritchie, P. F., and Purves, C. B., Pulp and Paper Mag. Can., 48, No. 12, 74 (1947).

12. Harris, E. E., D'Ianni, J., and Adkins, H., J. Am. Chem. Soc., 60, 1467 (1938).

13. Brickman, L., Pyle, J. J., McCarthy, J. L., and Hibbert H., J. Am. Chem. Soc., 61, 868 (1939).

14. Creighton, R. H. G., Gibbs, R. D., and Hibbert, H., J. Am. Chem. Soc., 66, 32 (1944).

15. Pennington, D. E., and Ritter, D. M., J. Am. Chem. Soc. 69, 187 (1947).

16. Brickman, W. J., Ph. D. Thesis, McGill University (1953).

17. Corey, A. J., and Maass, O., Can. J. Res., B13, 149 (1935).

18. Levitin, N., Ph. D. Thesis, McGill University (1951).

19. Cabot, I., Ph. D. Thesis, McGill University (1951).

20. Smith, M. A., Calmer, F., Kraut, J., Witkoski, F. C. and Zuckerman, P., T.A.P.P.I., 36, 342 (1953).

21. Russel, A., Science, 106, 372 (1947); J. Am. Chem. Soc., 70, 1060 (1948).

22. Doree, C., and Cunningham, M., J. Chem. Soc., 103, 677 (1913).

23. Friederich, A., and Diwald, J., Monatsch., 46, 31 (1925).

24. Urban, H., Cellulosechem., 7, 73 (1926).

25. Rassow, B., and Zickmann, P., J. Pract. Chem., 123, 189 (1929); C. A., 24, 720 (1930).

26. Gruss, J., Ber., deut. botan. Ges., 41, 53 (1925).

27. Grafe, V., Monatsch., 25, 987 (1904).

28. Kurschner, K., J. prakt. Chem., (2), 118, 238 (1938).

29. Phillips, M., and Goss, M. J., J. Am. Chem. Soc., 55, 3466 (1933).

30. Heuser, E., and Winswold, A., Ber., 56, 902 (1923); Cellulosechem., 4, 49, 62 (1923).

31. Freudenberg, K., Engler, K., Flickenger, E., Sobek, A., and Klink, F., Ber., 71, 1817 (1938).

32. Freudenberg, K., and Lautsch, W., Naturwissenschaften, 27, 227 (1939).

33. Freudenberg, K., Lautsch, W., and Engler, K., Ber., 73, 167 (1940).

34. Creighton, R. H. F., McCarthy, J. L., and Hibbert, H., J. Am. Chem. Soc., 63, 312 (1941).

35. Creighton, R. H. F., and Hibbert, H., J. Am. Chem. Soc., 66, 37 (1944).

36. Lautsch, W., Plankenhorn, E., and Klink, F., Angew. Chem., 53, 450 (1940).

37. Pearl, I. A., J. Am. Chem. Soc., 64, 1429 (1942); 72, 1427 (1950).

38. Lautsch, W., and Piazolo, G., Ber., 73, 317 (1940).

39. Aulin Erdtman, G., Svensk Kem. Tid., 55, 116 (1943); C. A., 38, 4127 (1944).

40. Brewer, C. P., Cook, L. M., and Hibbert, H., J. Am. Chem. Soc., 70, 57 (1948).

41. Richtzenhain, H., Acta Chem. Scand., 4, 589 (1950); 4, 206 (1950); Ber., 83, 488 (1950); Svensk Papperstidn., 53, 644 (1950).

42. Read, D. E., and Purves, C. B., J. Am. Chem. Soc., 74, 120 (1950).

43. Bone, W. A., Horton, L., Ward, S. G., Proc. Roy. Soc. (London) A127, 480 (1930).

44. Traynard, Ph., and Robert, A., Bull. assoc. tech. ind. papetiere, 5, No. 7, 401 (1951).

45. Holmberg, B., Svensk Kem. Tid., 53, 415 (1951); Ber., 75, 1760 (1942).

46. Gralen, N., J. Colloid Sci., 1, 453 (1946).

47. Whittal, N. S., M. Sc. Thesis, McGill University (1950).

48. Brounstein, C. J., Ph. D. Thesis, McGill University 1952).

49. Sohn, A. W., and Reiff, F., Papier-Fab., 40, 1, 105 (1942).

50. Jayme, G., and Hanke, G., Cellulosechem., 21, 127 (1943).

51. Barton, J. S., T.A.P.P.I., 32, 496 (1950).

52. Bublitz, W. J., Ph. D. Thesis, The Institute of Paper Chemistry, Appelton, Wis. (1949).

53. Wacek, A. V., and Schroth, D., Das Papier, 4, 410 (1950).

54. Jayne, J. E., Ph. D. Thesis, The Institute of Paper Chemistry, Appelton, Wis., (1953); Research Bulletin, The Institute of Paper Chemistry, 19, No. 2, 55 (1952); T.A.P.P.I., 36, 571 (1953).

55. Mellor, J. W., "Comprehensive Treatise on Inorganic and Theoretical Chemistry", pp. 287-290 , Vol. 2, Longman Green and Co., (1922).

56. Husband, R. M., Ph. D. Thesis, McGill University (1947).

57. Logan, C. D., Ph. D. Thesis, McGill University (1949).

58. Furst, E., Ann., 206, 78 (1881).

59. Schmidt, E., and Grauman, E., Ber., 54B, 1860 (1921).

60. Schmidt, E., and Braunsdorf, K., Ber., 55B, 1529 (1922).

61. Schmidt, E., Geisler, E., Arndt, P., and Ihlow, F., Ber., 55B, 23 (1922).

62. Schmidt, E., and Miermeister, A., Ber., 56B, 1487 (1923).

63. Schmidt, E., Haag, W., Abele, J., and Sperlinger, L., Ber., 58B, 1394 (1925).

64. Schmidt, E., and Duysen, F., Ber., 54B, 3241 (1921).

65. Fuchs, W., and Honsig, E., Ber., 59B, 2850 (1926).

66. Sarkar, P. S., J. Ind. Chem. Soc., 12, 470 (1935).

67. Jeans, A., and Isbell, H. S., Bur of Stand. J. of Research, 27, 125 (1941).

68. Blank, E. W., and Willard, M. L., J. Chem. Ed., 9, 1819 (1932).

69. Signer, R., Ann., 478, 246 (1930).

70. Yackerl, E. C., and Kenyon, W. O., J. Am. Chem. Soc., 64, 121 (1942).

71. Gladding, E. K., and Purves, C. B., Paper Trade J., 116, No. 14, 26 (1943).

72. Coombs, R. D., B. Sc. Thesis, M. I. T. (1941).

73. Schacherl, G., Ann., 206, 78 (1881).

74. Menzies, A. W. C., and Wright, S. L., J. Am. Chem. Soc., 43, 2314 (1921); 43, 2309 (1921).

75. Wedekind, E., and Katz, J. R., Ber., 62, 1172 (1929).

76. Hibbert, H., and Brauns, F. E., Can. J. Research, 2, 364 (1930).

77. Shriner, R. L., and Fuson, R. C., "The Systematic Identification of Organic Compounds", pp. 88 , John Wiley and Sons, Inc., New York (1948).

78. Kolthoff, I. M., and Stenger, V. A., "Volumetric Analysis", Vol 2., pp. 282, Interscience Publishers, Inc., New York (1947).

79. "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists", pp. 13-14, Seventh Edition, Washington, D. C. (1950).

80. Jones, E. J., T.A.P.P.I., 32, 311 (1949).

81. Stone, J. E., and Blundell, M. J., J. Anal. Chem., 23, 771 (1951).

82. Vieboch, F., and Schwappach, A., Ber., 63, 2818 (1930).

83. Barker, D. A., and Kennedy, E. P., Anal. Chem., 23, 1033 (1951).

84. Papa, D., Schwenk, E., and Whitman, B., J. Org. Chem., 7, 587 (1943).

85. Reference 77, pp. 115.

86. Reference 77, pp. 55.

87. Niederl, J. B., and Niederl, V., "Organic Quantitative Microanalysis", pp. 62 , John Wiley and Sons, Inc., New York, Second Edition, (1942).

88. Vieboch, F., and Brecher, C., Ber., 63, 3207 (1930).

89. Clark, E. P., J. Assoc. Official Agr. Chem., 15, 136 (1932).

90. Penniston, Q. P., and Hibbert, H., Paper Trade J., 109, 46 (1939).

91. T.A.P.P.I. Standards, T-222-M-50 (Aug. 1950).

92. "Peroxide Bomb, Apparatus and Methods", Manuel No. 121, p. 38, Parr Instrument Company, Moline, Ill. (1950).

93. Reference 87, pp. 101-107.

94. Clark, E. P., Ind. Eng. Chem., Anal. Ed., 8, 481 (1936); 9, 539 (1937).

95. Clark, E. P., Ind. Eng. Chem., Anal. E., 13, 820 (1941).

96. Beckman Bulletins 91G "Operating Instructions" and 89C "Instructions for the Use of the Ultra-Violet Accessory Set", Beckman Instrument Company, South Pasedina, Cal.

97. Lange, J., and Paris, R., J. pharm. Chim., 21, 403 (1935).

98. Fleury, P., and Lange, J., J. pharm. chim., 17, 107, 196 (1933).

99. Kurth, E. F., and Ritter, G. J., Ind. Eng. Chem., 25, 1250 (1933); J. Am. Chem. Soc., 56, 2720 (1934).

100. Reference 78, pp. 242.

101. Towers, G. H. N., M. Sc. Thesis, McGill University (1951).

102. Bray, W. C., and Miller, H. E., J. Am. Chem. Soc., 46, 2204 (1924).

103. Kolthoff, I. M., and Furman, N. H., "Volumetric Analysis", Vol. 2., pp. 394, John Wiley and Sons, Inc., New York (1929).