OXIDATIONS OF PERIODATE LIGNIN LEVITIN

OXIDATIONS OF PERIODATE LIGNIN WITH SODIUM CHLORITE AND CHLORINE DIOXIDE

A Thesis

by

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TABLE OF CONTENTS

	Page
GENERAL INTRODUCTION	1
HISTORICAL INTRODUCTION	3
Isolated Lignins	3
Chlorine Dioxide and Sodium Chlorite as Bleaching and Delignifying Agents	7
The Action of Chlorine Dioxide and Sodium Chlorite on Simple Compounds	18
DISCUSSION	27
Preliminary Oxidations with Sodium Chlorite	29
Oxidations with Chlorine Dioxide	42
Isolation of Products from Oxidations with Sodium Chlorite	44
Isolation of Products from Oxidations with Chlorine Dioxide	47
Preliminary Examination of Oxylignin B	57
Oxidation with Nitrobenzene	64
Experiments on Molecular Structure of Oxylignin B	65
Molecular Weight	65
Acid Equivalents	68
Carbonyl Determinations	77
Methylations of Oxylignin B and its Derivatives	78
Summary of Methylations	86
EXPERIMENTAL	88
Preparation of Periodate Lignin	88
Analyses of Periodate Lignin	89
Methoxyl	89
Klason Lignin	90
Holocellulose	90
Pulpability	90
Ash	91
Oxidations of Periodate Lignin with Sodium Chlorite	91
Sodium Chlorite Oxidations with Expulsion of Chlorine Dioxide	99
Oxidations with Chlorine Dioxide	105
Isolation of Products from Chlorite Oxidations	110
Isolation of Products from Chlorine Dioxide Oxidations	113

Page

Larger-Scale Oxidations with Chlorine Dioxide 115
Re-oxidations of Oxylignins A and B 120
Behaviour of Oxylignin B with Sulphuric Acid 121
Fractional Precipitation of Oxylignin B 123
Fractionation of an Isolated Oxylignin B 124
Dialyses of Oxylignin B in Caustic Soda 126
Dialysis of Oxylignin B in Dilute Alcohol 129
Oxidation of Oxylignin B with Nitrobenzene 130
Molecular Weight Determinations on Oxylignin B 132
Acid Equivalents 137
Effect of Alkali on Oxylignin B 142
Carbonyl Determinations 143
Hydrogenation of Oxylignin B 145
Methylation with Diazomethane 146
Methylation with Methanolic Hydrogen Chloride 150
Methylation with Dimethyl Sulphate 151
Saponification of Methylated Oxylignin B 153

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH 156

RECOMMENDATIONS FOR FUTURE RESEARCH 159

BIBLIOGRAPHY 161

LIST OF FIGURES

Figure		Page
1	Oxidation of Periodate Lignin by 0.025 Mole of Sodium Chlorite in 45 cc. at Initial pH 4.1	34
2	Oxidation of Periodate Lignin by 0.050 Mole of Sodium Chlorite at Initial pH 4.1 and 30°C.	36
3	Oxidation of Periodate Lignin by Sodium Chlorite with Removal of Chlorine Dioxide ...	39
4	Oxidation of Periodate Lignin by Sodium Chlorite with Removal of Chlorine Dioxide ...	41
5	Oxidation of Periodate Lignin by Chlorine Dioxide	43
6	Flow Sheet for Oxidation with Sodium Chlorite ...	52
7	Flow Sheet for Oxidation with Chlorine Dioxide	53
8	Potentiometric Titration of Oxylignin B against Barium Hydroxide	70
9	Potentiometric Titration of Oxylignin B against Sodium Hydroxide	71
10	Potentiometric Titration of Oxylignin B against Sodium Hydroxide	72
11	Potentiometric Titration of Oxylignin B against Sodium Hydroxide and Back Titration with Acid	73
12	Apparatus for Molecular Weight Determinations ...	134
13	Potentiometric Titration of Oxylignin B and Sodium Hydroxide against Hydrochloric Acid	140
14	Potentiometric Titration of Oxylignin B and Sodium Hydroxide against Hydrochloric Acid	141

LIST OF TABLES

TABLE		Page
I	Sodium Chlorite Oxidations of 1 gm. Samples of Periodate Lignin	46
II	Chlorine Dioxide Oxidations of Periodate Lignin	54
III	Acid Equivalents of Oxylignin B	76
IV	Saponification of Diazomethane Methylated Oxylignin B	82
V	Oxidation at 30°C. of 1 gm. Lignin by 0.0246 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1	93
VI	Oxidation at 23 to 25°C. of 1 gm. Lignin by 0.0254 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1	94
VII	Oxidation at 20.3°C. of 1 gm. Lignin by 0.0253 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1	95
VIII	Oxidation at 8 to 10°C. of 1 gm. Lignin by 0.0253 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1	96
IX	Oxidation at 30°C. of 1 gm. Lignin by 0.0508 Mole of Chlorite in 47 cc. of Solution at Initial pH 4.1	97
X	Oxidation at 30°C. of 1 gm. Lignin by 0.0492 Mole of Chlorite in 87 cc. of Solution at Initial pH 4.1	98
XI	Change with Time in pH of Solutions and Blank..	99
XII	Oxidation at 30°C. of 1 gm. Lignin by 0.0236 Mole of Chlorite in 47 cc. of Solution at Initial pH 4.1 (Nitrogen Used for 22 Hours)	100
XIII	Oxidation at 30°C. of 1 gm. Lignin by 0.0239 Mole of Chlorite in 47 cc. of Solution at Initial pH 4.1 (Compressed Air Used for 10 Hours)	101

TABLE		Page
XIV	Oxidation at 30°C. of 1 gm. Lignin by 0.0504 Mole of Chlorite in 47 cc. of Solution at Initial pH 4.1 (Nitrogen Used for 32 Hours)	102
XV	Oxidation at 30°C. of 1 gm. Lignin by 0.0501 Mole of Chlorite in 47 cc. of Solution at Initial pH 4.1 (Nitrogen Used for 8 Hours)	104
XVI	Oxidation at 30°C. of 1 gm. Lignin by 0.0171 Mole of Chlorine Dioxide in 78 cc. of Solution at Initial pH 4.1	107
XVII	Oxidation at 30°C. of 0.5 gm. Lignin by 0.0179 Mole of Chlorine Dioxide in 46.5 cc. of Solution at Initial pH 4.1	108
XVIII	Oxidation at 25°C. of 1 gm. Lignin by 0.0219 Mole of Chlorine Dioxide in 43 cc. of Solution at Initial pH 0.92	109
XIX	Oxidations of Periodate Lignin with Unbuffered Aqueous Chlorine Dioxide at 25 to 28°C.	122

Ph.D. Chemistry

Norman Levitin

OXIDATIONS OF PERIODATE LIGNIN WITH SODIUM CHLORITE AND CHLORINE DIOXIDE

Abstract

The action of chlorine dioxide and of sodium chlorite, two new bleaching agents used to remove dark coloured lignin impurities from wood pulps, was studied on a wood lignin that had been isolated without much chemical change. Both oxidants produced similar results, converting the insoluble, presumably non-phenolic lignin first into alkali-soluble phenolic substances. The latter were then oxidized to light coloured strongly acidic substances soluble in alcohol but insoluble in dilute acid, and the final product was a brown gum soluble in dilute acid. A detailed study of the intermediate product suggested that it contained 2 methoxyl and 3 or 4 carboxylic acid groups in a molecular weight of about 900, aldehyde and ketone, phenolic and aliphatic hydroxyl groups being absent.

GENERAL INTRODUCTION

The increasing use of sodium chlorite and of chlorine dioxide for delignifying wood, and for the commercial bleaching of wood pulps, makes it highly desirable to know as much as possible about the action of these oxidants on lignin. The Wood Chemistry Committee of the Technical Section, Canadian Pulp and Paper Association, expressed this view some years ago, and this Thesis is the latest of several devoted to the above objective.

Attempts by other workers to use wood itself as the source of lignin for the study encountered the difficulty that the initial oxidation products, which were labile substances of unknown nature and fairly high molecular weight, had to be separated by uncertain methods from the carbohydrate part of the wood. The present work made use of an isolated, carbohydrate-free periodate lignin which, as far as is known, retains all the properties of lignin in situ.

After noting the rates at which sodium chlorite and chlorine dioxide were consumed under various conditions of temperature and pH by the periodate lignin, the Thesis describes the isolation of three oxidation products called A, B and C. The one which appeared of intermediate complexity and which was prepared with chlorine dioxide was then studied in greater detail. The results suggest that neither the original periodate lignin nor the oxylignin B contained free phenolic groups, but

that the chlorine dioxide introduced carboxylic acid groups into the latter.

HISTORICAL INTRODUCTION

Isolated Lignins

One of the many difficulties encountered in the study of wood lignin is that with one or two exceptions the methods used for its isolation are so drastic that the isolated product could very well have been changed from the original material. A few brief references to standard methods of isolation are all that are required to emphasize this point. Klason (1) used 72% sulphuric acid to dissolve the cellulose fraction of wood, Willstatter and Zechmeister (2) 40 to 42% hydrochloric acid, Urban (3) a mixture of hydrochloric and phosphoric acids while hydrofluoric acid was used by Fredenhagen and Cadenbach (4). These strongly acidic conditions produced dark coloured products, whereas lignin *in situ* must be white or near white. Paloheimo (5) stated that using 70% sulphuric acid, a short treatment left unhydrolyzed carbohydrate material, whereas a long exposure to the acid caused the formation of resinified material or humus. Hilpert (6) went so far as to say that lignin did not actually exist in wood, but that it was merely a condensation product of carbohydrate produced during isolation. This argument has been defeated by a mass of evidence, but the formation of condensation products owing to harsh conditions of isolation, is a real danger. Also the carbon content of the lignin has been found to vary with the concentration of the acid used and with the reaction time. Under mild conditions the carbon content of spruce lignin isolated with sulphuric acid is around 63%; under more

severe conditions the carbon content increases to 65 or 66% while according to Wald, Ritchie and Purves (7) the value found by an indirect method was 68 ± 1%. The thesis by Read (8) reported that drastic oxidation of isolated spruce Klason and Willstatter lignins with alkaline permanganate yielded 3 to 4% of benzene tetra and penta carboxylic acids, whereas a similar oxidation of spruce wood gave only traces. As further evidence of change during the isolation, all the above products proved to be insoluble in a standard bisulphite cook, whereas the lignin in wood was definitely soluble under those conditions. Even the lignin isolated by Freudenberg (9)(10) using alternate extractions of wood with hot 1% sulphuric acid and with cuprammonium solution at room temperature, and that of Hibbert (11)(12) using alcoholic hydrogen chloride at 80 to 120°C. proved to be insoluble in bisulphite cooks.

In 1939 Brauns (13) isolated a lignin from spruce wood meal by the use of alcohol alone. This material retained its solubility in bisulphite cooks, and since no drastic conditions were used, could be identical with the lignin in wood. However, the yield was only 10% of theory, and Harris (14) pointed out that an alcohol extraction of freshly cut wood yielded none of Brauns' "native" lignin. Since Nord and his collaborators (15) increased the yield of what appeared to be "native" lignin to about 23% of theory by first rotting the wood meal with a cellulose destroying enzyme, it therefore now seems likely that this lignin was an artifact produced by incipient biochemical action.

Like the other isolated lignins mentioned, "native" lignin contained free phenolic groups.

Finally in 1947 Ritchie and Purves (16) isolated lignin by oxidizing the carbohydrates of wood at pH 4 and 20°C. with aqueous trisodium paraperiodate, the oxidized carbohydrates being removed at intervals by extractions with boiling water. "Periodate" lignin was a light brown coloured material which contained little or no holocellulose, dissolved normally in a standard bisulphite cook, retained much of the biological structure of the wood, and was isolated in about 95% yield. Since no harsher condition than boiling distilled water was used in its isolation, the chances of resinification were very slight. However, some oxidation probably occurred because the methoxyl content of the material ranged from 10 to 12% instead of the 16% calculated for lignin in spruce wood. Periodate lignin, unlike other isolated lignins, also resembled spruce lignin in situ in being insoluble in dilute alkali, thereby indicating that the phenolic groups always present in other isolated lignins, were substituted in the former material.

When wood is pulped by any of the technical pulping methods, which all employ acidic or alkaline systems under elevated temperature or pressure, a small amount of lignin almost always remains in the pulp. Such residual lignins must certainly be considered to have undergone drastic chemical change and to resemble lignins isolated by similar methods rather than the original lignin.

In 1922 Heuser and Samuelsen (17) first related the discoloration of wood pulps to the presence in them of residual lignin. The colour could be removed by boiling with water or treatment with acid or alkali and could also be extracted with solvents. However, oxidation in the air caused the colour to return and the action of an excess of a suitable oxidizing agent was necessary to bleach the pulp. Schwartz, McCarthy and Hibbert (18) claimed that phenolic materials in the pulp after exposure to air or oxidizing agents condensed to dark coloured quinones which caused the discoloration. These quinones could be removed by solvents, but the residual phenols still present would again be oxidized in the air, causing the colour to return. Thus, only by treating with a suitable bleaching agent could the phenols and the lignin from which the phenols were derived, be completely destroyed. The phenols, these workers claimed, were formed from the lignin during the pulping process.

Moerke (19) also thought that the lignin or its transformation products in pulps contained quinone-like substances which might be the cause of discoloration. Peill (20) noted that esterification or etherification could prevent the return of colour to bleached ligno-cellulosic materials. This process presumably prevented the formation of the coloured quinones by substituting the phenolic hydroxyl groups.

All these speculations support the view that residual lignin is primarily responsible for the discoloration in pulps although they do not exclude contributions from coloured extra-

neous components. Although the bleaching of ground wood with alkaline hydrogen peroxide fails to remove the lignin, the bleaching of pulps seems to involve the solubilization and removal of the residual lignin. This removal is accomplished by means of various oxidizing agents such as hypochlorites, chlorine, and more recently, sodium chlorite and chlorine dioxide.

Chlorine Dioxide and Sodium Chlorite as Bleaching and Delignifying Agents

Since Schmidt (21) first noted that chlorine dioxide did not attack the carbohydrate components of wood, the gas has been used to an increasing extent in the bleaching of pulps. The literature was reviewed briefly by Giertz (22).

Under acid conditions at 50 to 60°C. for 3 to 4 hours, chlorine dioxide bleached pulp readily with very little degrading effect on the cellulose. However, since most mill equipment was not designed to withstand the corrosive effect of acid solutions, alkaline conditions were much more suitable. Although chlorine dioxide reacts to form sodium chlorite and chlorate in alkali, this reaction is quite slow and the gas has the op-

$$2\ ClO_2 + 2\ NaOH \longrightarrow NaClO_2 + NaClO_3 + H_2O$$

portunity to react on the pulp before the chlorite is formed. In practice, to prepare the latter from the chlorine dioxide, such catalysts as silver, nickel, manganese, copper, mercury, iron, or their compounds, or active carbon are used (23).

Sodium chlorite alone in neutral or alkaline solution had no bleaching effect, but at pH 3.5 to 5.5 the effect was good. A temperature of 45°C. could be used, although for sulphate pulps 70 to 80°C. was necessary. Under these conditions chlorine dioxide was liberated and the general assumption was made that the latter was the reagent which had the greatest effect in bleaching. No chlorine, which in acid conditions would be destructive to cellulose, was liberated in this system.

$$4\ HClO_2 \longrightarrow H_2O + 2\ ClO_2 + HCl + HClO_3 \quad .$$

Sodium chlorite in the presence of sodium hypochlorite bleached satisfactorily in alkaline solution at pH 7 to 9 and there was much less degradation of the cellulose than when sodium hypochlorite was used alone. According to Taylor, White, and Vincent (24), hypochlorite alone had such a high oxidation potential that it oxidized the cellulose as well as the coloured bodies. In order to lower this oxidation potential, the solution would have to be made even more alkaline, but at the higher pH there was more danger of the cellulose being oxidized by the air. The chlorite, on the other hand, at a pH as low as 2, still had a lower oxidation potential than the hypochlorite in alkaline solution. By mixing the hypochlorite and chlorite at pH 8 to 9 the bleaching action which occurred was typical of the chlorite bleaching under acid conditions. The above workers claimed that the hypochlorite was consumed in reacting with the chlorite to form chlorine dioxide and that it was the latter which did the bleaching.

$$3\ NaClO_2 + 2\ HClO \longrightarrow 2\ NaCl + 2\ ClO_2 + NaClO_3 + H_2O$$

A mixture of chlorine and chlorine dioxide was used in bleaching (25) and found to give results of the same nature as the pure dioxide. Experiments conducted on kraft pulp at pH values of 6, 8, and 9 showed that the effect on the pulp varied only slightly at different pHs and temperatures. Thus, unlike treatments with hypochlorite itself, it was not necessary to control these factors very carefully. Also a high degree of brightness was attained without much loss in strength.

Since both chlorine dioxide and sodium chlorite are at present rather expensive, they are used only as final bleaching agents after hypochlorite treatments. They can bleach the pulp further without damaging it, whereas hypochlorite is quite destructive when most of the lignin has been removed.

About 30 years ago Schmidt and co-workers (21) made the first comprehensive study of the effect of chlorine dioxide on many substances. Using a saturated (8%) aqueous solution of the gas they found that the following were not affected in 24 hours: cellulose, oxycellulose, mannan, xylan, starch, glucose, fructose, xylose, and other sugars. Since these substances were resistant to the reagent, it was then tried on solvent-extracted wood, followed by a treatment with 2% aqueous sodium sulphite. This procedure gave them what they termed a "Skelettsubstanz", free of lignin but supposedly containing all the cellulose and hemicelluloses. A saturated (1.3 M) solution of chlorine di-

oxide in 50% acetic acid was also used to remove what Schmidt (26) termed "plant incrustations". In further work (28) it was found that polyhydric alcohols, carbohydrates, esters, aliphatic amino acids, and certain cyclic compounds were also stable to the reagent. However, aromatic compounds with amino or phenolic groups on the benzene nucleus were unstable and gave coloured products. Unsaturated and carbon-sulphur compounds also decomposed in chlorine dioxide solution, but with carboxyl or nitro groups on the unsaturated carbon atom, the substance was more stable.

Heuser and Merlau (27) compared the action of chlorine dioxide and chlorine on wood, and claimed that Schmidt (28) was not correct in assuming that his "Skelettsubstanz" contained all the carbohydrate portion. Heuser and Merlau dialysed the spent aqueous solution of the oxidant and found in the non-dialysable fraction approximately 2% of pentosan based on the weight of wood. Although they agreed with Schmidt that only a 0.2% solution of the chlorine dioxide was necessary to decompose the wood, they used 1.5% solutions for 48 hours prior to a treatment with a 2 to 3% solution of sodium sulphite. The use of alternate treatments of chlorine and alkali gave a smaller residue than that from chlorine dioxide. However, a smaller amount of hemicellulose was present in this residue and the yield of pure cellulose was almost the same.

Fuchs and Honsig (29) a few years later repeated much of Schmidt's work on wood and phenolic materials. They claimed

that the lignin portion of the wood was decomposed to some extent to carbon dioxide, oxalic acid, and other simple substances, and that such products accounted for the observed loss in carbon in the isolated lignin fractions. The latter, obtained from the chlorine dioxide solution after dialysis and evaporation of the liquid, had only 40% carbon as compared to 60% supposed to be present in lignin. The methoxyl content of these materials also dropped to values close to zero. When Willstatter lignin was treated with chlorine dioxide much of it was converted to water-soluble materials. The crude reaction mixture was evaporated to dryness and the residue extracted with hot absolute alcohol. Most of the product dissolved, leaving a 16% yield of a dirty white substance which appeared to be a polysaccharide. However, Fuchs and Honsig claimed that this residue was a polysaccharide of wood occluded in the lignin used, and not as Schmidt claimed, a "polysaccharide of lignin". They found that most phenols also decomposed to carbon dioxide, oxalic, maleic and even formic acids. Partially methylated phenols such as guaiacol, vanillin, pyrocatechol, vanillic acid, protocatechuic aldehyde and acid, were likewise found to be unstable to chlorine dioxide.

Finally, in 1931, Schmidt and co-workers (30) published a revised method of obtaining the "Skelettsubstanz" by treating the wood at room temperature with an aqueous solution of chlorine dioxide in pyridine at pH 6.8 for 17 to 28 days. The buffering action of the pyridine prevented hydrolysis and there was less

danger of loss of hemicelluloses from the residue. This method of obtaining what is now called the holocellulose portion of wood was used until Ritter and Kurth (31)(32) developed a new technique using repeated alternate treatments of moist extractive-free wood with chlorine and pyridine in alcohol. Van Beckum and Ritter (33) later used a 3% solution of ethanolamine in alcohol as the extractant. These methods of determining the percentage of holocellulose in wood are still in common use. By adding to this percentage the value obtained for Klason lignin, it is possible to account for about 100 ± 0.3% of the wood. This close concordance may be due merely to a compensation of errors, since some of the holocellulose was lost during the treatments, and on the other hand, some of the monoethanolamine remained in the product (34).

In 1942 Jayme (35) described a new method of isolating holocellulose using sodium chlorite. Thin sections of wood which were first extracted with methanol-benzene, were treated with 30 parts of sodium chlorite and 6 parts of acetic acid in an aqueous system at 60°C. for 12 hours. The residue was recovered on a filter, washed, and subjected to the sodium chlorite solution three more times. Approximately 2.8 to 3.5% of lignin still remained in the resulting holocellulose, and any attempt completely to delignify the material resulted in the destruction of some of the carbohydrate. This behaviour was also noted in some work done on the extraction of lignin from deciduous trees using chlorine dioxide and sodium chlorite (36). The holocellu-

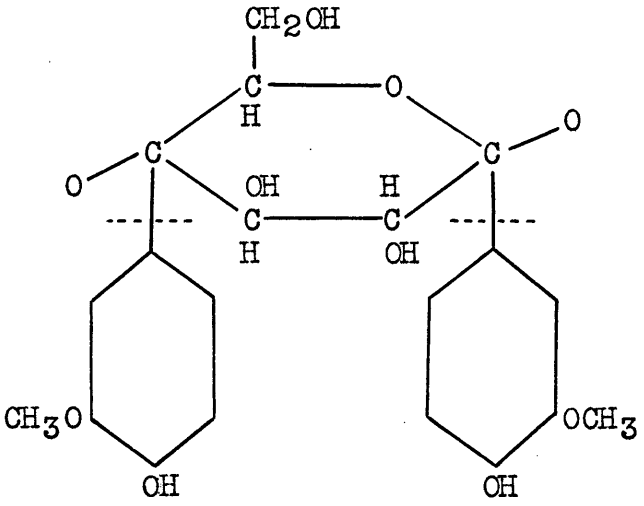
lose was quantitatively retained until the lignin content fell to 5%, but any attempt to remove the remainder resulted in a partial removal of pentosans and hexosans. Larinkari (36) accordingly thought that there might be a chemical linkage between lignin and hemicelluloses.

The following year (1943) Jayme and Hanke (37) continued the study of the action of sodium chlorite on spruce wood and concentrated on the material dissolving in the solution rather than on the residual holocellulose. Using an acetic acid solution of sodium chlorite at 60°C., they found that two 12-hour reaction periods, followed by a similar 8-hour treatment, gave them slightly more than the expected yield of holocellulose. The latter was calculated from the lignin content determined on the original wood. A lower holocellulose yield was obtained after a fourth 8-hour oxidation, indicating that some had dissolved in this step. To avoid contaminating the aqueous portion with any of this carbohydrate, they used only three treatments in subsequent work. A precipitate formed on acidification of the liquor from the first three stages, whereas no precipitate appeared from the fourth. Sohn and Reiff (38) had obtained a material in a similar way from a chlorite bleaching liquor and had assumed it to be a hemicellulose product. However, Jayme and Hanke, working with a solution from which they claimed the holocellulose was quantitatively removed, considered their precipitate to be a lignin degradation product. Based on the quantity of lignin in the wood, the yield

was only 8 to 12%, but purification of the liquor by dialysis, resulted in the isolation of up to 50% of the lignin as a similar product. The oxidized lignin was a green-to-yellow powder, contained 0.7 to 3.0% ash, was hygroscopic and soluble, when freshly isolated, in alcohol, acetone, dioxane, excess sulphuric acid and acetic acid. It dissolved readily in dilute alkali, and was reprecipitated by acid, but the yield of the resulting product was not given. After drying in vacuum at room temperature, the product remained soluble in alkali and sodium bicarbonate, but was no longer soluble in the organic solvents in which it had dissolved previously. It contained 8.6 to 9.8% methoxyl, 2.90 to 4.48% chlorine and probably some carboxyl groups. The carbon content varied from 46.2 to 52.7% and the hydrogen content from 4.75 to 5.53%. The product was evidently not homogeneous, but the authors assumed it was largely aromatic in nature. The fractions containing the higher carbon content also had higher methoxyl and chlorine values.

Hydrolysis of one of the isolated fractions with 3% nitric acid yielded a syrup which smelled of vanillin and gave positive colour reactions for vanillin but no attempt was made to isolate vanillin or any of its derivatives. Using 5% sulphuric acid as hydrolyzing agent, a residue of 38% remained insoluble. The filtrate with phenylhydrazine yielded the osazone of glucose in quantity corresponding to approximately 20% of glucose in the original fraction. These results induced Jayme and Hanke to postulate a formula for lignin composed of phenyl-

propane units linked together so as to form a hexose residue.



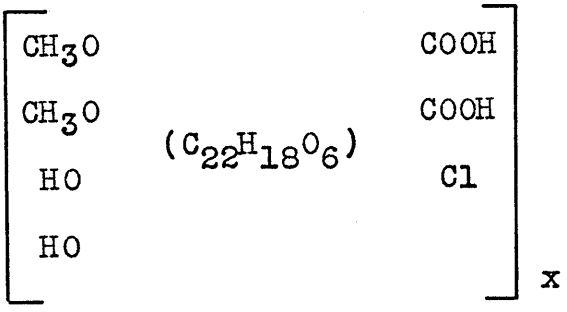
I

The production of a reducing sugar by the acid hydrolysis of such a structure seems, however, highly improbable because a cleavage of carbon-carbon bonds is required.

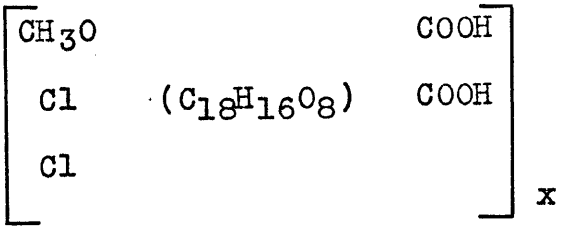
During the writing of this Thesis, there appeared a paper (39) on the reaction products of sodium chlorite and lignin. Extracted slash pine wood meal was treated for 4.5 hours at 70-80°C. with a sodium chlorite solution containing a small amount of acetic acid. After removal of the holocellulose portion, the clear filtrate was acidified with sulphuric acid and the resulting flocculent precipitate was collected. This chlorite lignin A was fractionated in dioxane and then precipitated again by ether. A second fraction, termed chlorite lignin B, was obtained when the acidified filtrate was concentrated and then acidified once more. These products were amorphous powders, white and light tan in colour respectively.

They were both soluble in acetone, methanol, butanol, pyridine, dioxane and dilute sodium bicarbonate and alkali solutions. They appeared to be homogeneous and were given the following formulae on the basis of elementary analyses and several methylation experiments.

Chlorite lignin A



Chlorite lignin B



Hydrolysis of the chlorite lignins gave a 92% recovery of the solid and the filtrates gave negative tests with Fehling's solution.

Jayme's (37)(35) method of isolating holocellulose from wood was later revised by Wise (40). Using less acetic acid, the pH of his solutions ranged from 3.2 to 3.9. Ten grams of unextracted air-dried wood meal was treated with 50 gm. of sodium chlorite in 500 cc. of water containing 50 cc. of acetic acid. The temperature was initially raised to 60°C. and the reaction was then allowed to proceed at 30°C. for 24 hours, after which time the lignin was almost completely re-

moved, and a nearly white residue of holocellulose remained in approximately quantitative yield. Wise, Murphy and D'Addieco (34) suggested using extractions with alcohol and ether before the chlorite treatment in order to remove extractives from resinous woods. Wood meal of approximately 60 mesh was found best for use, and short treatments at 70 to 80°C. Wise (40), Atchison (41) and Runkel (42) claimed that the removal of lignin in an acidified solution of sodium chlorite, was actually brought about by the chlorine dioxide liberated from the chlorite.

Lovell (43) prepared holocellulose from soft woods using a sodium chlorite solution buffered with acetic acid to a pH of 5 instead of the lower values used by other workers. Three stages of 6 to 7 hours at 60 to 70°C. were used, followed by a one-hour treatment at 70 to 74°C., and a thorough wash with hot water. The holocellulose was obtained in 92.5% yield using Ritter's method (31)(32) as the standard. This lower yield, according to Wise (34), was due to the removal of all the lignin, which took with it some of the polysaccharide portion. Runkel (42) further discussed the use of sodium chlorite for the preparation of wood pulp. After an alkali pretreatment, the wood was subjected to sodium chlorite in acetic acid solution at pH 4 to 5 and 60 to 70°C. Repeated treatments gave a pulp which was free of lignin and very little degraded.

Using Wise's method for extracting lignin from wood, Pearl (44) acidified the aqueous filtrate with dilute sulphuric acid and isolated some 6-chlorovanillin. This result was of

special interest since treatment of vanillin itself with sodium chlorite or chlorine dioxide yielded the 5-chlorovanillin (45) (46). Thus the lignin must have been chlorinated before it was degraded to vanillin. The fact that lignin halogenated in the 6 position was used as evidence that the para hydroxyl group, present in vanillin, was blocked by some substituent in lignin. Several workers (47)(48)(49) likewise found that when vanillin was directly chlorinated, the product was substituted in the 5 position, but with veratraldehyde (50) and acyl vanillin (49) (51)(52) halogenation occurred in the 6 position. When Lautsch and Piazolo (53) oxidized a brominated lignin in alkali with cobaltic hydroxide and obtained an 8% yield of 6-bromovanillin, they accordingly claimed that the units in lignin were united to each other by etherification at the 4 position. This argument was not decisive however, for Aulin Erdtman (54) compared the bromination of lignin with those of cresol and dihydroeugenol and showed that the formation of 6-bromovanillin did not necessarily indicate that the phenolic hydroxyl groups in lignin were etherified.

The Action of Chlorine Dioxide and Sodium Chlorite on Simple Compounds

The chemistry of chlorine dioxide and sodium chlorite was discussed in theses by Logan (46) and Husband (45) as well as in reviews by White, Taylor, Vincent and Cunningham (55)(56). In this work only reactions with compounds related in some way to wood chemistry will be reviewed. As previously mentioned, Schmidt and co-workers (21)(26) carried out extensive studies on

the effect of chlorine dioxide on many substances. Sarkar (57) continued this work, treating a great many compounds with unbuffered aqueous solutions of the gas and noting the rate at which they consumed the oxidant. All aromatic substances with free phenolic groups were readily attacked, but when the phenolic groups were protected by methylation or acylation, the resulting product was less readily affected by the chlorine dioxide. Benzene itself consumed no chlorine dioxide but toluene or xylene were slowly decomposed. On the other hand, carboxyl groups in the ring tended to stabilize the substances. Aliphatic hydroxyl groups on a side chain but adjacent to the benzene ring (e.g. benzyl alcohol) were oxidized supposedly to carboxylic acids. With such substituents as chloro, bromo or nitro groups in the ring, the phenols became more resistant. Small amounts of chlorolignin were found by Sarkar when lignin was treated with chlorine dioxide, and the chlorolignin was not susceptible to further action. No attention was paid to the pH of the solutions used, and no attempts were made to isolate the products.

Jeanes and Isbell (58) carefully studied the effect of sodium chlorite on carbohydrates. Glycosidic linkages such as in sucrose and α-d-glucopyranosides, were not affected in neutral or alkaline solutions which did not cause hydrolysis. Carboxylic groups on the carbohydrates were also found to be inert to the reagent. With a ketose such as fructose, only a slight decrease in reducing power was noted in 21 days, but aldoses were readily oxidized to the corresponding aldonic acids. At pH 4.2 to 4.9

about 31.5% of the glucose used was oxidized in 21 hours. This amount slowly decreased as the pH dropped, until at pH 2.0 to 2.1, 27.2% was oxidized in 23.5 hours. Even though the amount of oxidation produced by a given quantity of chlorite decreased slightly with decreasing pH, the actual rate of the reaction increased very markedly. At pH 2.1 most of the reaction occurred within the first few minutes, whereas at pH 4.2 the oxidation reaction continued over a period of several hours. The chlorine dioxide and chloric acid formed as by-products did not interfere in the reaction, the former reacting very slowly with aldoses and the latter being entirely inert. Thus the chlorous acid from the sodium chlorite appeared to be the oxidant which converted the aldoses to the aldonic acids. These workers finally presented the following equation as best fitting their results.

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

In neutral or alkaline solution there was no reaction for some time. However, after several days, chlorine dioxide was liberated and the oxidation slowly began. As it proceeded, the solution became more acidic and the rate of oxidation increased.

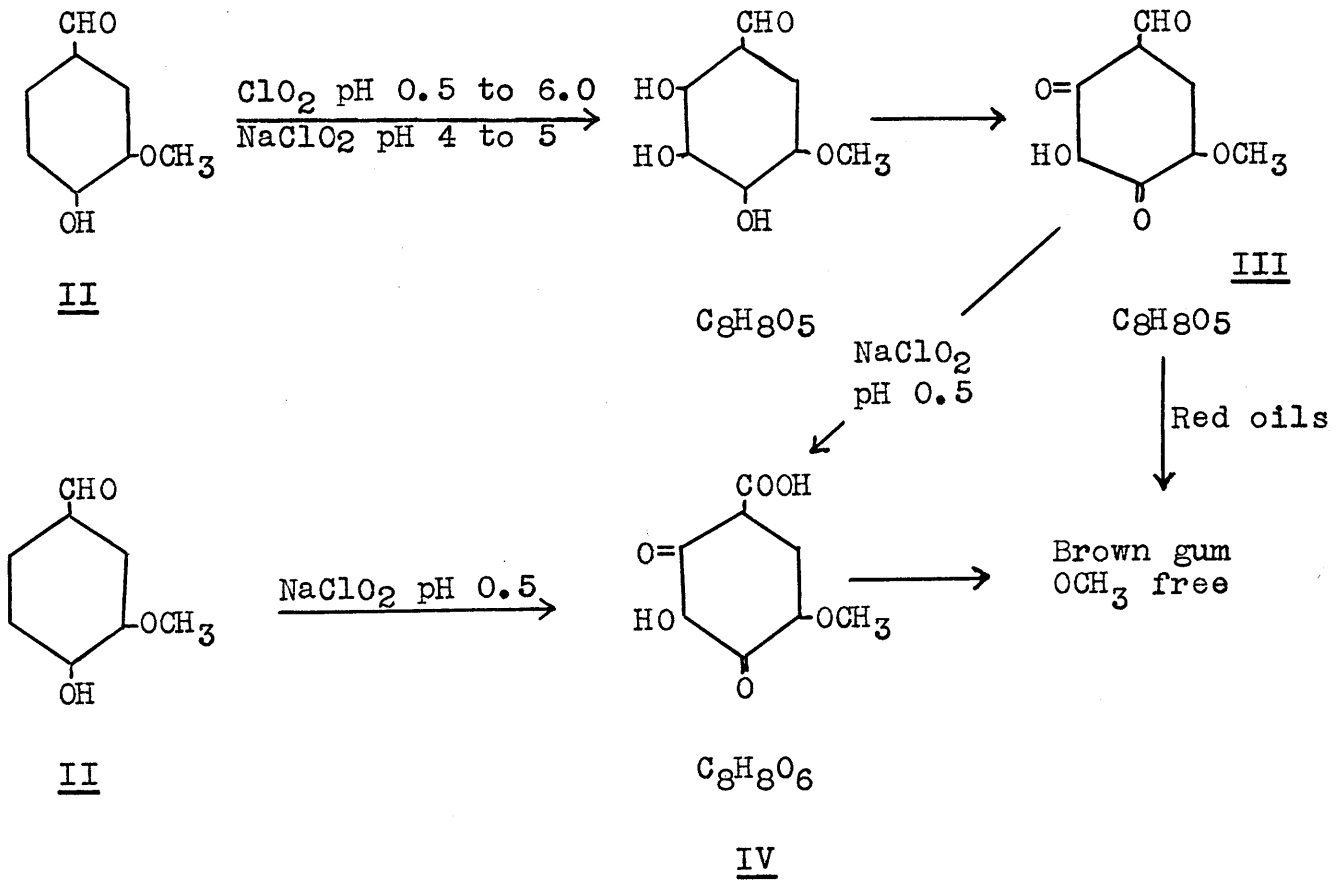
A change in pH was noted even in a blank solution of sodium chlorite. For example, within one hour a solution made up to pH 2.2 rose to pH 2.8 and then proceeded to decrease to pH 2.3. The initial decrease in acidity, the authors claimed, was due to the liberation of chlorine dioxide and the subsequent

increase was caused by the slow hydrolysis of the dioxide to hydrochloric and chloric acids. In the presence of oxidizable material a much greater release of chlorine dioxide occurred and the pH rose more rapidly. Within 10 minutes, in the presence of sucrose, the pH rose to 3.4 and then, after 7 days, dropped as low as 1.6. The lower pH eventually attained was probably caused by the greater amount of hydrochloric acid liberated, as well as by the acidic products formed. The liberation of larger amounts of chlorine dioxide in the presence of reducing agents was also noted by others (55)(56). For example, in weakly acid or even neutral solutions of sodium chlorite, the release of the dioxide was much greater when formaldehyde was present.

Logan (46) and Husband (45) carefully studied the effects of sodium chlorite and chlorine dioxide on simple phenols related to lignin. Chlorine dioxide was rapidly consumed by vanillin over the pH range of 1 to 6, giving an aldehydic compound with the formula $C_8H_8O_5$. At the lower pH levels, the consumption of the dioxide in excess of one mole per mole of vanillin was rather slow, but at pH 5.8 the uptake as far as 2.7 moles was fairly rapid. At pH 6.5 or more, the chlorine dioxide decomposed to chlorite and chlorate and became inactive to the vanillin.

The action of sodium chlorite, on the other hand, varied considerably as the pH and temperature was changed. For example, at pH 4.5, 0.5 mole was consumed at once, and 4 moles within 5 hours, while at pH 5 only 1.7 moles were used in 8 hours. A 20 to 35% yield of an acidic compound $C_8H_8O_6$, which proved to be

the acid of the aldehyde $C_8H_8O_5$ produced by chlorine dioxide, was obtained at pH 0.5. As the pH was raised, the oxidizing power of the chlorite decreased and at pH 4 to 5 only the above aldehyde was formed, while at pH 1 a mixture of the acid and aldehyde appeared. At pH 5 a very slow chlorination resulted and a 20% yield of 5-chlorovanillin was obtained. As the pH approached 6 the sodium chlorite became inactive towards vanillin. This loss in the oxidizing power of chlorite as the pH was increased to the region of 6 was also noticed with pyrogallol. The following equations illustrate the reactions of both oxidants with vanillin.



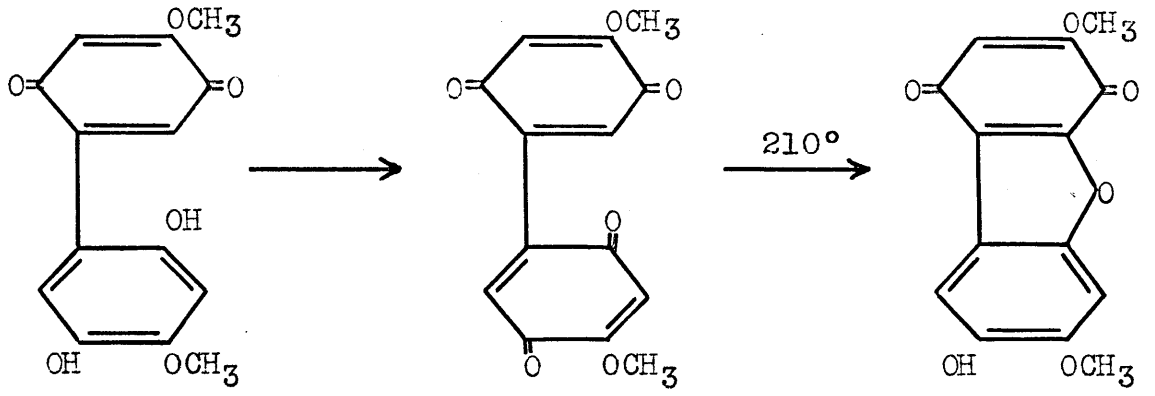
No trace of the acid $C_8H_8O_6$ was ever noted in a chlorine dioxide oxidation of the aldehyde, but the latter could be transformed quantitatively to the acid by treatment with chlorite at pH 0.5. This agreed with the observations of Jeanes and Isbell (58) on the ready oxidation of aldehydes by chlorite. The white crystalline aldehyde and acid derived from vanillin, rapidly gave red oils and methoxyl-free gums when dissolved in very dilute acids and bases, and the same obscure reactions occurred more slowly in aqueous solution. In the chlorite oxidation of the vanillin no liberation of chlorine dioxide was noted until 2 moles of the chlorite was consumed.

In the pH range from 1 to 6 a chlorine dioxide oxidation of methoxyhydroquinone proceeded rather slowly, but finally, in 8 hours, gave a quantitative yield of methoxyquinone. This observation enabled Logan to establish the following equation.

$$3 \text{ moles methoxyhydroquinone} + 3\ ClO_2 \longrightarrow 2\ HCl + HClO_3 + 3 \text{ methoxyquinone} + 3\ O + 3\ H$$

The effective oxidizing equivalent of chlorine dioxide in this case was thus found to be 2 instead of 5 as generally assumed by analogy from the results of iodometric titrations. Although the chlorine balance found by Logan was complete, the incomplete redox balance suggested that hydrogen peroxide was formed as a by-product in the oxidation of the hydroquinone.

Using sodium chlorite on methoxyhydroquinone in the pH range of 2.2 to 5, coloured condensation products of the following type appeared.



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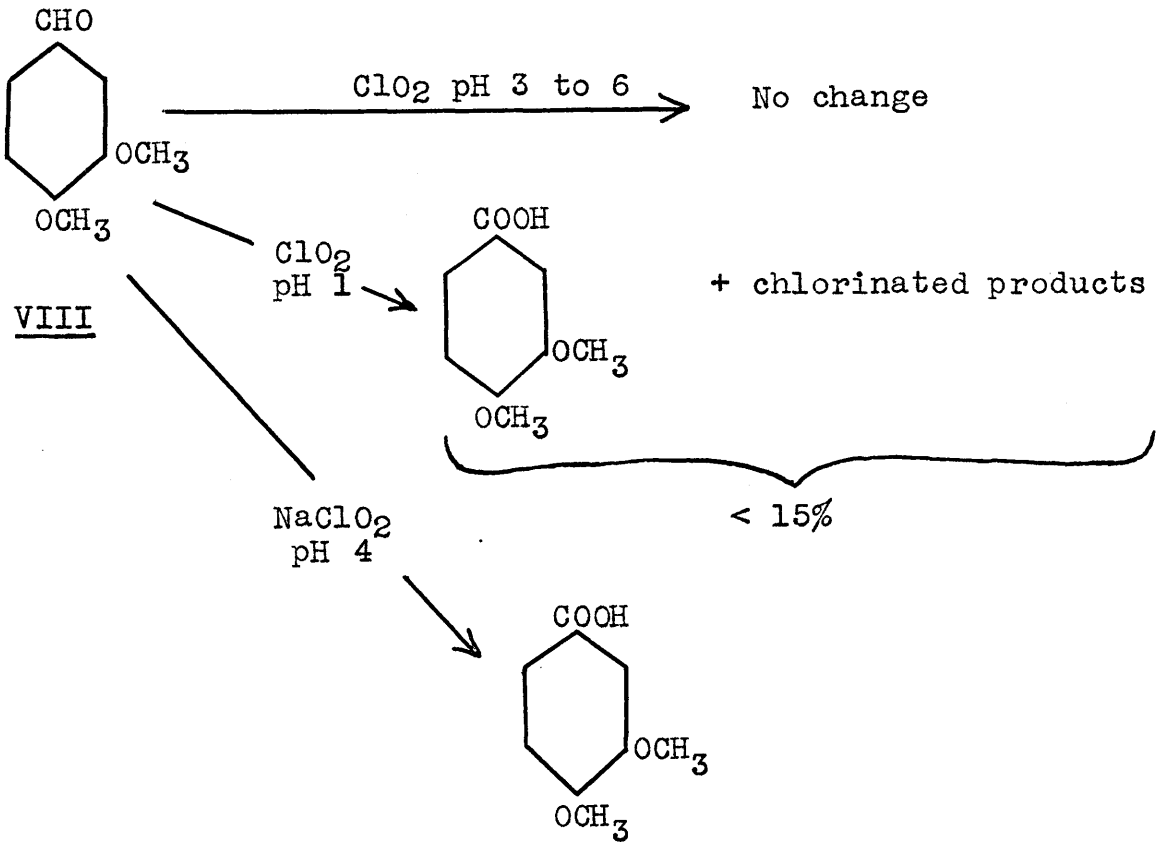
As the pH of the reaction was lowered to 1, the oxidation potential became high enough to transform the dimethoxyquinhydrone (V) to the methoxyquinone (VI) in 91% yield. This change occurred so rapidly that the quinhydrone intermediate did not appear. When heated to its melting point in the neighbourhood of 210°C., the yellow crystals, (VI), suffered further condensation to the red crystalline substance (VII).

The action of chlorine dioxide on veratraldehyde was very slow, and at pH 1 Logan was able to isolate only 15% of a mixture of veratric, 5,6-dichloroveratric and other chlorinated acids. The remaining solution appeared to contain quinoidal materials. In the pH range of 3 to 6, the veratraldehyde appeared to be completely stable to the dioxide. This circumstance enabled Logan to study the action of sodium chlorite on veratraldehyde without any interference from the chlorine dioxide formed as by-product. At pH 4 the reaction proceeded

best, giving a 90% yield of veratric acid. On analyzing the resulting products, the equation of Jeanes and Isbell (58) was verified.

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



The action of chlorine dioxide on acetylvanillin was in many ways anomalous. In the pH range between 2.3 and 4.5 there was no reaction, whereas at pH 6.00 to 6.25 a 25 to 35%

yield of acetylvanillic acid was produced. The fact that chlorine dioxide oxidized an aldehyde to an acid at a pH as high as 6.25 was not at all in agreement with previous experience. At pH 1, in contrast to the slow reaction of chlorine dioxide with veratraldehyde, an almost instantaneous reaction occurred with acetylvanillin. However, as with veratraldehyde, small yields of chlorinated acids resulted, 6-chlorovanillic acid and 6-chlorovanillin being isolated in a total yield of 17%. Since normally vanillin is chlorinated in the 5 position, the isolation of derivatives substituted in the 6 position with chlorine suggested that hydrolysis of the acetyl group took place after the oxidation and chlorination.

With sodium chlorite the reaction proceeded as expected. The consumption of oxidant was slow and a good yield (78%) of acetylvanillic acid was isolated at pH 4.2. The sodium chlorite as a rule seemed to be specific for converting aldehyde to acid groups, but there were exceptional cases in which chlorine dioxide accomplished this oxidation incompletely and slowly.

DISCUSSION

The periodate lignin used in this investigation was prepared from logs of black spruce by the method of Ritchie and Purves (16). In one preparation, the wood meal was ground to approximately 40 mesh in a Wiley mill, was solvent-extracted, and then oxidized with aqueous sodium paraperiodate. After five successive oxidations a fine light brown powder (lignin A) remained in 25% yield based on the air-dry weight of the wood meal, and analyzing 88.6% Klason lignin, 11.4% methoxyl and 2.3% ash. Values of 92 to 97% Klason lignin and 12% methoxyl were reported by Ritchie and Purves (16) but Ritchie (59) also quoted 86.0% to 95.8% for Klason lignin and methoxyls of 10.8% to 12.0%. In all of Ritchie's preparations the holocellulose content was close to zero.

The methods of Kurth and Ritter (31)(32) and Van Beckum and Ritter (33) were both used to determine the amount of holocellulose left in the lignin. The former method, employing pyridine-ethanol and considered to be quite reliable, proved to be very slow and tedious. Eleven successive chlorinations and extractions finally gave a holocellulose content of 4.1%. Further treatments lowered this value slightly but at the same time decomposition of the residual holocellulose appeared to take place. The second method, although now adopted as standard by TAPPI, was criticized by Wise (34) on the ground that the holocellulose retained small amounts of the ethanol-

amine used to extract the chlorinated lignin debris. On the other hand, this solvent dissolved carbohydrate to a small extent, thus balancing the retention of ethanolamine. Chlorinated lignin dissolved in the latter more readily than in the pyridine-alcohol solution. From all these determinations it was established that the holocellulose content was 4.1% or lower, the lower values being approximately 2.5%.

The fact that the Klason lignin content added to the maximum holocellulose content did not add to 100% of the periodate lignin was not of great importance since the lignin determination using 72% sulphuric acid, is rather empirical. Ritchie (59) found, as noted previously, Klason lignin contents ranging from 86.0% to 95.8% and yet in every case his holocellulose content was reported as being close to zero.

Only 9% of the periodate lignin remained insoluble in a standard calcium bisulphite cook, thereby confirming the observation of Ritchie and Purves (16).

Instead of using wood meal finely ground in the Wiley mill, a preparation of periodate lignin was attempted using the chips approximately 1 mm. by 5 mm. in size from a Mead mill (preparation B). This procedure eliminated the time necessary for drying the chips before the Wiley mill could handle them and the latter process was quite slow even when the wood was dry. After the usual five oxidations with sodium periodate, the holocellulose content was high, but a sixth treatment yielded a

product similar in analysis to the periodate lignin (A) prepared from wood meal. The lignin retained the morphological form of the original chips. The fact that the use of coarsely ground wood made necessary at least one more tedious oxidation more than offset the saving in effort accomplished by not grinding the wood more finely.

Preliminary Oxidations with Sodium Chlorite

In order to determine the best conditions for oxidizing the periodate lignin with aqueous sodium chlorite, many experiments were undertaken using 0.5- or 1-gm. samples. To follow the course of the reaction, the residual oxidant in aliquots of the solution was permitted to liberate iodine from an acidified potassium iodide solution and the iodine was titrated by standard sodium thiosulphate. This estimation included not only sodium chlorite, but also the chlorine dioxide formed as a by-product during the reaction. Any quinones formed by the oxidation of phenolic groupings in the lignin would also liberate iodine from acidified potassium iodide. However, as will be shown later, there were indications that quinoidal substances were not present in these reactions.

For the purpose of calculation, the residual oxidant was assumed to be entirely sodium chlorite, even though the presence of unknown and changing amounts of chlorine dioxide was realized. However, the error in calculating the total remaining oxidant was not great, since one mole of sodium chlorite released 4 equivalents of iodine, and the figure for chlorine di-

oxide was 5 equivalents. Since the amount of chlorite was in excess, the use of the value 4 gave approximately correct answers. It seemed safe to assume that the figures for the consumption of oxidant were at least comparable and showed the trend of the reaction under varying conditions of time, pH and temperature.

Since the aqueous sodium chlorite gradually decomposed at a rate dependent on the temperature and pH, each estimation was accompanied by a parallel blank titration, and the difference in residual oxidant was calculated as the amount used by the periodate lignin. Here again the calculations were somewhat in error because the amount of chlorine dioxide liberated in the blank run was small compared to the amount noted in the sample. In the latter, the green-yellow colour and distinctive odour of the dioxide were noted within 5 minutes of the start of the oxidation. Thus, as observed by others (56)(58) chlorine dioxide was formed from sodium chlorite in the presence of an oxidizable material, in this case the lignin. The reaction was probably as shown in the following equation, using an aldehyde as an example.

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

Chlorine dioxide was also known to oxidize periodate lignin actively.

A method used by Husband (45) was also tried in the hope of following the course of the reaction without the possibility of any interference from quinones. Husband determined

the amount of chlorite left in his solutions by the amount of sulphate they formed in a bisulphite solution. According to Jackson and Parsons (60) this oxidation occurred quantitatively.

$$HClO_2 + 2\ NaHSO_3 \longrightarrow HCl + 2\ NaHSO_4 \quad .$$

The bisulphite on the other hand would not be oxidized by quinones, but would form soluble complexes (61) which it was hoped would not interfere with the sulphate determination. After a few brief attempts which were unsuccessful, the method was abandoned since the danger of quinone interference was small and the rapid iodometric method appeared to be reasonably satisfactory for comparative purposes.

The absence of quinone formation was indicated by the lack of the colour changes which usually occur when quinones are exposed to air for any period of time. Throughout the whole reaction the colour remained light yellow and even after several weeks' exposure to air or heat there was no change. If no excess of sodium chlorite was used, it was possible to continue the oxidation until no oxidizing agent remained. Any interfering quinone would have liberated iodine from an acidified solution of potassium iodide, but in these cases the thiosulphate titration was close to zero. With chlorine dioxide oxidations, the excess oxidizing agent could be removed after any specified time by bubbling nitrogen through the reaction mixture. The resulting solution was almost colourless and liberated no iodine from acidified potassium iodide, again indicating no quinones.

Several small-scale oxidations were started at pH 4.0 to 4.1 with disodium phosphate and citric acid as buffer. However even when using six times the recommended strength (62), the pH of the mixtures rose to 5.1 in 22 hours. At this point all of the lignin had dissolved and then the solution slowly became more acidic, until after 82 hours the pH was 4.4. In the blank a maximum pH of 5.0 was reached in 22 hours, but thereafter this value remained constant. Jeanes and Isbell (58) suggested that the initial rise in pH was caused by the conversion of chlorous acid to neutral chlorine dioxide. This conversion was slow in the blank, but rapid in the solution containing the lignin. Then, after a time, the chlorine dioxide hydrolyzed, liberating hydrochloric acid. In the blank an equilibrium was reached keeping the pH constant. With the sample, hydrochloric acid was also liberated by the reaction of the chlorite on the lignin, and the soluble product formed from the lignin was found to be highly acidic. These two factors caused the sample solution eventually to become much more acidic than the blank.

In Figure 1, plot 1, is shown the consumption of oxidant when 1 gm. of periodate lignin was treated with 0.025 mole of sodium chlorite in 45 cc. of solution at 30°C. and starting at pH 4.1. All the solid dissolved in 12 hours and by 18 hours the uptake of oxidant appeared to come almost to a halt. Plot 2, representing the moles of oxidant left in the blank, showed that most of it was still present at the 18-hour stage, but from plot 3, it can be seen that very little remained in the sample. Under

these conditions the blank might have eventually decomposed more rapidly than the other solution, causing an apparent levelling of the consumption plot before it actually should have occurred. The fact that the oxidant was still being consumed after 12 hours, when all the solid had dissolved, was of considerable interest because it showed that the soluble product was not stable to sodium chlorite.

At 23 to 25°C. the lignin took longer to dissolve, a small amount still being present after 22 hours, but the curves were practically identical with those at 30°C. and are therefore not shown in Fig. 1 (but see Table VI). Even after 59 hours at 20°C., 38% of the solid remained undissolved, although it had changed to a faintly yellow powder, unlike the original brown material. Plots 4, 5 and 6 show that the consumption of oxidant and the decomposition of blank and sample all occurred at a slower rate than at 30°C.

The oxidation was very slow at 8 to 10°C. and after 96 hours 25% of the original material was present as a light yellow solid. The blank decomposed very slowly as seen in plot 8 and the consumption rose steadily up to 96 hours. It was only after 48 hours that the solid appeared to change in appearance, having retained its original brown colour until that time. At the end of the 96 hours, approximately the same amount of chlorite (0.019 mole) was consumed (plot 7) as in 30 hours at 25 or 30°C. (plot 1). The reaction at 20°C. was discontinued after 0.017 mole had been consumed (plot 4).

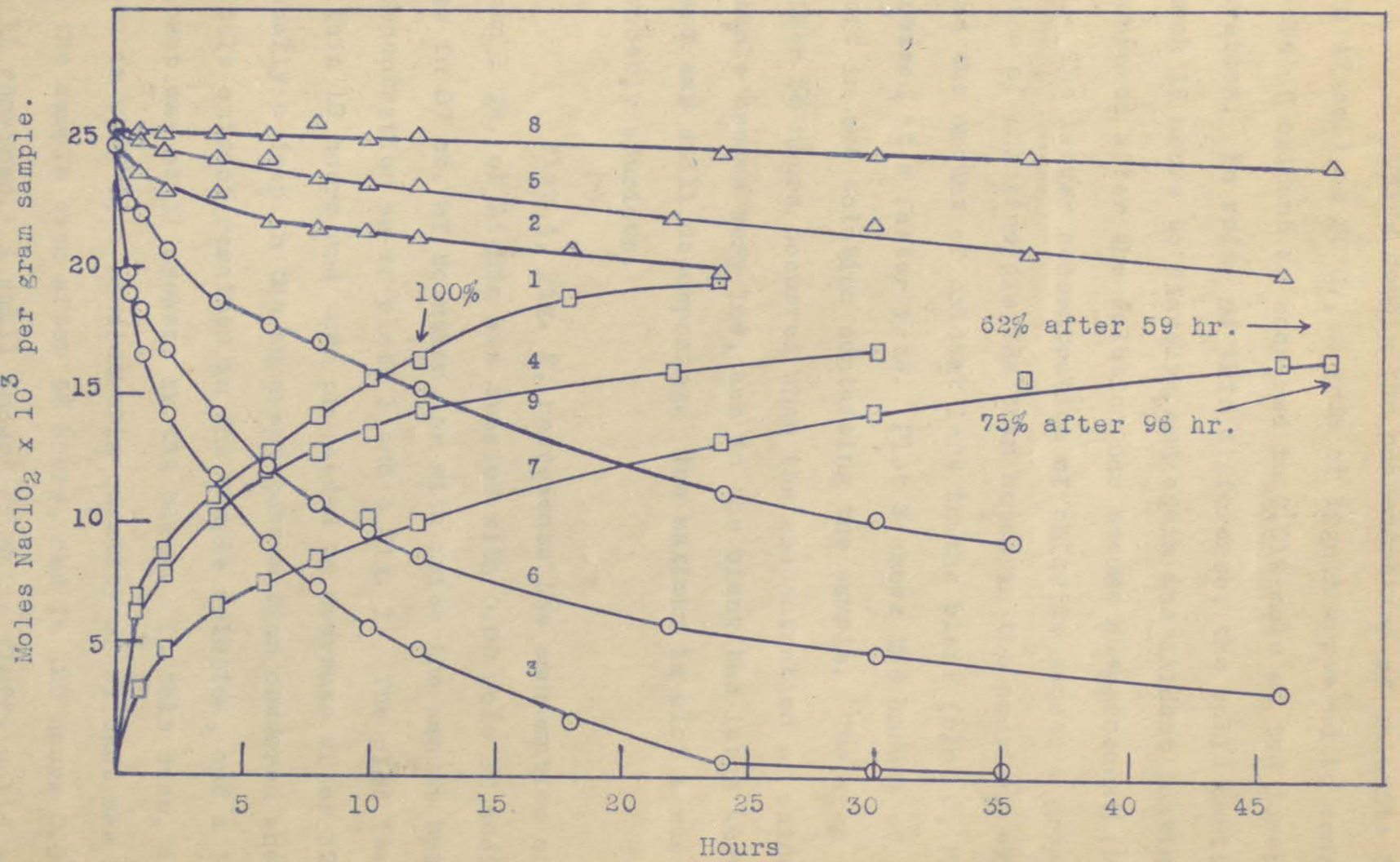


Fig. 1. Oxidation of 1 gm. of periodate lignin by 0.025 mole of sodium chlorite in 45 cc. at initial pH 4.1. Plots 3, 6, 9, residual oxidant at 30°, 20°, and 10°C; plots 2, 5, 8, blanks; plots 1, 4, 7, consumption of oxidant at the same temperatures. See Tables V, VII, and VIII.

Using double the concentration of chlorite (0.05 mole in 47 cc.) at 30°C., a gram of lignin appeared to consume 0.028 mole of oxidant as compared to 0.019 mole at the lower concentration. In spite of this difference, the solid particles still took 16 hours to dissolve, and again the oxidant continued to be consumed after the solution had become homogeneous. (plot 1, Fig. 2). The larger concentration of chlorite caused a greater evolution of chlorine dioxide than noted in the previous experiments, and the amount of oxidant left in the blank (plot 2, Fig. 2) decreased at a faster rate. Plot 3 shows the amount of oxidant left in the solution containing the sample. The drop in plot 1 after 36 hours occurred when the concentration of oxidant in the sample became very low, and in the blank the large amount of oxidant was still decomposing. The maximum in plot 1 was therefore probably spurious.

Plot 4, Fig. 2, represents the consumption of oxidant when 1 gm. of lignin was treated with 0.05 mole of sodium chlorite in 87 cc. of solution, or with twice the amount but the same concentration as in plots 1 and 4 Fig. 1. The plot levelled off within 12 hours and then proceeded to decrease after 32 hours. Usually a drop in the apparent consumption occurred when very little oxidant remained in the sample solution, and a large amount was still present in the blank. In this case, as can be seen in plot 6, a considerable quantity of oxidant was present in the sample even after 48 hours, and in 120 hours 0.0067 mole still remained. A small amount of white fluffy solid still remained after 32 hours but by 48 hours all the lignin had gone

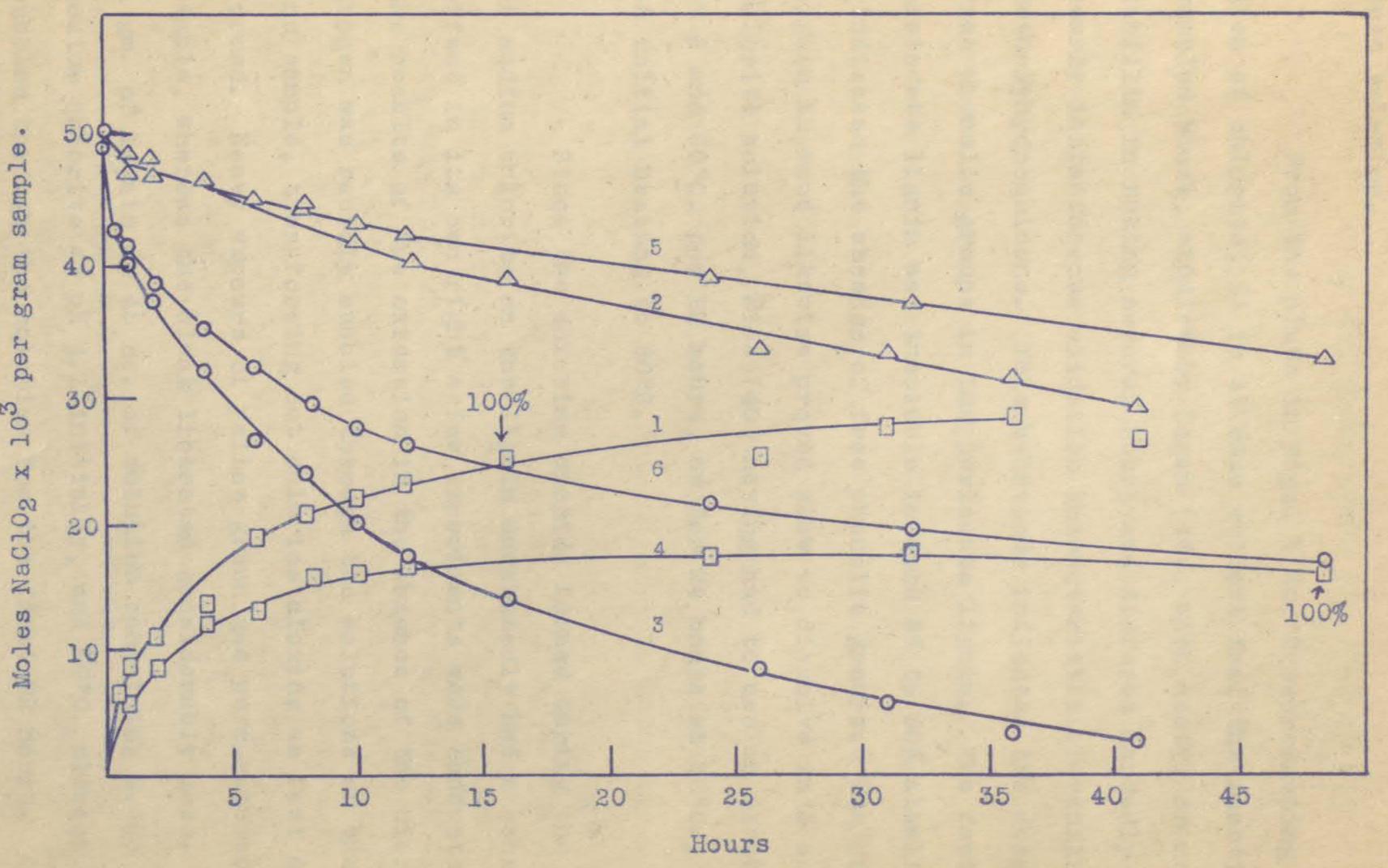


Fig. 2. Oxidation of 1 gm. of periodate lignin by 0.050 mole of sodium chlorite at initial pH 4.1 and 30°C. Plots 3 and 6 residual oxidant in 47 cc. and 87 cc. respectively; plots 2 and 5 blanks; plots 1 and 4 consumption of oxidant in these dilutions. See Tables IX and X.

into solution.

From the plots in Figs. 1 and 2 representing consumption of chlorite, it is at once evident that the reactions resembled those, studied by Logan (46), with acetyl or methyl vanillin in taking several hours and differed markedly from the nearly instantaneous oxidation characteristic of vanillin or methoxyhydroquinone. This behaviour indicated the absence of free phenolic groups in the periodate lignin. The fact that periodate lignin was insoluble in cold 5% to 50% alkali also indicated the absence of free phenolic groups. The original lignin in wood likewise proved slow to dissolve in a sodium chlorite solution, Wise (40) having had to use an excess at pH 3 and 60°C. for 12 hours, or for 24 hours at 30°C., after an initial heating to 60°C.

Since the chlorine dioxide formed during the reaction of sodium chlorite on the lignin undoubtedly had a considerable effect in its own right a few experiments were conducted to note the results of the oxidation in the absence of the dioxide. Nitrogen was rapidly bubbled through the solutions of the blank and sample, thus forcing out chlorine dioxide as fast as it formed. Heavy vapours of yellow-green gas were removed from the sample, whereas the blank liberated considerably less. Using 1 gm. of lignin in 45 cc. of solution containing 0.024 mole of sodium chlorite at pH 4.1 initially, and 30°C., nitrogen was bubbled through the solution for the first 22 hours. As seen from Fig. 3 plot 1, there was little or no change in the rate

of decomposition of the blank after the flow of nitrogen was discontinued. With the sample (plot 2) after 22 hours there was a decided change, and the oxidant disappeared at a rapid rate. The apparent consumption (plot 3) remained constant after 10 hours but rose rapidly when the chlorine dioxide was allowed to remain in the solution. The blank solution remained colourless as long as the chlorine dioxide was being removed, but turned yellow when the gas was allowed to remain. On the other hand, the solution containing the sample and also the undissolved solid became slightly yellow within one hour and only turned darker in colour after the 22-hour period. Thus, a little of the lignin dissolved rapidly even in the absence of chlorine dioxide. The initial fast reaction dissolved a small amount of the lignin and bleached the remainder but it was only after the chlorine dioxide was allowed to accumulate that the solid began to dissolve extensively. After 96 hours, however, 25% still remained insoluble, but by this time all the oxidant had been consumed. These observations indicated that the chlorine dioxide was the principal active agent in a sodium chlorite oxidation of lignin and therefore agreed with the current views on the bleaching of pulps with these reagents. Sodium chlorite alone evidently reacted sufficiently with the lignin, to decolourize it, but for a more extensive action the accompanying dioxide was necessary. This may be a chemical effect, or, on the other hand, it may be merely a physical one, the dioxide being better able to penetrate the insoluble lignin.

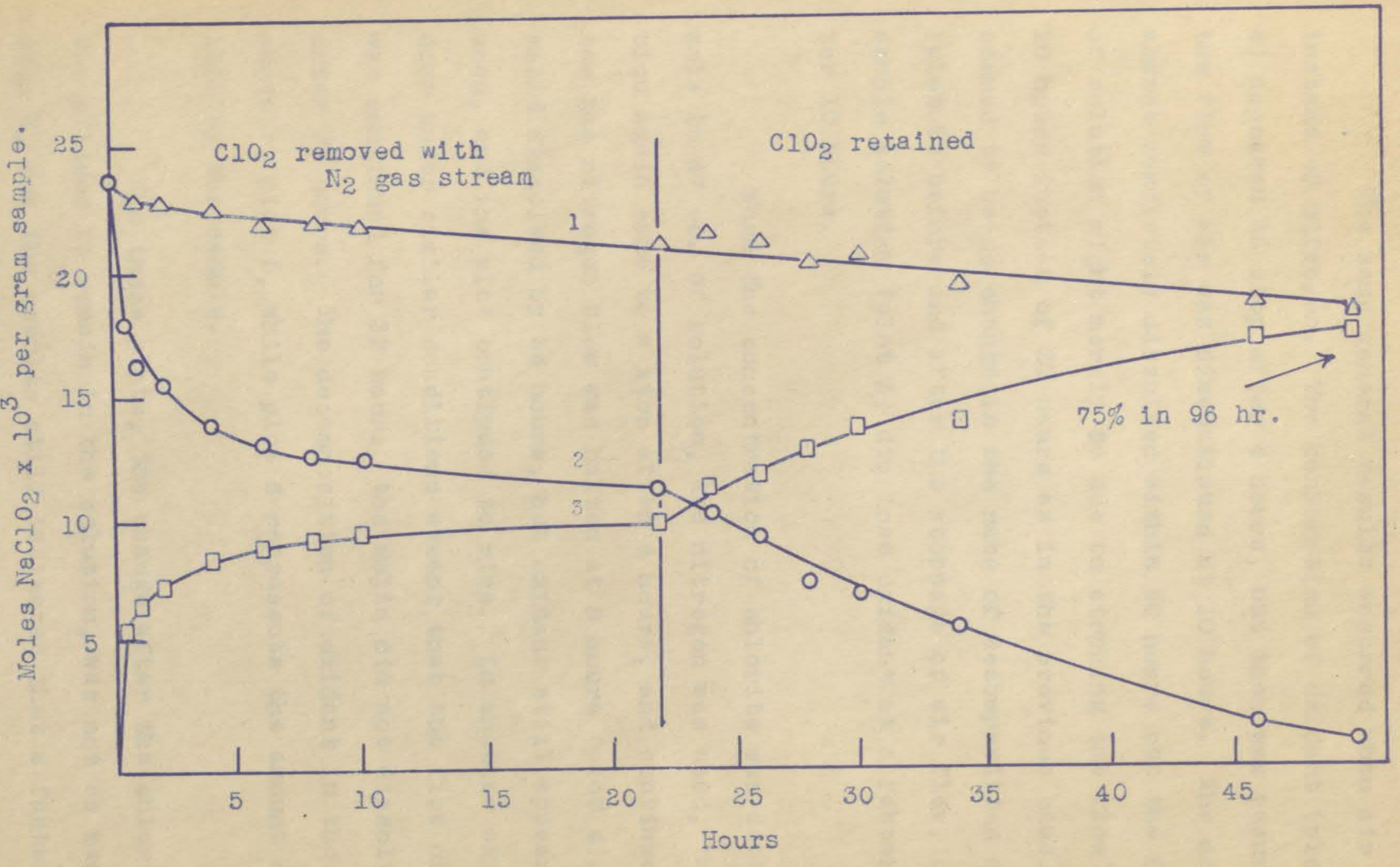


Fig. 3. Oxidation of 1 gm. of periodate lignin by 0.024 mole of sodium chlorite in 45 cc. at initial pH 4.1 and 30°C, the chlorine dioxide being removed with nitrogen for the first 22 hours. Plot 1 blank; plot 2 residual oxidant; plot 3 consumption of oxidant. See Table XII.

The same general results occurred when air was used instead of nitrogen. The consumption of oxidant (plot 1, Fig. 4) appeared to stop after 4 hours, but then was resumed when the flow of air was discontinued at 10 hours. The solid was almost completely dissolved within 50 hours but the faster rate of solution might merely be due to stopping the flow of gas at 10 hours instead of 22 hours as in the previous case. There seemed to be no change in the rate of decomposition of the blank (plot 2) before and after the stoppage of air flow, but the sample solution (plot 3) did lose oxidant at a faster rate after 10 hours.

When the concentration of chlorite was doubled, 0.05 mole in 47 cc. of solution, and nitrogen was used, the consumption again came to a stop after 4 hours, and continued only after the nitrogen flow was halted at 8 hours (plot 4). The solid dissolved by 34 hours, but oxidant still appeared to be used, and the plot continued to rise. In another experiment done under similar conditions except that the flow of nitrogen was continued for 32 hours, the solid did not dissolve until after 70 hours. The decomposition of oxidant in the blank is shown by plot 5, while plot 6 represents the amount of oxidant left by the sample.

In these plots, the change after the chlorine dioxide was allowed to remain in the solution, was not as marked as in Fig. 3, but the results still indicated that a faster consumption of oxidant occurred when chlorine dioxide was present.

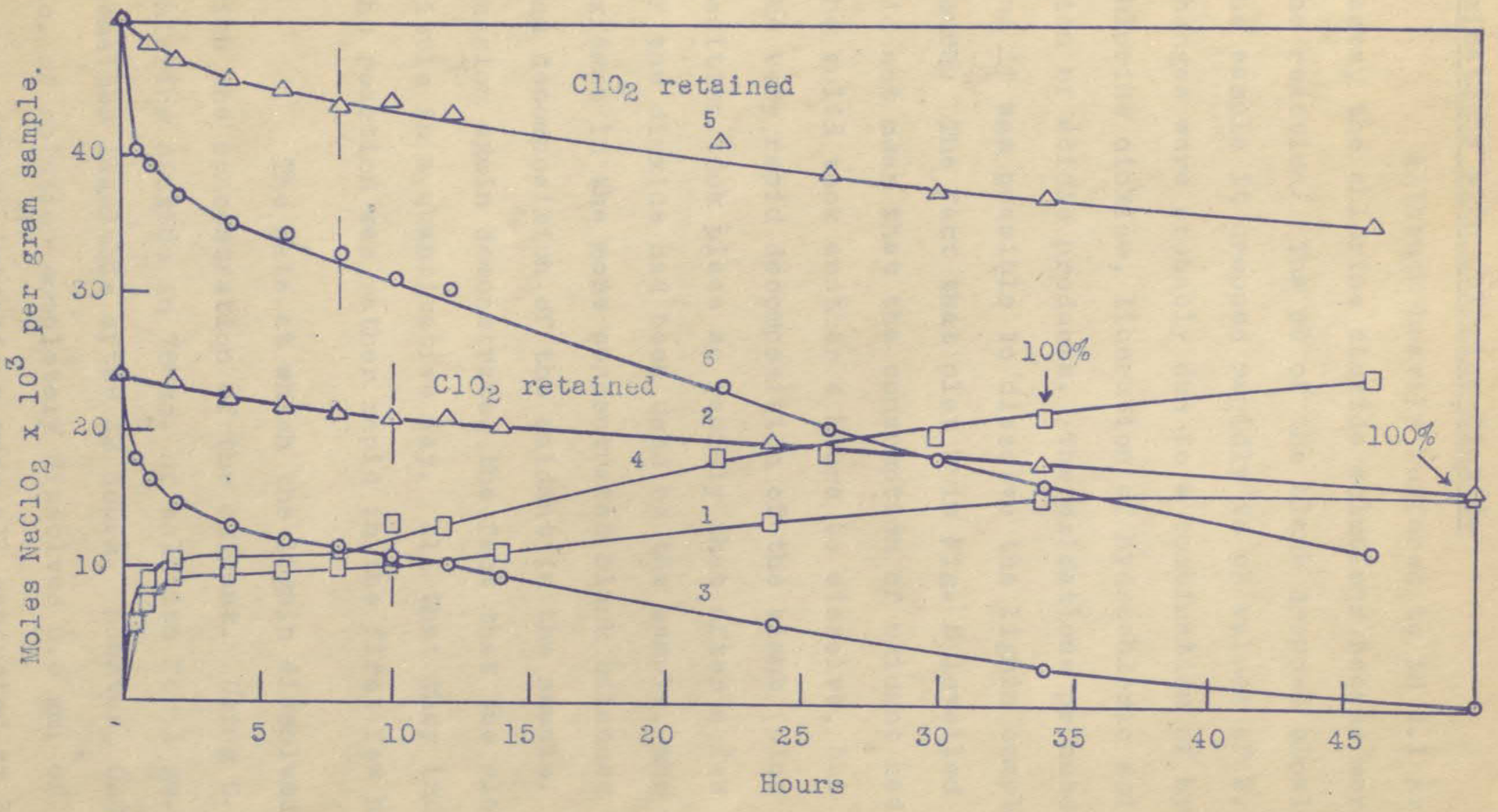


Fig. 4. Oxidation of 1 gm. of periodate lignin with sodium chlorite at initial pH 4.1 and 30°C. Plot 1 consumption of oxidant; plot 2 blank; plot 3 residual oxidant when using 0.024 mole in 45 cc, the chlorine dioxide being removed with compressed air for 10 hours. See Table XIII. Plot 4 consumption of oxidant; plot 5 blank; plot 6 residual oxidant when using 0.050 mole in 47 cc, the chlorine dioxide being removed with nitrogen for 8 hours. See Table XV.

Oxidations with Chlorine Dioxide

Although heavily buffered to pH 4.1 as in previous cases, the chlorine dioxide solutions became more acidic during the reaction. The pH of the blank dropped slowly, whereas in the sample it dropped rapidly to pH values of 2.8 to 3.5. These changes were probably due to a combination of hydrolysis of the chlorine dioxide, liberation of hydrochloric acid, and the formation of acidic products. The oxidations proceeded quite rapidly and it was possible to dissolve the lignin completely with 8 hours. The fact that plot 2 in Fig. 5 levelled off after 4 hours did not mean that the consumption of oxidant had ceased, since the solid took another 4 hours to dissolve, but rather reflected the very rapid decomposition of the blank (plot 1). This decomposition took place so rapidly that after a few hours, when some of the dioxide had been used by the sample, the disappearance of oxidant in the more concentrated blank balanced the consumption and decomposition of the oxidant in the sample. This brief discussion again demonstrates the fact that the plots mean very little in a quantitative way. All that they indicate is that the reaction was rather rapid in the first few hours.

The rate at which the lignin dissolved varied greatly with the concentration of the oxidant. Using 0.017 mole of chlorine dioxide in 78 cc. of solution for 1 gm. of lignin, 7.5% remained insoluble after 32 hours. However, 0.018 mole in 46.5 cc. of solution completely dissolved 0.5 gm. of lignin within 8 hours. These oxidations were all conducted at 30° C. and were

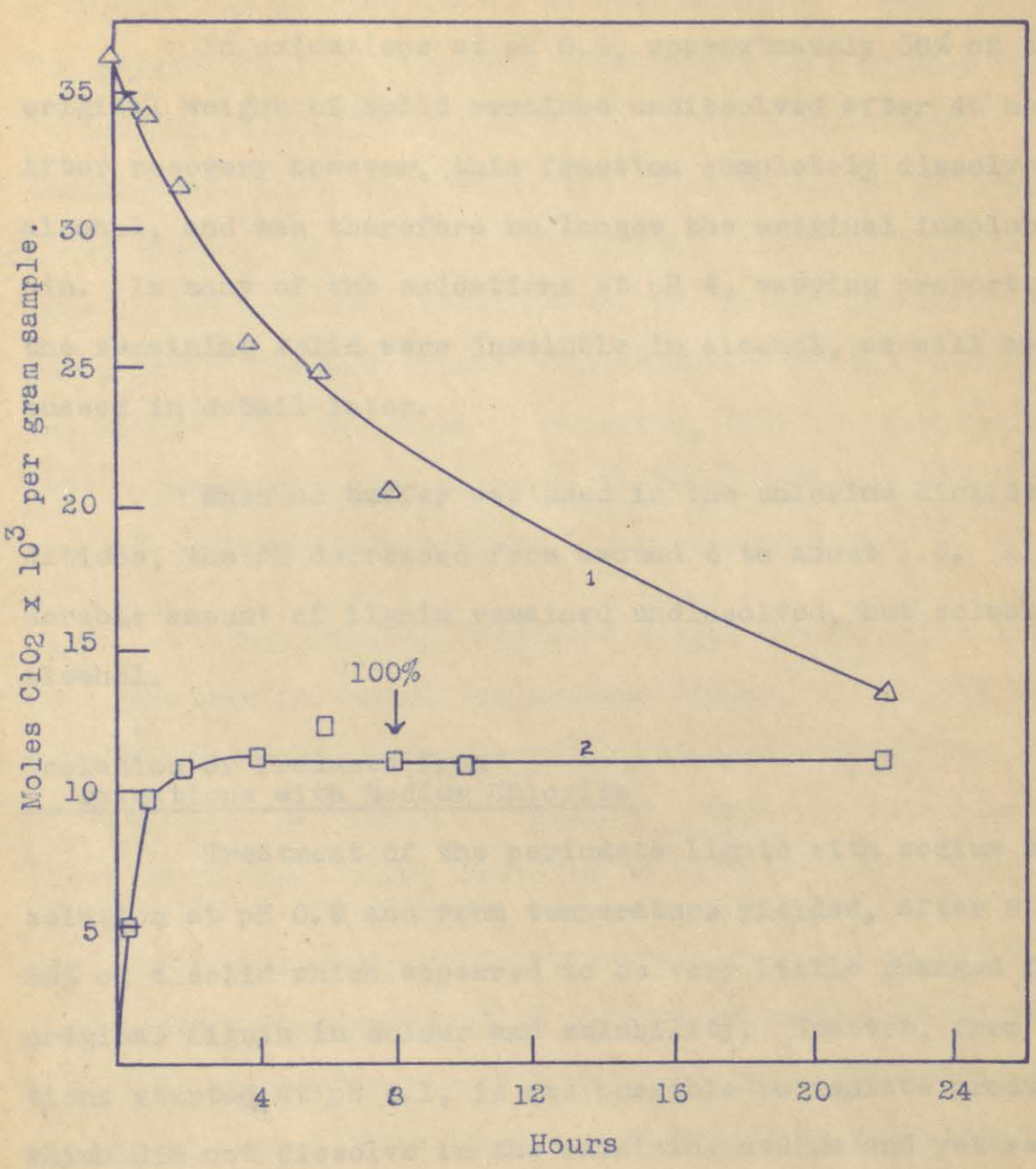


Fig. 5. Oxidation of 0.5 gm. of periodate lignin by 0.0179 mole of chlorine dioxide in 46.5 cc. at initial pH 4.1 and 30°C. Plot 1 blank ; plot 2 consumption of oxidant. See Table XVII.

started at pH 4.1. (Tables XVI and XVII)

In oxidations at pH 0.9, approximately 58% of the original weight of solid remained undissolved after 45 hours. After recovery however, this fraction completely dissolved in alcohol, and was therefore no longer the original insoluble lignin. In many of the oxidations at pH 4, varying proportions of the remaining solid were insoluble in alcohol, as will be discussed in detail later.

When no buffer was used in the chlorine dioxide oxidations, the pH decreased from around 4 to about 1.5. A considerable amount of lignin remained undissolved, but soluble in alcohol.

Isolation of Products from Oxidations with Sodium Chlorite

Treatment of the periodate lignin with sodium chlorite solution at pH 0.9 and room temperature yielded, after 8 hours, 86% of a solid which appeared to be very little changed from the original lignin in colour and solubility. However, from oxidations started at pH 4.1, it was possible to isolate products which did not dissolve in the oxidizing medium and yet were highly bleached and readily soluble in 1% alkali. This product was named "oxylignin A" because its easy solubility in dilute alkali differentiated it sharply from the original periodate lignin. Acidification of the solution to pH 1 with sulphuric acid, in many cases yielded another fraction of light coloured solid which differed from the oxylignin A in being alcohol-soluble.

This fraction was precipitated from the alcohol by the addition of ether, and was designated as "oxylignin B". The isolation and differentiation of similar products from chlorine dioxide oxidations will be discussed later and in more detail. In the larger-scale oxidations where the relative amount of oxylignin A was greater, the yield of B was small, but the reverse was true in the smaller oxidations where a larger amount of chlorite per gram of lignin was used. In other words, the more drastic the conditions, or length of reaction, the less oxylignin A was left. The latter thus appeared to be an intermediate degradation product between the periodate lignin and oxylignin B.

Ether readily extracted slight amounts of a brown viscous gum from the acidified aqueous liquor, but if the latter was evaporated to dryness and the solid extracted with dioxane, a distillable oil could be obtained. The initial action of the chlorite on the lignin proved to be slightly exothermic.

As shown in Table I, treatment of the lignin for 120 hours with approximately 0.05 mole of chlorite (run 5) gave a 27% yield of oxylignin B with no trace of A, the latter disappearing in 48 hours. When the reaction was stopped in 12.5 hours, even though 27.2% of the oxylignin A remained, the yield of B was slightly higher (run 6). Thus, the continued action of the oxidant appeared to have destroyed some of B as well as A. The best yield would probably have been attained in 48 hours, when all of A had just disappeared and before the chlorite had had time to decompose too much of the other fraction. Using a more

TABLE I

Sodium Chlorite Oxidations
of 1-gm. Samples of Periodate Lignin

No.	Moles of Chlorite	Volume of Solution (cc.)	pH	Temp. (°C.)	Duration of Oxidation (hr.)	% Oxylignin A	% Oxylignin B
1	.0340	47	0.92	25	8	86.1	0.0
2	.0246	45	4.05	30	35	0	14.8
3	.0508	47	4.10	30	55	0(a)	36.7
4	.0253	45	4.10	20.3	59	38.4	0
5	.0492	87	4.10	30	120	0(b)	27.0
6	.0492	87	4.10	30	12.5	27.2	29.8

(a) after 16 hours
(b) after 48 hours

concentrated solution of chlorite (run 3) all the solid material dissolved in a shorter time (16 hours) and in 55 hours a 36.7% yield of B remained. With half this quantity of oxidant, (run 2) only 14.8% of B was recovered in 35 hours. Actually this yield was low since the work was done before the technique of isolating B was improved. Temperature seemed to be important in this work also, as seen by comparing runs 2 and 4. With a 10° lowering of temperature, a considerable amount of oxylignin A (38.4%) remained, whereas otherwise this decomposed in a shorter time. At initial pH 0.9 and 25°C., the 86% of alcohol-insoluble material remaining (run 1) was very slowly soluble in dilute caustic soda, but was insoluble in sodium bicarbonate solution. This product was

classed as oxylignin A, although the usual A products were more readily soluble in alkali. Thus the reaction with lignin at a low pH and 25° was much less extensive than at pH 4 and 30°, and it seems unlikely that the 5° difference in temperature was responsible for the whole of the difference. The reaction in run 1 could not be continued more than 8 hours since at the end of that time the oxidant was completely exhausted.

Little further work was carried out on sodium chlorite oxidations, because the presence of massive amounts of inorganic salts from the oxidant and buffers made the quantitative isolation of the products difficult. The oxidations were also difficult to reproduce partly because the pH could not be held sufficiently constant during the reactions.

Isolation of Products from Oxidations with Chlorine Dioxide

Treatment of the periodate lignin with chlorine dioxide buffered at pH 4.1 also gave two products designated as oxylignins A and B. After several hours of treatment, depending on the conditions used (Table II), varying amounts of insoluble material remained. This oxylignin A, readily removed from the supernatant liquor on a filter, was bleached and, unlike the original lignin, was soluble in dilute alkali. However, it was insoluble in a dilute sodium bicarbonate solution, and thus probably contained phenolic rather than the more acidic carboxylic acid groups. It was also insoluble in alcohol and other common solvents. When the oxidations at pH 4.1 were carried to the stage where no oxy-

lignin A remained, the solution was still not clear, and on centrifuging, a grey film of solid separated. This solid dried to a brittle powder unlike the brown or cream coloured oxylignin A. A methoxyl content of only 3.9%, insolubility in alcohol, and very slow solubility in aqueous alkali or bicarbonate, suggested that this small fraction was mainly composed of the small amount of carbohydrate originally left in the periodate lignin. When oxylignin A remained, it was probably contaminated with this product.

On addition of either dilute sulphuric or hydrochloric acid to the clear aqueous supernatant liquor from oxylignin A no change occurred until a pH of 1.5 was reached. At this point a faint turbidity appeared, which on addition of more acid became a distinct precipitate. By pH 1.2 the precipitation appeared to be complete. This white fluffy material could not be readily removed by filtration as was oxylignin A because it formed a gel which clogged the filter. It was therefore removed on the centrifuge and was washed in the cups with dilute sulphuric or hydrochloric acids. If washed with distilled water, a certain amount dissolved and was lost. The gel left after the acid wash was dissolved in alcohol and any remaining insoluble grey solid was removed on the centrifuge. Yields of the grey solid never exceeded 2%. Addition of the alcohol centrifugate to about three volumes of ether usually precipitated most of the oxylignin B as a cream coloured solid, but reprecipitations were sometimes necessary, as described in the Experimental Section. Evaporation

of the ether-alcohol mother liquors gave a brown powder which behaved like the other oxylignin B fractions. The colour of these products, which varied from red-brown to a light cream, depended on the particle size. Large coarse particles were much darker in colour than the fine powder. The concentrations of solid, solvent, and precipitant affected the particle size. The oxylignin B differed from the oxylignin A in being soluble in alcohols, in aqueous sodium bicarbonate, and pyridine. Its solubility in 1% or weaker sodium bicarbonate indicated the presence of strong acidic groups.

In solutions buffered to pH 1, or with no buffer at all, in which the final pH was close to 1, the solid left after the oxidation consisted of a mixture of oxylignins A and B and the small amount of presumed carbohydrate. This mixture was removed by the centrifuge, washed as before, and then alcohol was added. The oxylignin B dissolved, leaving the insoluble A product and impurity. From the clear supernatant liquid, B was reprecipitated as described above.

From the remaining solutions, containing products soluble in water at pH 1 and designated as oxylignin C, a continuous ether extraction over a long period of time removed a large quantity of brown gum. In one case, (experiment number 1, Table II) a 48-hour extraction removed 35.9% by weight of the lignin used, from the aqueous solution remaining from the first oxidation. A further 68.5% was obtained in a similar way from the second oxidation. However, this very high yield (104.4%)

was rather unusual, the usual recovery being in the range of 40%. Some of these results are given in more detail in the Experimental Section. Separation of the ethereal solution into carbonyl, carboxylic, and phenolic constituents by extractions with aqueous sodium bisulphite and alkali proved to be impossible because of the extractability of the product by water.

In an attempt to remove any peroxides formed in the ether during the prolonged extraction, the solution was washed with ferrous ammonium sulphate, but here again the aqueous solution removed so much of the sample that a back extraction with ether took back a clear viscous oil in 15.9% yield. In this case, the material extracted by the ether was almost colourless, whereas in the other extractions a brown gum was obtained. Thus, the reducing action of the ferrous ammonium sulphate seemed to have had some effect. The oils, however, in spite of the precautions, still appeared to contain peroxides.

A butanol extraction of the aqueous oxylignin C solution was complete after only a few minutes of shaking in a separatory funnel. Difficulty was encountered in attempting to remove the last of the butanol from the remaining brown gum, which very slowly distilled during the process. Adding together the yields of this gum and oxylignin B gave a total recovery of 100% or more in cases in which no oxylignin A remained. When the aqueous solution of oxylignin C was extracted with ether until as much as 15.5% was removed, and then extracted with butanol, the product from the latter was a dark red-brown glass very much un-

like the extract obtained without a previous ether extraction. A dry weight could be obtained readily since there was no distillable material in this residue.

Figures 6 and 7 summarize the method of isolating oxylignins A, B and C using chlorite and chlorine dioxide.

Table II shows the yields of oxylignins A and B obtained by systematically varying the conditions of the oxidations with chlorine dioxide. In all cases, none or practically none of the alkali-insoluble lignin remained. The conditions stated in the first line of run 1 produced a large amount of oxylignin A which was not isolated and weighed, but was re-oxidized as in the second line. The disappearance of A and the recovery of a 41.4% yield of oxylignin B showed conclusively that the latter could be produced at pH 4 by the oxidation of the former. Doubling the amount of chlorine dioxide (run 2) produced in half the time substantially the same amount of oxylignin B as the two-stage run 1, but intermediate yields of both oxylignins A and B were obtained under the milder conditions of runs 3 and 4. The highest yield of oxylignin B occurred in a solution buffered with sulphuric acid to pH 0.92 (run 5) but the time was still long.

The temperature was raised from 25° to 30° C. in the later runs in order to decrease the time required for the oxidation at pH 4. The conditions of run 6 caused all of the oxylignin A to go into solution after 8 hours and only 19.7% re-

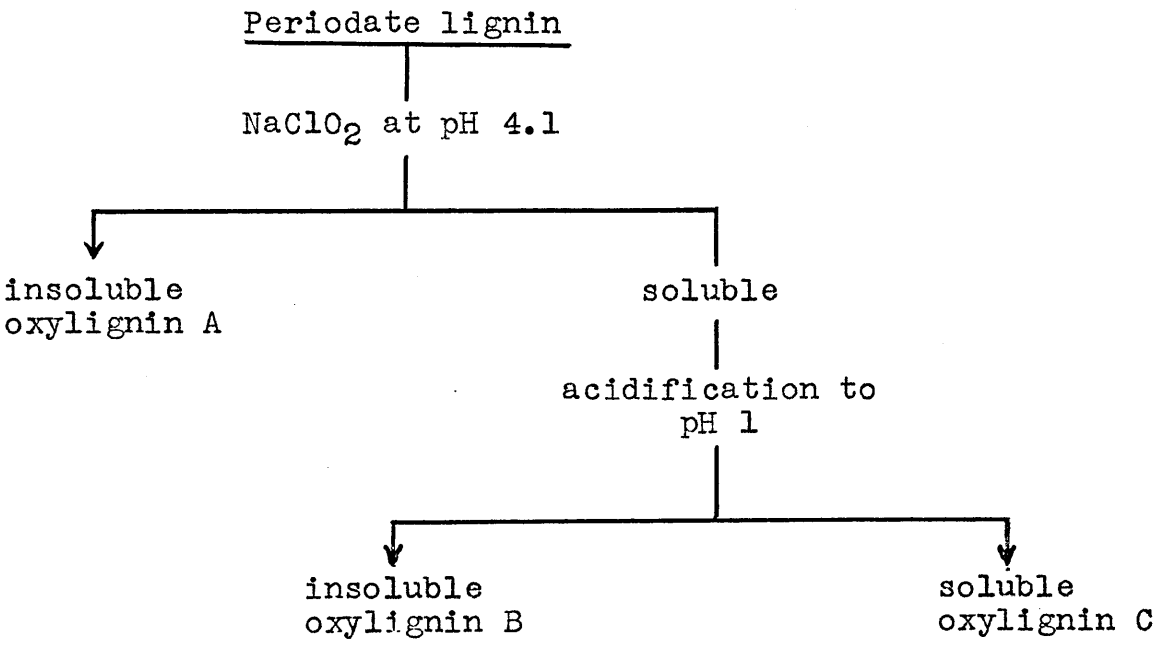


FIGURE 6

Flow Sheet for Oxidation with Sodium Chlorite

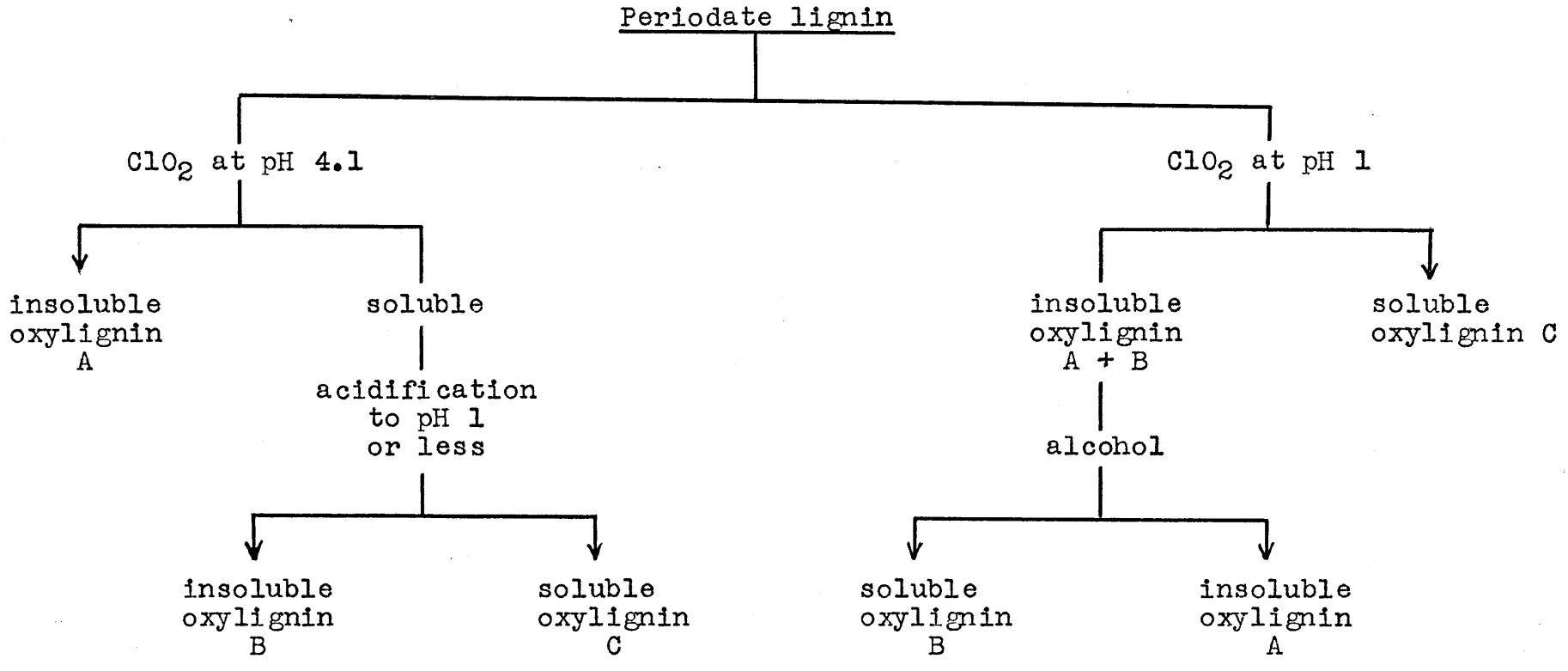


FIGURE 7

Flow Sheet for Oxidation with Chlorine Dioxide

TABLE II

Chlorine Dioxide Oxidations of Periodate Lignin[a]

Run No.	Moles ClO_2 Added per gram	Soln. (cc.)	pH	°C.	Hours	% Oxylignin A	% Oxylignin B	% Oxylignin A + B
1	0.0134	43	4.10	25	21.5	..[b]	0)	..
	0.0147[c]	41	4.15	25	25	0[c]	41.4)	41.4
2	0.0333	63	3.70	25	21	13.7	38.1	51.8
3	0.0286	93	4.06[d]	25	8	25.3	19.2	44.5
4	0.0222	93	4.06[d]	25	8	27.6	18.4	46.0
5	0.0219	43	0.92	25	45	0	57.6	57.6
6	0.0358	93	4.10	30	26.5	0[e]	19.7	19.7
7	0.0330	103	4.06[f]	30	8	8.5	33.6	42.1
8	0.0165	43	4.10	30	8	56.0	13.7	69.7
9	0.0171	78	4.10	30	32	7.5	22.6	30.1
10	0.0370	71	3.6 -1.4	30	8	4.8	34.2	39.0
11	0.0322	113	1.4 -1.3	30	10	0	36.4	36.4
12	0.0420	68	4.15-1.45	30	9.5	0	45.2	45.2
13	0.0408	72	4.35-1.5	30	4	8.1	35.2	43.3
14	0.0408	72	4.35-1.55	30	2	36.2	26.2	62.4

(a) Samples were either 1.0 or 0.5 gm.
(b) Oxylignin A recovered on centrifuge in large yield and re-oxidized.
(c) Oxylignin A from (b) re-oxidized.
(d) Final pH 3.0.
(e) After 8 hours.
(f) Final pH 2.75.

mained as B after 26.5 hours. Interruption of the similar run 7 after 8 hours did in fact leave only a minor amount of A and an improved yield of B. The use of half-quantities of oxidant at about the same concentration (run 8) gave the highest combined yield of A + B recorded, but oxylignin A was greatly favoured. An attempt to increase B by quadrupling the time of oxidation (run 9) was not successful, although A was almost eliminated. When no buffer was present in the system, the initial pH of 3.6 to 4.4 rapidly changed to a pH of 1.3 to 1.6 as the chlorine dioxide underwent reduction. Comparison of runs 10 and 11 suggested that oxidation with this drifting pH ran a course similar to that buffered to the final pH of 1.4 with sulphuric acid. Increasing the amount and concentration of the oxidant somewhat (run 12) increased the yield of B to 45.2%, a figure which was probably near the optimum since decreases in the time of oxidation to 4 and 2 hours (runs 13 and 14) decreased B, although the total A + B was favoured.

No detailed comparisons were possible between the oxidations of periodate lignin with sodium chlorite, (Table I), and chlorine dioxide, (Table II), partly because of the undetermined physical and chemical nature of the oxylignins A, B and C. As far as solubilities and appearance were concerned, however, the products from both oxidants were very similar, and in both cases, the products A, B and C represented increasing levels of oxidation.

This conclusion was checked in separate experiments by treating oxylignin A with a chlorine dioxide solution for 12 hours. The resulting material obtained in 49.5% yield, proved to be the alcohol-soluble oxylignin B and the remaining product was the dilute acid-soluble oxylignin C. No trace of the original alcohol-insoluble material could be found. When isolated oxylignin B was treated with either chlorine dioxide or sodium chlorite solution it was recovered in yields of only 45.6 and 57.3% respectively. The remaining material had thus been transformed into oxylignin C. These experiments also explain the continued consumption of oxidant even after all the periodate lignin had dissolved as oxylignin B in the solutions at pH 4.

Of the oxylignins A, B, and C, it has already been noted that the first appeared to be of a phenolic rather than an acidic character and was insoluble in common organic liquids. As the initial product of the oxidation, it presumably had a rather high molecular weight. Oxylignin C, on the other hand, was a brown gum freely soluble in water, dilute acid and butanol, was partly volatile, and was formed from A and B. These features suggested that C consisted of highly oxidized fractions of low molecular weight, perhaps derived from the phenyl propane lignin monomers. The decision was therefore made to concentrate attention on the intermediate oxylignin B, which had convenient solubilities, and whose examination might throw more light on the structure of lignin. Several larger-scale oxidations with un-

buffered chlorine dioxide were therefore carried out (Table XIX) with conditions chosen to give a 40 to 50% yield of oxylignin B. These conditions were similar to those of run 12, Table II.

Preliminary Examination of Oxylignin B

Samples prepared from the same batch of periodate lignin with different conditions of oxidation always had less than 0.5% of ash and a methoxyl content of 6.8 to 7.3%, while the chlorine content varied within somewhat greater limits, 7.3 to 9.3%.

Although readily soluble in cold ethanol when freshly prepared and never dried, oxylignin B, after being dried at room temperature under vacuum, required continued shaking in ethanol at room temperature for several hours to redissolve. A trace of a white fluffy residue remained insoluble. Evaporation of the clear alcoholic solution at 30°C. and reduced pressure, left a very light brown solid with the original methoxyl content, which again would not completely dissolve in fresh alcohol. In all cases the insoluble portion was very small and the failure to dissolve completely after drying appeared to be a physical effect, rather than a fractionation due to chemical differences.

Isolated oxylignin B was slowly soluble in cold methanol, but its ability to dissolve in alcohols decreased greatly from ethanol to butanol. In acetone and dioxane the material slowly dissolved if the solvent was heated but there was no sign of reprecipitation as the solution cooled. In each

case the colour of the solution was yellow. However, when dissolved in cold sodium bicarbonate, dilute caustic soda, or pyridine, the colour was a distinct red-brown, and only became yellow as the pH was lowered below 7. This change in colour as the pH varied was reversible and indicated the possible presence of a keto-enol or other tautomeric system. In ether, petroleum ether, benzene and chloroform the oxylignin was completely insoluble.

Oxylignin B was surprisingly stable to heat, and its light cream colour was not noticeably altered after several weeks at 105°C. A colour change to brown set in at 170°C. and the brown was fairly dark at 300°C., although no signs of softening were apparent. This drastic treatment, however, had brought about some sort of chemical change, for the sample was no longer soluble in cold caustic soda.

Treatment of an alcoholic solution of oxylignin B with ferric chloride produced a green colour so faint in comparison with that developed in a control test with vanillin that the presence of phenolic groups in the oxylignin was considered doubtful. In testing the reducing power of the oxylignin with boiling Fehling's solution, a red precipitate of copper oxide formed readily within 2 minutes, but the presence of carbonyl groups was of course by no means proven. Other groupings, such as certain phenolic substances also readily reduce Fehling's solution.

When oxylignin B was heated with a solution of 1% calcium oxide in 6% sulphurous acid at 135°C. for 6 hours, as in a standard wood pulping procedure, all but 8.7% of the sample dissolved. This solubility was in the same range as that of the original periodate lignin in a similar treatment. However, unlike the residue from the latter, which was a light brown, the insoluble material from the oxylignin B was white. Although the reaction afforded no evidence for the presence of a carbonyl group, the normal dissolution in a bisulphite cook showed the preservation of a property peculiar to lignin in wood and to periodate lignin. Cabott (63) has shown that periodate lignin readily loses this property when treated with aqueous buffers at 120°C. and upward.

It will be remembered that Jayme and Hanke (37) removed the lignin from wood with aqueous sodium chlorite, hydrolyzed the extract with 5% sulphuric acid, and recovered a 20% yield of glucosazone from the hydrolysate. Oxylignin B when treated exactly as Jayme and Hanke described, gave no trace of an osazone. Although Jayme and Hanke inferred the presence of a glucose grouping within the lignin building unit, it is now much more plausible that in dissolving the lignin from the wood a certain amount of the carbohydrate portion was also removed. The fact that Jayme and Hanke's yield of holocellulose when added to the Halse lignin content of the wood accounted for 100% of the wood, was actually no proof that the carbohydrates had been left untouched, and only the lignin re-

moved. The lignin determination is empirical and their results could have owed much to a balancing of errors. In the present investigation the trace of carbohydrate possibly present in the periodate lignin was removed as insoluble material when the chlorite solution containing oxylignin was centrifuged, and also when the isolated oxylignin B was dissolved in alcohol. It seems likely, therefore, that Jayme and Hanke's glucosazone originated in polysaccharides from which his crude chlorite oxylignin had not been freed, rather than from a glucose-containing lignin unit of the kind they postulated.

After 90 minutes of heating the oxylignin B under reflux, a 52% yield of brown powder remained insoluble in the 5% acid solution. In addition to being darker in colour, this powder was insoluble in sodium bicarbonate solution and in alcohol, and dissolved only very slowly in 1% sodium hydroxide. It thus appeared as if the acid treatment had chemically altered the oxylignin B. Even when a 2.1% suspension of the latter in distilled water was boiled for 100 minutes, a 40% yield of insoluble material remained which, like the residue from acid hydrolysis, was insoluble in bicarbonate and alcohol. The corresponding yield was 42.6% for a 3% suspension boiled for 4 hours. Another water suspension containing 1.4% of oxylignin B, after 2 hours of boiling left 51.8% of insoluble material. These observations appeared to indicate the formation of a condensed material during extractions with hot water, rather than a fractionation of the original substance.

Considerable work was done in attempts either to prove the homogeneity of the oxylignin B or to separate it into fractions. When dissolved in bicarbonate or alkali, extractions with ether or butanol even for extended periods removed no material. From an acidified suspension of oxylignin B, however, butanol did eventually remove as much as 89% of the original sample. When a very dilute alkaline solution was acidified, only a small amount of oxylignin appeared as a suspension, the remainder staying in solution. Owing to this solubility of the oxylignin in water it was not possible to recover all of it in the butanol, but there did not appear to be any butanol-insoluble fraction. Non-acidic products were therefore probably absent since they would have been removed from the alkaline solution by ether or butanol. It is well to remember in all this work, that the dry powdered oxylignin did not have the same solubilities as the material in solution. The powder itself is very poorly soluble in butanol, and, as noted previously, only a portion dissolves in water after considerable heating.

A sodium bisulphite extraction very readily removed all the colour from the butanol solution. Acidification of the bisulphite solution and removal of the sulphur dioxide made it possible to recover the original oxylignin B in butanol, but again in reduced yield owing to its solubility in water. After removal of inorganic salts and concentration of the butanol to a small volume, a fraction with a chlorine content of 6.7% was precipitated by ether. This was lower than the 7.9% chlor-

ine in the original oxylignin, but the difference could be accounted for to some extent in the traces of solvent and ash left in the material. To sum up, although soluble to some extent in water, the oxylignin was much more readily extracted by a sodium bisulphite solution thus indicating the possible presence of a carbonyl fraction.

Fractional precipitation seemed to indicate the oxylignin B to be relatively homogeneous. A typical sample was precipitated in three fractions by the addition of increasing amounts of ether to the alcoholic solution and all three fractions contained approximately the same amount of chlorine: 7.9, 8.1 and 8.1% respectively. Their appearance and solubilities were also very similar.

Dialysis of the oxylignin B again appeared to indicate the product to be homogeneous. Since it was not possible to dissolve the dried powder readily in water, 0.5% aqueous sodium hydroxide was used with the same concentration of sodium hydroxide outside the membrane. Even after several days, with periodic changes of the outer solution the yellow colour appeared in the dialysate at approximately the same rate as it did during the first day. There appeared to be no slackening of the diffusion other than that caused by increased dilution of the sample, which behaved as a uniform substance that had a molecular weight high enough to cause difficulty in diffusion.

After several days of dialysis the chlorine content (7.3%) of the product remaining within the membrane was practically the same as that of the original oxylignin. Hence the

chlorine present was not associated with some low molecular weight impurity that would have diffused more rapidly and left the dialysed material with a lower chlorine content. It also followed that the halogen was combined in the oxylignin securely enough to resist removal as dialysable sodium chloride by the prolonged action of the dilute alkali.

The dialysate also yielded products with chlorine contents of 5.4 and 6.2% or close to that of the oxylignin used. However, as typical of all of this work, acidification of the alkaline solution resulted in recoveries of not more than 50% even from relatively concentrated solutions. More dilute solutions gave smaller yields. In all cases no precipitate appeared before a pH of 1.7 was attained and the precipitation was complete by pH 1 or slightly less. To avoid the danger of interference in the chlorine content from hydrochloric acid, sulphuric acid was used in the acidifications. The difficulty which then arose was to eliminate completely the sulphuric acid during the isolation of the product. Its presence with the powder caused considerable charring and rather large errors in the chlorine determination.

In order to obtain better recovery of the oxylignin, the acidified solution was extracted with butanol. It was interesting to note that although the dry oxylignin B was not very easily soluble in butanol, it readily dissolved in the solvent when taken from the aqueous solution. However, this procedure also presented difficulties because of the solubility of the

sulphuric acid and inorganic salts in the butanol. Removal of the acid by washing with water also removed much of the sample, and the use of barium chloride resulted in the formation of barium salts of the oxylignin which precipitated with the barium sulphate.

To overcome these difficulties of isolation, dialysis in dilute alcohol was tried since it was easy to isolate the products by removing the alcohol under reduced pressure. However the diffusion was very slow and only 10% of the original oxylignin was obtained from the dialysate after 6 days (Cl, 7.65%) In another 8 days, 11% was recovered (Cl, 7.25%) and still another 10% in 6 more days (Cl, 6.65%). Since all these fractions contained substantially the same percentage of chlorine as the material remaining within the membrane (Cl, 7.33%) no fractionation was apparent. The slowness of the diffusion might be due to the partial closing of the pores of the cellophane in the alcohol solution, whereas in the dilute caustic solution swelling opened the pores and allowed a greater rate of diffusion.

Oxidation with Nitrobenzene

A nitrobenzene oxidation at 160 to 175°C. of periodate lignin suspended in caustic soda will give vanillin in 10 to 12% yield (59). However, after treatment with chlorine dioxide, the resulting oxylignin B yielded neither vanillin nor its 2,4-dinitrophenylhydrazone using the standard oxidation. However, one crude product had a melting point of 164 to 167°C., close

to the 167 to 168°C. reported for 6-chlorovanillin (44) and the 164 to 165°C. for 5-chlorovanillin (45). A sodium fusion test of the crude product showed the presence of chlorine but lack of material prevented any further attempt at identification. With the exception of this trace of what was possibly chlorovanillin, it appeared that the vanillin nucleus in the periodate lignin had been destroyed during the oxidation with chlorine dioxide. This inference was not improbable because many experiments by Schmidt (28) showed that phenolic, as opposed to aliphatic substances, were readily oxidized in similar circumstances.

Experiments on Molecular Structure of Oxylignin B

Molecular Weight

Although it had been assumed that oxylignin B was heterogeneous, attempts at fractionation by dialysis, by precipitation from cold dilute alkali or from organic solvents, or by extractions of butanol solutions with water or aqueous solutions had failed to demonstrate heterogeneity. These failures, it is true, might have been caused by an inability to recognize differences in the fractions other than those of solubility, of methoxyl or chlorine content. Nevertheless, the only practicable course was to proceed on the assumption that the oxylignin B was chemically homogeneous.

Difficulty was encountered in attempting to determine the molecular weight of the oxylignin B. The usual ebullioscopic or cryoscopic methods were not tried since the oxylignin was evidently of high molecular weight and these methods were increasingly inaccurate in the higher range. The Rast method (64) often used successfully for molecular weights of the order of 1000, could not be employed since the oxylignin was not soluble in the camphor. The best methods for the present case were evidently those which depended upon differential vapour pressure or isothermal distillation.

According to the latter principle, separate solutions of different substrates in the same liquid, kept in communication through their vapour phase, will adjust their concentrations by evaporation and condensation until each has the same vapour pressure. The attainment of equilibrium can be followed by noting the changes in weight or volume of the two solutions. If the two solutions of volume V_1 and V_2, contain W_1 and W_2 grams of solutes of molecular weight M_1 and M_2 respectively, then when equilibrium is attained

$$\frac{M_1}{M_2} = \frac{W_1 V_2}{W_2 V_1} \quad .$$

A knowledge of all these quantities except M_2 enables the value of M_2 to be calculated.

The first method tried was that of Blank and Willard (65) as adapted from Blackman (66). Methanol at approximately

65°C. was used as the solvent, since it was the best one known for oxylignin B. Trial experiments, using recrystallized azobenzene as the standard, gave the molecular weight of pure resorcinol as 110.9, 110.0, 110.5, 109.2, in comparison to the correct value of 110.1. The attainment of equilibrium took several hours even when the volumes of the solutions were initially chosen to be near the final equilibrium ratio. If this initial choice was badly made, the method was tedious to the point of being impracticable.

When attempts were made to equilibrate methanol solutions of oxylignin B against azobenzene, the great difference in the molecular weights required a large volume of solvent with the azobenzene and a volume with the oxylignin so small that there was danger of precipitation. The results after periods of up to one week, 309 and 487, for the molecular weight of the oxylignin, did not represent equilibrium conditions and were regarded as worthless. Sucrose octaacetate, having a molecular weight of 678, was next used as the standard but the results were very unreliable. In one case the calculated molecular weights rose from 4700 without reaching an equilibrium, whereas in another case they dropped from 4400 to 4000 and continued to descend. The method was therefore abandoned for a slower but more reliable adaptation of the isothermal distillation type originated by Signer (67) and described by Clark (68).

This method operated on the same principle as that just discussed, but the vapours from the two bulbs came into

equilibrium in a closed evacuated system at room temperatures instead of at atmospheric pressure and an elevated temperature. To attain this equilibrium required several days rather than the several hours suggested by Blackman for his method. However, using a sealed system there was no loss and once the equilibrium was reached the volumes of the solutions remained constant. Sucrose octaacetate in methanol was used as the standard solution and equilibrium with a methanol solution of oxylignin B was attained after several days. The molecular weights of 895 and 980 obtained for the oxylignin were considered to be satisfactory.

Acid Equivalents

The plot shown in Fig. 8 represents a potentiometric titration of oxylignin B against standard barium hydroxide. Although the titration proceeded normally from the initial pH of about 3.5 to 7, in the alkaline range the pH recorded immediately after the addition of an increment of the alkali (full line) was not stable but drifted within 60 seconds to the lower value shown on the dotted line. Repetition of the experiment in a nitrogen atmosphere showed that this behaviour was not caused by the absorption of atmospheric carbon dioxide, and the precipitate that appeared in the solution after pH 8 was not barium carbonate but a barium salt of the oxylignin. If pH 7.1, the point at which "drifting" was first observed and approximately half-way between the parallel parts of the plot, was taken as the end-point, the acid equivalent of the oxylignin was 448. The equivalent corresponding to pH 9 was 364.

Titrations using sodium hydroxide gave plots with no distinct breaks and the alkali was consumed at a relatively steady rate (Figs. 9 and 10). Again drifting occurred between pH values of 8 and 9, and the only end-point that might be used was the place at which the drifting first appeared. This gave a value of 354 for Fig. 9 and 352 for Fig. 10. The values corresponding to pH 9 were approximately 346 and 356.

When the oxylignin was dissolved in an excess of standard alkali and the solution was back titrated after a time with acid, a good plot with a sharp end-point at pH 7.5 was obtained and acid equivalents of approximately 170 to 180 could be duplicated. The equivalent for pH 9 was about 165. In these cases the reversible colour change from red-brown in alkaline to light straw yellow in acid solution was readily observed. In one case in which the oxylignin was dissolved in dilute alcohol and titrated with alkali, a value of 432 was calculated from the result at pH 7.9 where the drifting started. The equivalent for pH 9 was 376. On back titrating with acid, an equivalent of 212 was obtained. This would probably have been even closer to the 180 obtained in other cases, if a larger excess of alkali and a longer reaction period had been employed. These results are depicted in Fig. 11.

The above results suggested that the oxylignin contained groups as acidic as acetic acid and presumably of a carboxylic acid nature. In addition, alkali readily produced an approximately equal number of weakly acidic units which might

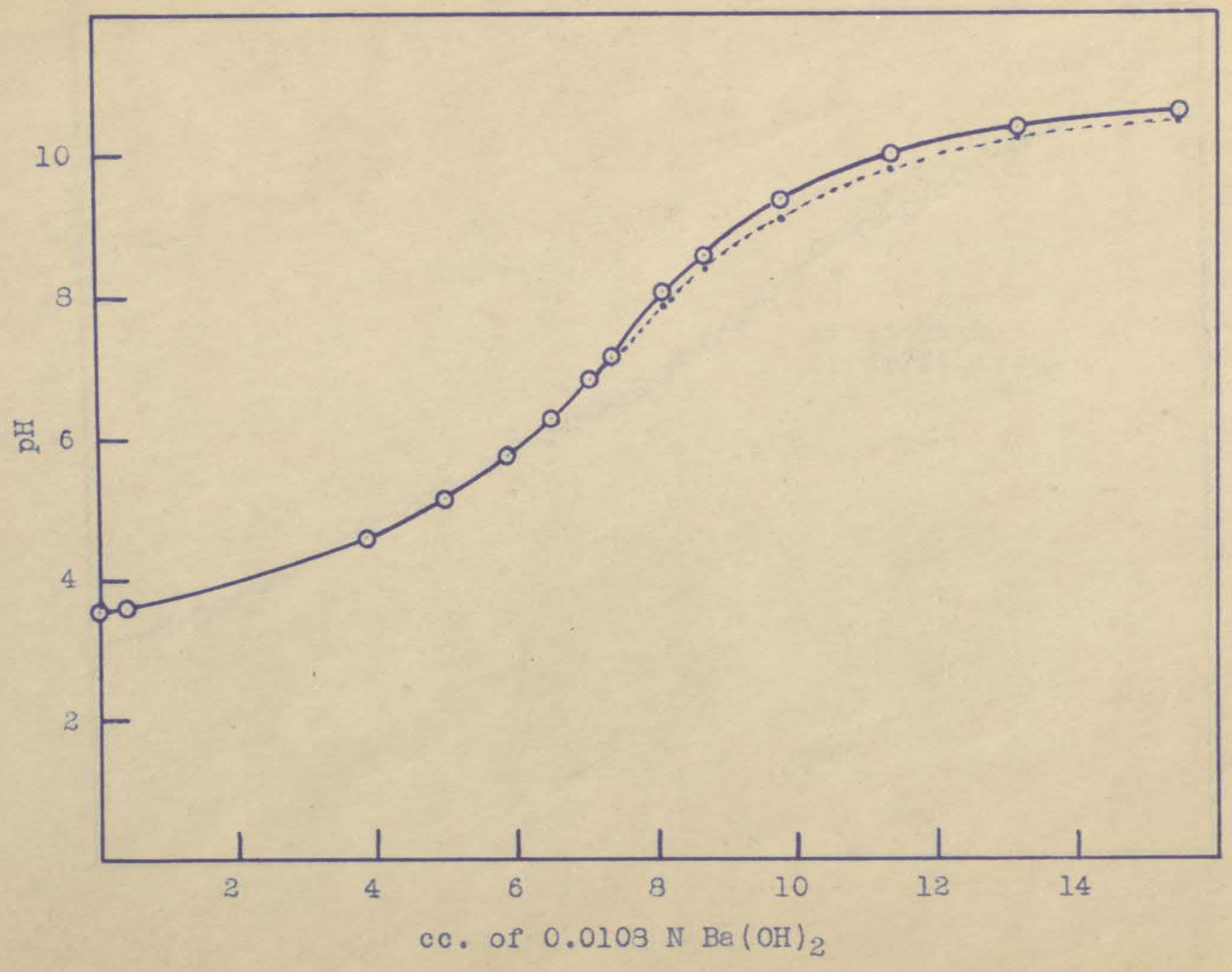


Fig. 8. Potentiometric titration of 0.0354 gm. of oxylignin B with barium hydroxide.

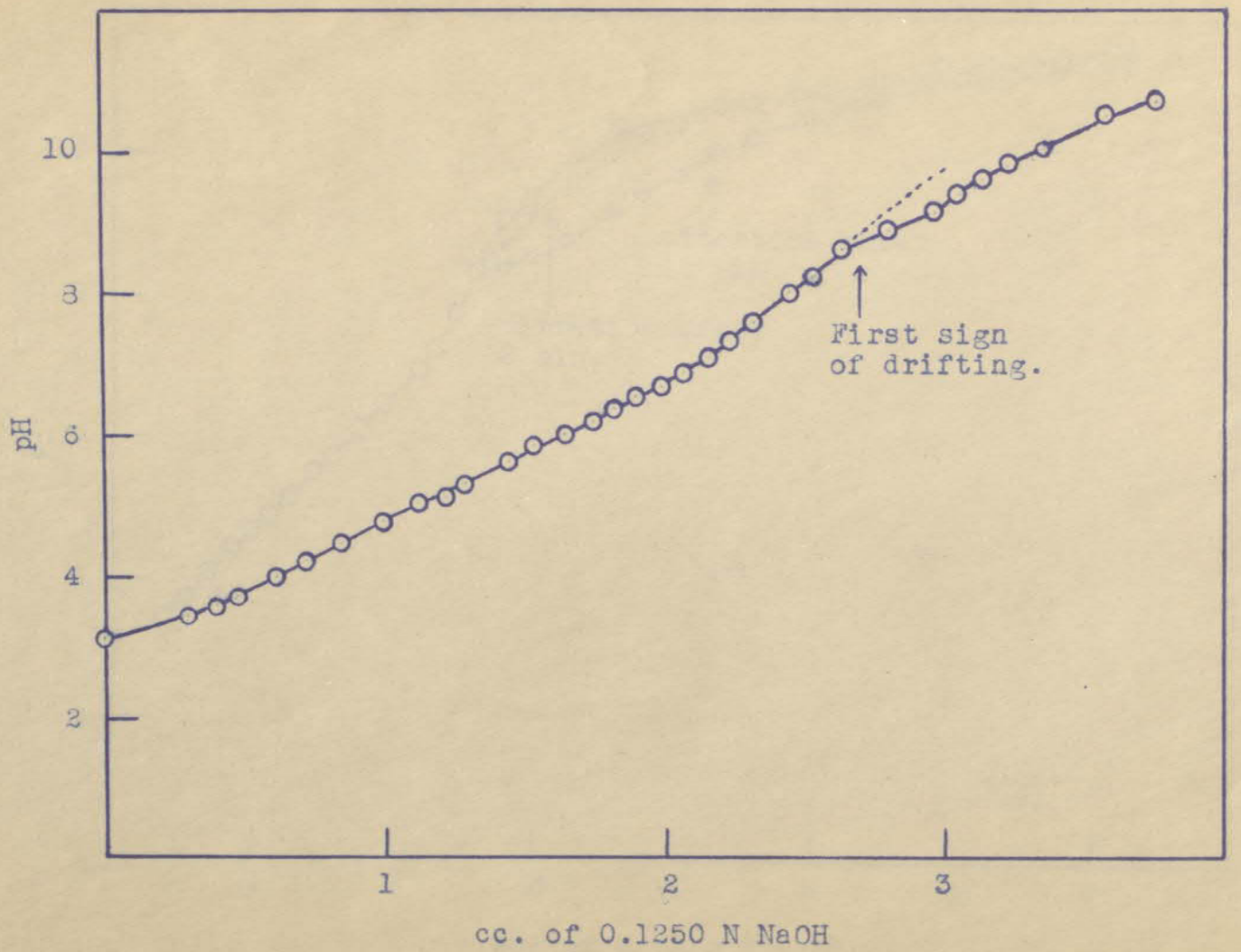


Fig. 9. Potentiometric titration of 0.1191 gm. of oxylignin B in dilute alcohol with 0.1250 N alkali.

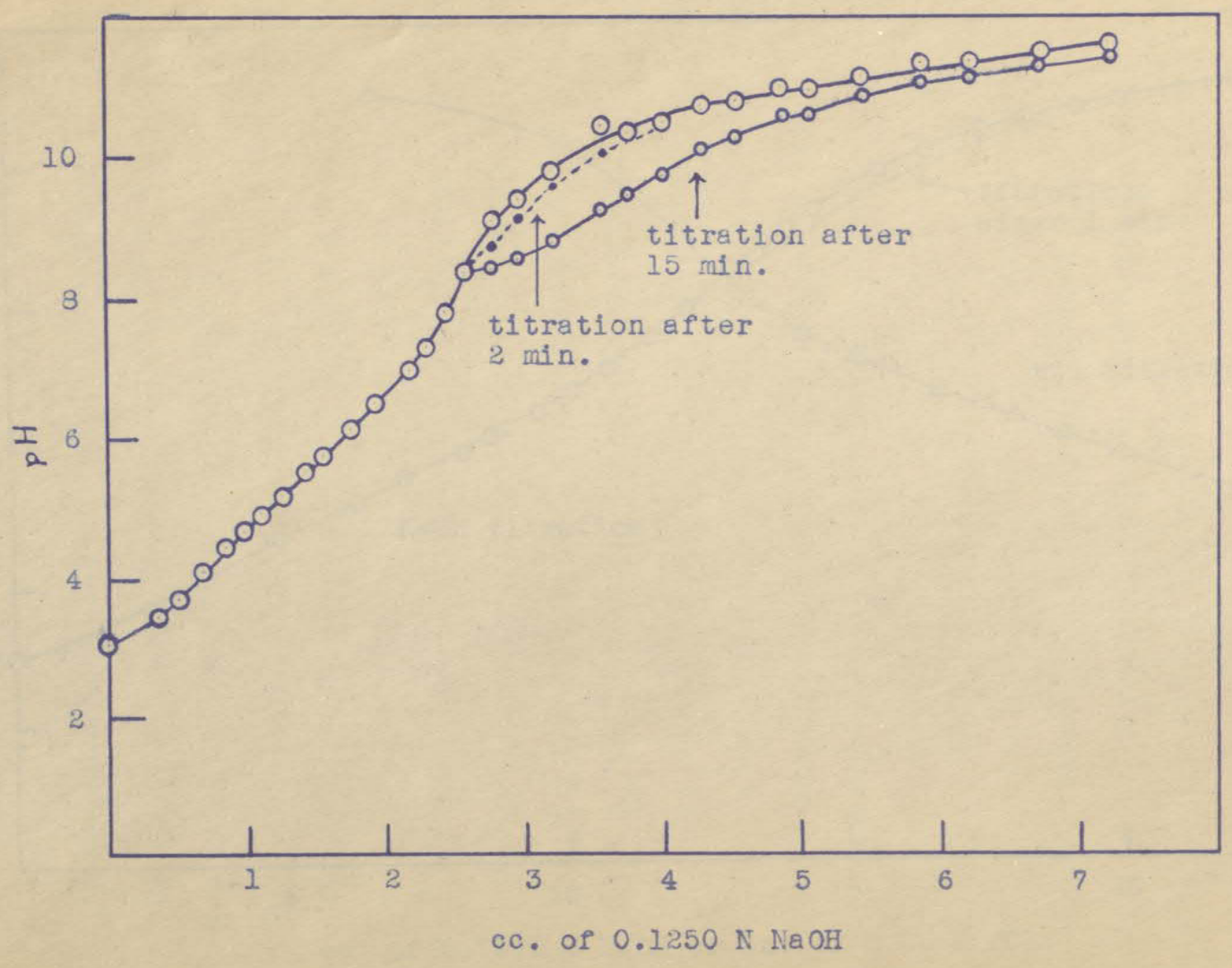


Fig. 10. Potentiometric titration of 0.1210 gm. of oxylignin B in dilute alcohol with 0.1250 N alkali.

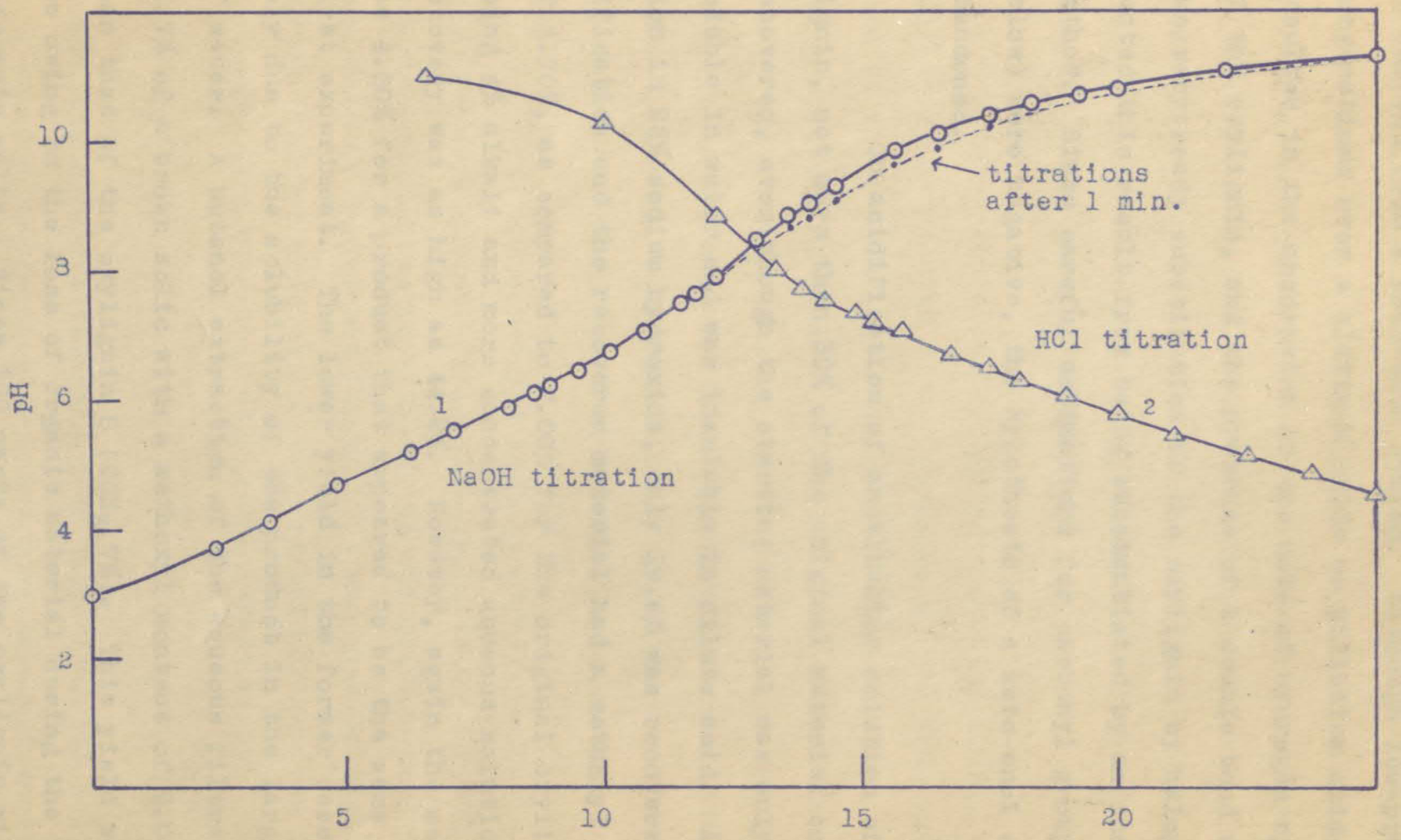


Fig. 11. Potentiometric titration of 0.1976 gm. of oxylignin B in dilute ethanol, o with caustic soda; △ back titration with acid.

be derived from a keto-enol system. Although low-pressure hydrogenations over a platinum oxide or palladium oxide catalyst resulted in the absorption of one mole of hydrogen by 1020 gm. of the oxylignin, and the presence of a double bond was possible, the very ready substitution of the oxylignin by halogens prevented this result from being substantiated by an independent method. Since careful estimations for carbonyl groups (see below) were negative, the hypothesis of a keto-enol system was abandoned.

On acidification of an alkaline solution of the oxylignin, not more than 50% of the original material could be recovered, even though the starting material was only slightly soluble in water and was insoluble in dilute acid. After solution in 25% sodium hydroxide, only 19.6% was recovered on acidification and the recovered material had a methoxyl content of 4.70%, as compared to 7.00% for the original oxylignin. Using 4% alkali and more concentrated aqueous solutions, the recovery was as high as 44.2%. However, again the methoxyl value was 4.80% for a product that appeared to be the same as in the first experiment. The lower yield in the former case was probably due to the solubility of the product in the larger amount of water. A butanol extraction of the aqueous filtrate yielded 21.7% of a brown solid with a methoxyl content of 9.0%, higher than that of the oxylignin B (OCH_3, 7%). This yield was probably low owing to the loss of organic material during the removal of inorganic salts. Since 100 parts of the oxylignin with 7%

methoxyl yielded 44.2% with 4.8% methoxyl, the remainder by the law of mixtures had 8.8% methoxyl. Although difficulties with the separation of inorganic salts resulted in a low recovery of this remainder, the observed methoxyl content of 9.0% suggested that the alkali had cleaved the oxylignin B without over-all loss of methoxyl, into fractions of greater and less solubility and methoxyl content.

When the acid-precipitated product (OCH_3, 4.80%) was repeatedly methylated with diazomethane, the methoxyl content rose to about 17.7%, or higher than when oxylignin B itself was completely methylated (OCH_3, 17.0%). The difference was small, but it was possible that the alkali had hydrolyzed some labile phenolic or carboxylic grouping that was then available for methylation. The uptake of hydrogen during hydrogenolysis might have been connected with a similar cleavage.

The above results made it highly desirable to determine the acid equivalent of the oxylignin by a method that did not involve the use of alkali. In the calcium acetate method described by Yackel and Kenyon (70) and then by Meesook and Purves (69) the acid equivalent was calculated from the amount of acetic acid liberated during the reaction.

$$2\ RCOOH + Ca(CH_3COO)_2 \longrightarrow Ca(COOR)_2 + 2\ CH_3COOH \quad .$$

The acetic acid was determined by titration with sodium hydroxide to a pH of 8.3. Such estimations gave apparent equivalents of 245 and 241 whereas micro determinations of the calcium ash

corresponded to equivalents of 332 and 356. The reason for the discrepancy was not determined.

TABLE III

Acid Equivalents of Oxylignin B

Method		Acid Equivalent
(a)	Back titration with acid - pH 7.5	170 - 180
	pH 9.0	165
(b)	Direct titration with alkali - pH 9	346 - 356
	pH 9	376
	pH 7.9	432
(c)	Direct titration with $Ba(OH)_2$ - pH 9	364
	pH 7.1	448
(d)	Calcium acetate method	241, 245
(e)	Ashing of calcium acetate	332, 356

Table III summarizes some of the acid equivalents obtained by various methods. The most significant point noted was the fact that a value about 350 was obtained by direct titration with alkali, and a value about 175 when an alkaline solution of the oxylignin was back titrated with acid. The latter value, which was easily duplicated, was obtained at pH 7.5 which the titration curve appeared to indicate as the endpoint. The value around 350 was obtained at pH 9 where the drifting usually began in these direct titrations. The curve showed no definite break, but some change definitely occurred

at this point. The values over 400, obtained when drifting began at pH values of 7.1 and 7.9, seemed to be out of line, and also, in titrating a relatively weak acid (oxylignin B) against a strong base, an end-point around 9 would be expected.

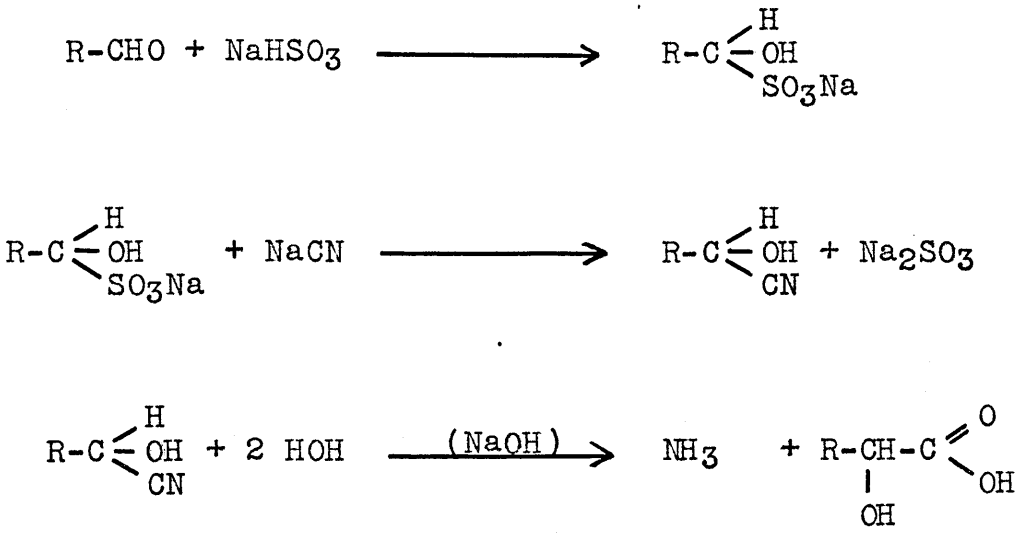
Carbonyl Determinations

The method of Gladding and Purves (71) depends on the addition of hydroxylamine hydrochloride and the subsequent liberation of one mole of hydrochloric acid for each mole of carbonyl group.

$$\geq C{=}O + NH_2OH{\cdot}HCl \longrightarrow \geq C{=}NOH + H_2O + HCl$$

The liberated acid is determined by titration back to the original acidic pH of 5.3 and the method has the advantage of not requiring an alkaline system. With oxylignin B, however, the pH of the solution did not change over 1.5 hours and no consumption of hydroxylamine was indicated. Carbonyl groups therefore appeared to be entirely absent.

Another method used to determine carbonyl was developed by Brach (72) and Coombs (73) and used by Perlin (74). This method depends on the condensation of sodium cyanide with the carbonyl group, and decomposition of the resulting cyanohydrin by sodium hydroxide to yield ammonia quantitatively. Perlin allowed his product to react first with sodium bisulphite and then with sodium cyanide in an attempt to stabilize any aldehyde groups during the cyanohydration.



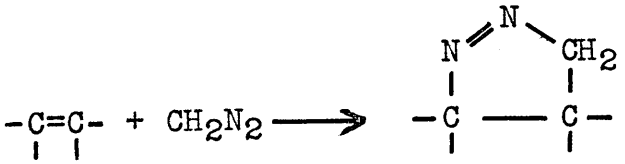
Thus from the amount of ammonia liberated it was possible to determine the amount of carbonyl originally present. When applied to oxylignin B, without and with the bisulphite pretreatment, the presence of one carbonyl group in 2380 gm. and 2880 gm. was indicated. These amounts were considered to be negligibly small.

Methylations of Oxylignin B and its Derivatives

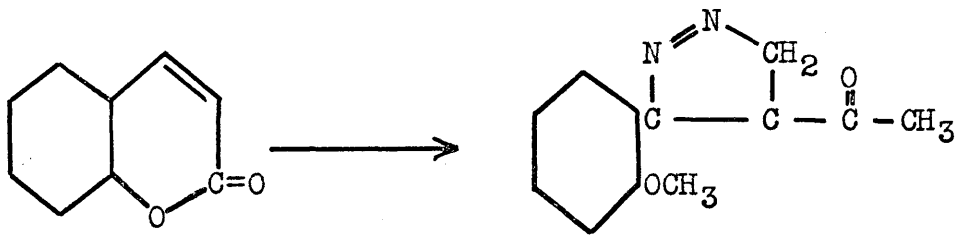
In an anhydrous medium, diazomethane will methylate acidic units such as phenolic and carboxylic without affecting aliphatic hydroxyl groups. The most common solvent for the gas is dry ether, but precipitation occurred when such a solution was added to oxylignin B dissolved in dioxane. Repeated methylations of this kind failed to raise the methoxyl content of the oxylignin beyond 12.8%, whereas diazomethane in dioxane yielded products of methoxyl content about 17%. The discrepancy might have originated in the fact that the heterogeneous system pre-

valent in the former case caused an incomplete reaction. An alternative explanation might be that diazomethane in ether fails to methylate as many kinds of acidic hydroxyl groups as diazomethane in dioxane solution. This explanation was put forward by Brauns (75) to account for some of his results when methylating certain lignin preparations.

After several methylations with diazomethane in dioxane, the percentage methoxyl actually began to decrease slightly from its maximum value of 17.0%. This decrease was coupled with an increase in nitrogen to 2.1% which might be responsible for the apparent change in methoxyl. A single treatment with diazomethane in preliminary work raised the methoxyl content from 7% to the vicinity of 16% without the appearance of any nitrogen as shown by a sodium fusion test. It is well known that diazomethane will add to double bonds. Logan (46) in methyl-



ating vanillin oxidized to $C_8H_8O_5$ with chlorine dioxide, found that 2 moles of diazomethane were consumed, one to add to a double bond in the ring and the other to methylate a hydroxyl group. Spencer and Wright (76) claimed that certain ketones were split by diazomethane in the following way. This type of reaction, they claimed, occurred in lignin and was responsible for erroneous results when diazomethane was used to determine



acidic hydroxyl groups. Either of these chemical reactions could also explain the presence of nitrogen in the methylated oxylignin. As already mentioned, the hydrogenation of oxylignin B was not inconsistent with the presence of a double bond, although not of a reactive type.

The fully methylated material isolated in practically quantitative yield and having 17.0% methoxyl, was insoluble in sodium bicarbonate and very slowly soluble in 3% sodium hydroxide. In the latter case the dissolving effect was probably connected with saponification after long standing in the alkali. The resulting solution was yellow, instead of the usual red-brown colour obtained when the original oxylignin B dissolved in alkali. Thus the grouping which was responsible for the colour change was evidently not reconverted by the alkali under these conditions. As opposed to oxylignin B, the methylated sample was insoluble in methanol, ethanol and butanol, and readily soluble in dioxane.

As noted in the Experimental Section, closely agreeing analysis assigned the formula

$$C_{32.8}\ H_{32.9}\ O_{20.2}\ Cl_{2.28}\ (OCH_3)_2$$

to the original oxylignin B on the assumptions that its molecular weight was of the order 893 and that two methoxyl groups were present in the molecule. The analysis of the fully methylated material, when calculated for five methoxyl groups, and a molecular weight of 907 became

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

while the assumption of six methoxyl groups and 1091 as the molecular weight yielded

$$C_{41.6}\ H_{42.9}\ O_{16.85}\ N_{1.63}\ Cl_{1.94}\ (OCH_3)_6 \qquad (B)$$

Even after minor and dubious corrections were made for the chlorine and nitrogen contents, it was obvious that the methylated product was either poor in oxygen (formula A) or was rich in carbon and hydrogen (formula B). The latter alternative was perhaps to be preferred, because there seemed to be no reason for oxygen to be eliminated during methylations with diazomethane. Moreover, the crude yield of the methylated product was 116% by weight, whereas the increase calculated from the increase from 6.9% to 17.1% in methoxyl content was 112%. It thus appeared likely that the product had undergone side reactions of an unknown nature with the diazomethane, or was contaminated in spite of the careful attempts made to purify it by exhaustive extraction with n-propane.

If both phenolic and carboxylic acid groups were present in the oxylignin, they would have formed methyl ethers and esters respectively during the exhaustive treatments with diazomethane. Saponification, it was hoped, would change the methyl ester groups to soluble salts and leave the methyl ethers intact.

The results of the saponifications are shown in Table IV.

TABLE IV

Saponification of Diazomethane Methylated Oxylignin B (OCH_3, 17.0%)

Run	Aq. NaOH per gm. sample		Saponification Conditions		Saponification Number(a)	Recovered % (b)
	N	cc.	hours	°C.		
1	0.1118	47.4	21	25	328	45
2	0.433	44.4	22	25	282	..
3	0.1118	52.4	8 10	100) 25)	223.5	44(c)
4	0.1118	69.4	5	100	181.5	46

(a) Grams required to neutralize one equivalent of alkali.
(b) By acidification of saponified liquor to about pH 0.5.
(c) A further 26%, with OCH_3 4.3%, recovered from mother liquor.

The methylated lignin slowly dissolved in dilute caustic soda at room temperature (Table IV, run 1) and the amount of alkali consumed was not greatly increased by nearly a four-fold increase in concentration (run 2). The use of boiling dilute alkali reduced the saponification number from about 300 to about 200, but also caused a colour change from light yellow to red-brown. This change, reminiscent of the similar colour change of the unmethylated oxylignin B in cold dilute alkali, suggested that a reaction more deep-seated than the mere hydrolysis of methyl ester groups had occurred.

Acidification of the saponified liquors precipitated about 45% of a cream coloured solid with a methoxyl content of 10% from run 1, 9% from run 3 and 7.7% from run 4. Extraction of the aqueous liquor from run 3 recovered a further 26% having 4.34% methoxyl. Thus the alkali split the methylated ester into two parts corresponding in yield and methoxyl content, 4.8% and 9.0%, to the fractions obtained by the alkaline cleavage of the unmethylated oxylignin. Taking yields into account, the average methoxyl of these two fractions from the methylated oxylignin was 7.34% or close to that of the unmethylated substance (7.0%). These analogies were explained by the assumption that the diazomethane methylated only carboxylic acid groups in oxylignin B, and that the product OCH_3, 17.0%) on saponification first reverted to oxylignin B (OCH_3, 7.0%) and then cleaved into two fractions differing in solubility and methoxyl content (OCH_3, 9% and 4.3% respectively).

If the assumption that oxylignin B contained carboxylic but not phenolic groups was correct, then esterification with methanolic hydrogen chloride should increase the methoxyl content to the level (about 17%) produced by methylation with diazomethane. Trial showed that practically all of the oxylignin dissolved in methanolic hydrogen chloride under reflux and that dilution of the solution with cold water precipitated a 58% yield of a grey substance with a methoxyl content of 16.3%. A further 22%, with the same methoxyl content, was recovered from the mother liquors as a brown solid. The result not only supported the above assumption but also demonstrated a sharp chemical difference between the oxylignin and periodate lignin. Alcoholysis of the latter was shown by Ritchie (59) to yield a substantial fraction of water-soluble propiovanillone derivatives, together with insoluble dark resins, and thus to resemble very closely the behaviour of lignin in wood as elucidated by Hibbert and his colleagues (77)(78)(79). The phenolic units responsible for the behaviour of periodate and wood lignin, however, were presumably destroyed during oxidation with chlorine dioxide, as also evidenced by the failure to obtain vanillin by further oxidation with alkaline nitrobenzene.

The results of methylating oxylignin B with dimethyl sulphate and strong caustic soda were variable and unreliable. In one case the product isolated in 63% yield by acidification and filtration, had a methoxyl content of 10.3%, but repetition of the methylation yielded a very small precipitate when acid

was added, and the methoxyl content dropped to 7.3%. Using a more concentrated solution of the oxylignin, 0.5 gm. in 4 cc. of 15% alkali, methylation and subsequent acidification produced only 0.22 gm. (44%) of material with a methoxyl content of 9.52%.

The poor recoveries after acidification agreed with the view that the basic change occurring in these methylations was cleavage or rearrangement of the oxylignin into more soluble products in the strongly alkaline conditions. Since this method of methylation and isolation would not affect carboxylic acid groups, the increase of methoxyl content from the original value of 7.0% to 10.3% probably reflected the methylation of a phenolic or aliphatic hydroxyl group in a cleavage or re-arrangement product.

With the hope that the diazomethane methylation had stabilized the oxylignin toward alkali, the methylated product (OCH_3, 17.0%) was treated with dimethyl sulphate and dilute 2% cold alkali in such a way as to keep the pH between 11 and slight acidity. In this way, on strong acidification the resulting precipitate was obtained in 89.5% yield, but the methoxyl value had dropped from 17.1% to 14.5%. The product was now easily soluble in sodium bicarbonate solution, whereas the fully methylated oxylignin had shown no acidic properties. Further methylation with diazomethane raised the methoxyl value again to 16.85% or close to that of the original methylated sample. The presence of traces of nitrogen caused the variations in

methoxyl value of the samples treated with diazomethane.

This experiment indicated the absence of aliphatic hydroxyl groups in the oxylignin. Since diazomethane would not have methylated these groups, they would have been free to react with the dimethyl sulphate in the later methylation. Then diazomethane, used to remethylate the highly acidic groups exposed by the dimethyl sulphate and alkali would have raised the methoxyl content to a value greater than that (17.1%) of the original methylated oxylignin. The fact that approximately the same level was again attained suggested that the action of the alkaline methylation had been restricted to a partial saponification of the ester groups previously introduced by diazomethane.

Summary of Methylations.

The methylations just discussed may be summed up as follows.

1. Original Oxylignin B - 6.93% methoxyl - 2 methoxyls in 896 gm. This agrees with molecular weight determinations of 900 and 980.

2. Diazomethane methylated - 17.06% methoxyl or 17.42% calculated on nitrogen-free basis - 5 methoxyls in 890 or 6 in 1068. This does not fit in with the values for oxylignin B and indicates the possibility of some other reaction with the diazomethane.

3. Methanolic-HCl methylated - 16.20% methoxyl on a portion representing 82% of the original oxylignin - 5 methoxyls in 956. This indicates the addition of 3 methoxyls to highly acidic (carboxyl) groups.

4. Dimethyl sulphate on No. 2 above - 14.54% methoxyl - 5 methoxyls in 1065 which indicates the loss of one group assuming that the 6 in 1068 had been correct. Thus one methoxyl group was present as an ester which was readily saponified by the alkali.

5. Diazomethane methylation on No. 4 - 16.87% methoxyl - 6 methoxyls in 1104. This indicates that the methoxyl lost above was readily replaced by the diazomethane. No nitrogen was determined on 4 and 5.

6. Saponification of No. 2 - The results varied according to conditions used. The more drastic treatments gave products (in poor yields) with methoxyl values close to the oxylignin B. The most drastic cook gave saponification value of 181.5 - 5 carboxyls in 908 and the recovered product contained 7.68% methoxyl.

7. Diazomethane Methylation of alkali treated oxylignin - Oxylignin B treated with 4% alkali and then methylated with diazomethane gave a product with 18.02% methoxyl calculated on a nitrogen-free basis. This corresponds to 6 methoxyls in 1032 gm.

EXPERIMENTAL

Preparation of Periodate Lignin

Logs of black spruce approximately 70 years of age and 4 feet in length were cut into sticks about 2 inches square which were chipped, and the chips reduced in a Mead mill to particles about 1 mm. by 5 mm. in size. After air-drying for a week, the batch was ground in a Wiley mill to a powder of approximately 40 mesh. To remove fats, waxes, and other extraneous materials, the wood meal was extracted for 48 hours in a large Soxhlet extractor using the constant boiling mixture 1:2 of alcohol-benzene. This treatment removed 1.9% of a brown gum. The residue, after drying in the air for one day, lost another 0.5% by weight when extracted for 24 hours with alcohol.

(A) The resulting wood meal was dried in the air for several days and was then oxidized with aqueous trisodium paraperiodate as outlined by Ritchie and Purves (16) but on a larger scale. The sodium paraperiodate was prepared as given in the paper, by passing bromine into a hot 10% caustic soda solution of sodium iodide.

Sodium paraperiodate (204 gm.) contained in a 12-liter round-bottom flask, was mixed with 6050 gm. of distilled water and 300 cc. of glacial acetic acid to give a pH of 4.5. Another 80 cc. of acetic acid was added to give a pH of 4.2.

The solution, milky at first, became quite clear after several hours of stirring. Extracted wood meal (500 gm.) was then added and the mixture was stirred at room temperature for 24 hours. The recovery of iodate and any unused periodate, the washing of the wood residue and its extraction with boiling water were conducted as described in the original paper (16). Five such treatments gave a lignin relatively free of holocellulose in 25% yield (125 gm.). The material was dried through methanol and benzene, and then for a week in vacuum at room temperature. The odour of benzene remained, but was finally removed under vacuum at 50°C.

(B) Using the same method of preparation, periodate lignin was also made from the small chips about 1 mm. by 5 mm. taken from the Mead mill. In the preliminary solvent extraction, 3.8 gm. (1.5%) of a brown, very viscous oil was removed from 255 gm. of wood by the alcohol-benzene mixture. Since the subsequent 24-hour extraction with alcohol only removed 0.5 gm. (0.2%) of oil, the total extractives (1.7%) were considerably less than the value (2.4%) found for the same wood in a finer state of division. Six periodate oxidations were then necessary to achieve a lignin relatively free of holocellulose.

Analyses of Periodate Lignin

Methoxyl

The method of Vieböck and Schwappach (80) as modified by Clark (81) gave values of 11.35, 11.37 and 11.42% for the periodate lignin A prepared from the finely ground wood. On

the preparation from coarsely ground wood (periodate lignin B) the values were 11.28, 11.30 and 11.38%.

Klason Lignin

The method using 72% sulphuric acid as described in the TAPPI Standards (82) gave 88.4 and 88.8% Klason lignin in the periodate lignin A.

Holocellulose

Using the method described by Kurth and Ritter (31), eleven successive brief chlorinations and extractions with alcohol-pyridine finally gave a holocellulose content of 4.00% and 4.12% on the lignin preparation (A). The lignin (B) from the coarse wood meal, after five periodate oxidations, contained 10.60% and 10.75% of holocellulose. After one more oxidation with sodium paraperiodate the value, found by the alcohol-ethanolamine method of Van Beckum and Ritter (33) was 2.46% and 3.23%. Nine chlorination-extraction cycles were necessary in this case.

Pulpability

To check the solubility of the periodate lignin in a standard bisulphite cook, an 0.3 gm. sample was weighed into a 20 mm. glass tube, and to this was added a solution containing 5% of free sulphur dioxide and 0.90% combined as a calcium salt. These values were determined by the method described by Palmrose (83). In this method the total sulphur dioxide is calculated from a titration with potassium iodate and then the free acids are titrated with sodium hydroxide to give the free sulphur di-

oxide. The concentration of combined sulphur dioxide was obtained by the difference of these two values. The glass tube was sealed, placed in a metal bomb, surrounded by water, and the cap screwed onto the bomb. The assembly was then agitated for 6 hours in an oil bath thermostatically controlled at 135°C. Since the lignin residue left after the cook was a gel which could not be recovered by filtration, the suspension was centrifuged and the residue washed several times to remove all the inorganic salts. The remaining material, after maceration in alcohol, could be readily filtered through a tared sintered glass crucible, and the light brown residue was dried *in vacuo* at room temperature. Insolubles found: 9.2, 8.8%.

Ash

The micro procedure described by Niederl and Niederl (84) was used. Found for lignin (A), 2.4, 2.2%.

Oxidations of Periodate Lignin with Sodium Chlorite

All oxidations carried out in the vicinity of pH 4.1 were buffered with the McIlvain Standard Buffer Solution (62) of citric acid and disodium phosphate. In an attempt to keep the pH constant, the buffer was used in six times the recommended strength.

The small-scale experiments were conducted on 1 gm. or 0.5 gm. samples using the quantities of buffer and oxidant shown in the Tables. A blank containing everything but the lignin was treated under identical conditions. Both 125 cc.

Erlenmeyer flasks were placed side by side in a thermostatically controlled bath. At regular intervals, 0.5 cc. aliquots were withdrawn, 1 gm. of potassium iodide and 5 cc. of 10% sulphuric acid were added, and the liberated iodine was titrated with 0.05 N sodium thiosulphate. In a typical experiment, an 0.5 cc. aliquot required x cc of 0.05 N sodium thiosulphate, whereas the blank at the corresponding time required y cc. If the weight of the sample was w gm. in a volume of 47 cc. and since 4 moles of thiosulphate equal 1 mole of chlorite, the consumption of chlorite by 1 gm. of sample was

$$\frac{(y - x) \times 0.05 \times 47}{w \times 4 \times 0.5 \times 1000} \text{ moles} .$$

However, as previously discussed, there were certain complicating factors which made this calculation only of semi-quantitative significance.

TABLE V

(See Fig. 1, plots 1, 2, 3.)

Oxidation at 30°C. of 1 gm. Lignin by 0.0246 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1

	Millimoles of $NaClO_2$		
Hours	Blank	Sample	Consumed
0.0	24.6	24.6	0.0
0.5	23.9	18.8	5.1
1.0	23.5	16.5	7.0
2.0	22.9	14.2	8.7
4.0	22.8	11.8	11.0
6.0	21.7	9.1	12.6
8.0	21.5	7.4	14.1
10.0	21.3	5.7	15.6
12.0	21.1	5.0	16.1
18.0	20.8	2.1	18.7
24.0	19.7	0.4	19.3
30.0	18.5	0.3	18.2
35.0	17.7	0.3	17.4

In 12 hours the solid had completely dissolved, leaving a light brown solution with a pH of 4.18 at the end of 35 hours. The blank at the same time had a pH of 4.58. At the end of this reaction period, an 0.5 cc. aliquot corresponded to only 0.25 cc. of 0.0488 N thiosulphate, but the blank needed 16.0 cc. of thiosulphate and contained a considerable amount of chlorine dioxide. The value for millimoles of chlorite used after 30 hours was spurious for there was such a small amount of oxidant left that the additional amount consumed in the oxidation was less than the spontaneous decomposition of the blank.

TABLE VI

Oxidation at 23 to 25°C. of 1 gm. Lignin by 0.0254 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1

	Millimoles of $NaClO_2$		
Hours	Blank	Sample	Consumed
0.0	25.4	25.4	0.0
0.5	25.1	18.4	6.7
1.0	25.0	17.5	7.5
2.0	24.3	15.4	8.9
4.0	23.8	12.2	11.6
6.0	23.4	10.0	13.4
8.0	22.5	8.4	14.1
10.0	22.4	7.1	15.3
12.0	22.4	5.6	16.8
22.0	21.2	2.4	18.8
30.0	19.9	1.0	18.9

A small amount of yellow powder was still present after 22 hours, but was almost completely dissolved by 30 hours. The number of millimoles of chlorite consumed in 30 hours was spurious for the same reason as in the previous Table.

TABLE VII

(See Fig. 1, plots 4, 5, 6.)

Oxidation at 20.3°C. of 1 gm. Lignin by 0.0253 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1

	Millimoles of $NaClO_2$		
Hours	Blank	Sample	Consumed
0.0	25.3	25.3	0.0
0.5	24.9	19.6	5.3
1.0	24.6	18.2	6.4
2.0	24.5	16.6	7.9
4.0	24.2	14.1	10.1
6.0	24.1	12.0	12.1
8.0	23.3	10.7	12.6
10.0	23.1	9.6	13.5
12.0	23.1	8.7	14.4
22.0	21.8	6.0	15.8
30.0	21.6	4.9	16.7
46.0	19.6	3.3	16.3
59.0	19.3	2.4	16.9

A faintly yellow solid was centrifuged out of the solution after 59 hours. It was washed with alcohol, then ether, and finally recovered on a sintered glass crucible. Weight, 0.38 gm.

TABLE VIII

(See Fig. 1, plots 7, 8, 9.)

Oxidation at 8 to 10°C. of 1 gm. Lignin by 0.0253 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1

	Millimoles of $NaClO_2$		
Hours	Blank	Sample	Consumed
0.0	25.3	25.3	0.0
0.5	25.3	22.2	3.1
1.0	25.0	21.9	3.1
2.0	25.3	20.5	4.8
4.0	25.2	18.6	6.6
6.0	25.1	17.6	7.5
8.0	25.6	17.0	8.6
10.0	25.0	14.9	10.1
12.0	25.1	15.1	10.0
24.0	24.4	11.2	13.2
30.0	24.4	10.1	14.3
36.0	24.2	8.6	15.6
48.0	24.0	7.7	16.3
54.0	23.4	6.8	16.6
72.0	23.9	5.4	18.5
96.0	23.0	3.5	19.5

At the end of 12 hours the solid particles in the solution appeared very little changed in appearance and quantity. Within 48 hours the brown colour changed to light yellow, but even after 96 hours as much as 25% of this powder remained insoluble and was isolated as described previously.

TABLE IX

(See Fig. 2, plots 1, 2, 3.)

Oxidation at 30°C. of 1 gm. Lignin by 0.0508 Mole of Chlorite in 47 cc. of Solution at Initial pH 4.1

	Millimoles of $NaClO_2$		
Hours	Blank	Sample	Consumed
0.0	50.8	50.8	0.0
0.5	50.4	43.8	6.6
1.0	48.9	40.2	8.7
2.0	48.6	37.6	11.0
4.0	45.7	32.0	13.7
6.0	45.3	26.3	19.0
8.0	44.7	23.8	20.9
10.0	41.9	19.9	22.0
12.0	40.4	17.2	23.2
16.0	38.9	13.9	25.0
26.0	33.7	8.4	25.3
31.0	33.4	5.7	27.7
36.0	31.5	3.4	28.1
41.0	29.2	2.7	26.5
55.0	26.2	0.4	25.8

In calculating the number of moles of chlorite used in 41 and 55 hours, there was an apparent drop, for reasons previously explained. The aliquots withdrawn from the blank and sample solutions for titrations were 0.25 cc. Within 8 hours a very small amount of solid remained, and by 16 hours the solution was homogeneous. At the end of 55 hours the blank was red-brown in colour, had a pH of 4.88, and contained a considerable amount of chlorine dioxide. The other solution was yellow, had

a pH of 4.32 and contained less chlorine dioxide than the blank. Nitrogen was bubbled through both solutions until the odour of chlorine dioxide was no longer present. The blank became completely colourless, whereas the other solution became darker in colour. After standing for a time the yellow coloration returned to the blank.

TABLE X

(See Fig. 2, plots 4, 5, 6.)

Oxidation at 30°C. of 1 gm. Lignin by 0.0492 Mole of Chlorite in 87 cc. of Solution at Initial pH 4.1

	Millimoles of $NaClO_2$		
Hours	Blank	Sample	Consumed
0.0	49.2	49.2	0.0
0.5	48.9	43.1	5.8
1.0	47.4	41.8	5.6
2.0	47.2	38.8	8.4
4.0	46.9	35.2	11.7
6.0	45.4	32.3	13.1
8.2	45.0	29.3	15.7
10.0	43.5	27.5	16.0
12.0	42.7	26.2	16.5
24.0	39.1	21.6	17.5
32.0	37.2	19.5	17.7
48.2	32.9	16.9	16.0
120.0	19.7	6.7	13.0

The solution was homogeneous after 48 hours.

Using concentrations and conditions identical with those shown in Table IX, the pH of both the blank and the sample

solutions were noted at regular intervals.

TABLE XI

Change with Time in pH of Solutions and Blank(a)

Hours	pH of Sample	pH of Blank
0.0	4.10	4.10
0.5	4.40	4.18
1.0	4.49	4.21
2.0	4.54	4.30
4.0	4.67	4.39
6.0	4.74	4.46
8.0	4.78	4.50
10.0	4.79	4.54
12.0	4.80	4.60
22.0	5.10	5.00
32.0	4.90	4.90
46.0	4.78	5.00
57.0	4.46	4.98
82.0	4.40	4.97

(a) One gram lignin, 0.0508 mole of sodium chlorite in 47 cc. at 30°C.

After 12 hours there was very little solid left. By 22 hours the solution was homogeneous, and the pH at its maximum.

Sodium Chlorite Oxidations with Expulsion of Chlorine Dioxide

All these experiments were conducted with 1 gm. of lignin at 30°C. and initial pH of 4.1 just as in previous cases save that a rapid stream of air or nitrogen was bubbled through the

mixture for the periods desired. The compressed air used was first filtered through two towers containing absorbent cotton to remove any suspended materials. Either gas stream removed chlorine dioxide as soon as it was formed. The results are given in the following Tables.

TABLE XII

(See Fig. 3, plots 1, 2, 3.)

Oxidation at 30°C. of 1 gm. Lignin by 0.0236 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1

	Millimoles of $NaClO_2$		
Hours	Blank	Sample	Consumed
Nitrogen Used for 22 Hours			
0.0	23.6	23.6	0.0
0.5	23.5	17.8	5.7
1.0	22.7	16.1	6.6
2.0	22.6	15.2	7.4
4.0	22.4	13.8	8.6
6.0	21.7	13.0	8.7
8.0	21.8	12.6	9.2
10.0	21.7	12.3	9.4
22.0	20.8	11.0	9.8
Nitrogen Discontinued			
24.0	21.4	10.1	11.3
26.0	20.8	9.2	11.6
28.0	20.2	7.4	12.8
30.0	20.4	6.8	13.6
34.0	19.2	5.5	13.7
46.0	18.6	1.6	17.0
50.0	18.0	0.9	17.1
71.0	16.5	0.3	16.2
95.0	14.4	0.3	14.1

After 71 and 95 hours only 0.0003 mole of oxidant was present in the sample solution, whereas in the blank 0.0165 and 0.0144 moles were still present. The continued decomposition of the blank solution caused the apparent drop in consumption noted for 71 and 95 hours. A cream coloured powder (0.25 gm.) remained insoluble at the end of the experiment.

TABLE XIII

(See Fig. 4, plots 1, 2, 3.)

Oxidation at 30°C. of 1 gm. Lignin by 0.0239 Mole of Chlorite in 45 cc. of Solution at Initial pH 4.1

	Millimoles of $NaClO_2$		
Hours	Blank	Sample	Consumed
Compressed air used for 10 hours			
0.0	23.9	23.9	0.0
0.5	23.5	17.7	5.8
1.0	23.4	16.3	7.1
2.0	23.5	14.6	8.9
4.0	22.3	13.0	9.3
6.0	21.6	12.0	9.6
8.0	21.4	11.5	9.9
10.0	20.8	10.7	10.1
Air discontinued			
12.0	20.8	10.3	10.5
14.0	20.3	9.3	11.0
24.0	19.3	5.9	13.4
34.0	17.8	2.6	15.2
50.0	15.9	0.3	15.6

Only a trace of an almost white powder remained undissolved after 50 hours.

TABLE XIV

Oxidation at 30°C. of 1 gm. Lignin by 0.0504 Mole of Chlorite in 47 cc. of Solution at Initial pH 4.1

Hours	Millimoles of $NaClO_2$ Blank	Sample	Consumed
Nitrogen used for 32 hours			
0.0	50.4	50.4	0.0
0.5	47.9	40.6	7.3
1.0	47.3	39.4	7.9
2.0	46.7	36.4	10.3
4.0	44.6	34.8	9.8
6.0	43.0	33.8	9.2
8.0	41.6	31.9	9.7
10.0	41.2	30.9	10.3
12.0	40.5	29.8	10.7
22.0	38.8	27.6	11.2
32.0	36.7	24.9	11.8
Nitrogen discontinued			
46.0	35.5	23.4	12.1
47.0	35.4	21.6	13.8
52.0	34.8	20.8	14.0
58.0	34.0	18.8	15.2
70.0	33.3	15.8	17.5
82.0	31.6	12.4	19.2
95.0	30.3	10.5	19.8
118.0	28.1	7.3	20.8

After 46 hours the pH of the blank had risen to 5.00 and that of the other solution to 5.10. The solid particles in the oxidizing

solution had almost completely dissolved by 70 hours.

As long as nitrogen or air was bubbled through the blanks they remained colourless, but when the gas flow was discontinued, the solutions attained a green-yellow colour and the odour of chlorine dioxide was soon noted in abundance. The sample solutions remained faintly yellow in colour as long as the chlorine dioxide was being removed as described, but became much darker when the gas was allowed to accumulate.

TABLE XV

(See Fig. 4, plots 4, 5, 6.)

Oxidation at 30°C. of 1 gm. Lignin by 0.0501 Mole of Chlorite in 47 cc. of Solution at Initial pH 4.1

	Millimoles of $NaClO_2$		
Hours	Blank	Sample	Consumed
Nitrogen used for 8 hours			
0.0	50.1	50.1	0.0
0.5	48.8	40.4	8.4
1.0	48.0	39.3	8.7
2.0	47.0	37.0	10.0
4.0	45.6	35.0	10.6
6.0	44.8	34.3	10.5
8.0	43.6	32.8	10.8
Nitrogen discontinued			
10.0	44.1	31.0	13.1
12.0	43.3	30.4	12.9
22.0	41.3	23.2	18.1
26.0	38.9	20.1	18.8
30.0	37.7	17.9	19.8
34.0	37.2	16.2	21.0
46.0	35.3	11.3	24.0
58.0	32.9	8.6	24.3
70.0	30.8	7.4	23.4
80.0	29.2	5.1	24.1

A slight amount of solid remained insoluble at 30 hours, but disappeared completely by 34 hours.

Oxidations with Chlorine Dioxide

The method of Schacherl (85) as used by Husband (45) and Logan (46) was employed to prepare chlorine dioxide solutions. Potassium chlorate (25 gm.) was mixed with 20 gm. of crystalline oxalic acid dihydrate contained in a 250 cc. round-bottom flask, and a cooled solution of 33% sulphuric acid (80 cc.) was added. The reaction proceeded according to the equation

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

The carbon dioxide evolved decreased the danger of an explosion, which was quite possible if the chlorine dioxide had been generated alone. Using crystalline oxalic acid dihydrate the reaction flask could be heated to 50°C. to start the evolution of the gas, but a certain amount of precaution was necessary to prevent too violent a reaction. In several preparations, in the absence of the crystalline oxalic acid, the anhydrous powder was used. In these cases the mixture had to be cooled in an ice bath and kept at this temperature until most of the reactants had been consumed. Even at room temperature the evolution of gas became so violent that in one preparation the apparatus was blown apart.

After passing through a trap to catch any foam, the gas was bubbled through a concentrated solution of sodium chlorite to convert any chlorine to chlorine dioxide.

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

Another trap was arranged to catch any sodium chlorite solution

which might be driven over by a sudden rush of gas. The latter was finally collected in a 250 cc. jar containing ice-cooled distilled water. The all-glass apparatus was blackened to prevent the decomposition of the chlorine dioxide by light.

To analyze the solution of chlorine dioxide, 1 cc. was added to 25 cc. of a citric acid - sodium phosphate buffer at pH 6.9. Approximately 1 gm. of potassium iodide was added, and the solution was titrated with 0.05N sodium thiosulphate, using 2 drops of starch indicator (titration A). Then, after the addition of 5 cc. of 10% sulphuric acid, the liberated iodine was further titrated to the end-point in the presence of more starch indicator (titration B). The difference between A and B gave a value C which, when multiplied by five-fourths, was equivalent to the chlorine dioxide content (45). The chlorine content was calculated from the difference between A and one-quarter of C. Since the chlorine concentration was found to be very low in all preliminary trials, in later work the neutral titration was eliminated and the quantity of chlorine dioxide was calculated from the amount of iodine liberated in an acid solution. In iodometry the equivalent of 5 for chlorine dioxide was used. The following Tables indicate the course of the reaction with chlorine dioxide under varying conditions.

TABLE XVI

Oxidation at 30°C. of 1 gm. Lignin by 0.0171 Mole of Chlorine Dioxide in 78 cc. of Solution at Initial pH 4.1

	Millimoles of ClO_2		
Hours	Blank	Sample	Consumed
0.0	17.1	17.1	0.0
0.5	15.1	11.7	3.4
1.0	14.9	10.3	4.6
2.0	14.9	7.1	7.8
4.0	14.1	5.6	8.5
6.0	14.5	4.9	9.6
8.0	12.7	2.9	9.8
10.0	14.7	3.3	11.4
12.0	12.9	2.6	10.3
24.0	11.4	0.6	10.8
32.0	11.5	0.5	11.0

A light brown solid (0.075 gm.) was left at the end of this time. The remaining solution was acidified to pH 1 with 10 cc. of concentrated sulphuric acid previously diluted to 50 cc. with water. The resulting white precipitate after solution in alcohol and re-precipitation by ether gave a fawn coloured solid in 22.7% yield.

TABLE XVII

(See Fig. 5, plots 1, 2.)

Oxidation at 30°C. of 0.5 gm. Lignin by 0.0179 Mole of Chlorine Dioxide in 46.5 cc. of Solution at Initial pH 4.1

	Millimoles of ClO_2		
Hours	Blank	Sample	Consumed
0.0	17.9	17.9	0.0
0.5	17.0	14.5	5.0(a)
1.0	16.9	12.0	9.8
2.0	15.8	10.4	10.8
4.0	13.0	7.4	11.2
6.0	12.4	6.2	12.4
8.0	10.3	4.9	10.8
10.0	9.7	4.3	10.8
22.0	6.7	1.3	10.8
26.5	5.7	0.9	9.6

(a) Since an 0.5 gm. sample was used, the millimoles of chlorine dioxide consumed per gm. is calculated as twice the difference between the number of millimoles in the blank and the sample.

In 4 hours a yellow solid was still present in the solution; in 6 hours very little was left, and by 8 hours the solution was homogeneous. At the end of the experiment, after forcing out the residual chlorine dioxide by bubbling nitrogen, the titration of the iodine liberated in acid solution by a 1 cc. aliquot amounted to 0.10 cc. of 0.05 N sodium thiosulphate. Four ether extractions in a separatory funnel removed 0.032 gm. of a brown oil. Acidification of the aqueous portion yielded the cream coloured solid, which, after the usual treatment

with alcohol and ether gave a 19.7% yield of oxylignin B. Another ether extraction, as above, on the acidified solution, extracted 8% of a brown gum.

TABLE XVIII

Oxidation at 25°C. of 1 gm. Lignin by 0.0219 Mole of Chlorine Dioxide in 43 cc. of Solution at Initial pH 0.92

	Millimoles of ClO_2		
Hours	Blank	Sample	Consumed
0.0	21.9	21.9	0.0
0.5	19.4	13.0	6.4
1.0	19.1	11.4	7.7
2.0	16.9	8.7	8.2
4.0	16.0	7.3	8.7
6.0	15.4	5.4	10.0
10.0	12.8	3.8	9.0
12.0	12.2	3.4	8.8
24.0	8.5	1.1	7.4
30.0	7.8	0.5	7.3
45.0	5.3	0.1	5.2

At the end of this time 0.5756 gm. of yellow-brown solid was at once recovered on a sintered glass filter. The material was readily soluble in alcohol, indicating it to be an oxylignin B. Further acidification of the filtrate yielded no more precipitate. After 6 hours the drop in apparent consumption in the above Table as in the other Tables, merely showed that the blank was decomposing at a faster rate than the oxidant

was being used by the sample.

Isolation of Products from Chlorite Oxidations

(a) Five grams of periodate lignin was stirred in a 500 cc. Erlenmeyer flask with a solution of 100 cc. of citric acid - sodium phosphate buffer at pH 4.1, and 10 gm. of sodium chlorite dissolved in 100 cc. of water. Within 5 minutes the brown lignin began to turn yellow and chlorine dioxide was evolved in large quantities from the solution. A much smaller evolution occurred from the blank. The reaction was slightly exothermic and the temperature rose spontaneously from 25 to 29°C. After 24 hours the remaining solid, which had become almost white in colour, was removed by centrifuging at 3000 r.p.m. A previous attempt to isolate the material by filtration through a sintered glass funnel was unsuccessful because of the formation of a gel. The product was washed in the centrifuge with a small amount of cold water, and the solid again isolated by centrifuging. The resulting gel was broken up in alcohol and the solid allowed to settle. The clear solution, which had dissolved a small amount of material, was decanted and the remaining powder stirred up with ether. After recovery on a medium porosity sintered glass crucible and drying for 5 days at room temperature under 0.2 mm. pressure, 2.75 gm. of a cream coloured powder remained. This powder, insoluble in the buffer at pH 4.1 and also in ethanol, was designated as oxylignin A.

The aqueous solution from the above was extracted three

times with 50 cc. volumes of ether. The faintly yellow ethereal solution was dried for 24 hours over anhydrous sodium sulphate and then concentrated to dryness. A small amount (1%) of brown gum remained after evaporation of the ether.

The aqueous layer was then taken to dryness under vacuum, and the brown residue washed several times with dioxane which removed a brown viscous oil. After removal of the solvent under reduced pressure, the residue was distilled at 85 microns pressure and 105°C. A faint yellow oil was collected in the receiver at first, but when the distillation temperature reached 120°C., the distillate began to turn brown and the pressure rose to 110 microns, indicating decomposition. Approximately 0.4 cc. of the faintly yellow oil was collected. It had a methoxyl content of 9.45% and a refractive index of 1.449.

(b) In a second experiment, using three times the quantities just described, with an initial pH of 3.98 and at 28 to 30°C., the solid dissolved completely in 12 hours.

(c) A water bath at 24 to 26°C. controlled the temperature in another oxidation. Three grams of lignin was used with 10 gm. of sodium chlorite in 100 cc. of water and 108 cc. of buffer of pH 4.13. After 30 minutes, 2, 8 and 25 hours the pH was observed to be 4.32, 4.50, 4.60 and 4.70 respectively. Oxylignin A was isolated as a cream coloured solid in 76% yield, and when the aqueous solution was acidified to pH 1 with sulphuric acid, only a slight precipitate appeared.

(d) Oxylignin A was also obtained in varying yields from several of the small-scale experiments described in the preceding pages and by the plots in Figs. 1 to 4.

The clear aqueous solutions from the chlorite oxidations, usually ending near pH 4.3, were acidified with dilute sulphuric acid (50 cc. containing 10 cc. of concentrated acid). to a pH of 1 or less. When the yield of oxylignin A was low, the acidification caused a larger amount of oxylignin B to precipitate, and a high yield of A corresponded to a low yield of B. The methods of isolation of this oxylignin B are given in the Discussion.

(e) The oxidation described in Table IX yielded 0.37 gm. (37%) of oxylignin B. The powder, dried for 48 hours in a vacuum desiccator at 0.2 mm. pressure, had a methoxyl content of 10.00, 9.85%.

(f) The oxidation described in Table X yielded 0.1040 gm. of oxylignin B by the usual technique of isolation. Another 0.1660 gm. was isolated when the alcohol-ether solution was concentrated to 5 cc. and again diluted with ether. Evaporation of the remaining solution left 0.0260 gm. of white powder.

(g) After 1 gm. of lignin had been treated for 12 hours with 0.0492 mole of sodium chlorite in 87 cc. of solution at pH 4.1 and 30°C., 0.2716 gm. of oxylignin A was isolated, and 0.1874 gm. of oxylignin B was removed in the usual way. Concentration of the alcohol-ether solution and further precipitation

with ether gave another 0.1108 gm. of oxylignin B.

(h) One gram of lignin buffered with sulphuric acid to pH 0.9 was treated with 0.034 mole of sodium chlorite in 47 cc. of solution at 25°C. The sodium chlorite decomposed very rapidly and within 8 hours titrations showed no more oxidant present in the solution. The brown coloured solid left undissolved was removed by the centrifuge, washed with water, then alcohol, and finally separated from the liquid on a sintered glass crucible. After washing with ether and drying at room temperature under 0.1 mm. pressure the product (0.86 gm.) looked exactly like the original brown lignin powder and was likewise insoluble in alcohol. The aqueous solution on further acidification did not yield any more solid.

Isolation of Products from Chlorine Dioxide Oxidations

(a) At initial pH 4.1 the isolation of oxylignins A and B from some of the small-scale experiments has already been mentioned. More of these experiments will now be described. In all cases where 0.5 or 1.0 gm. samples were used, the reaction was conducted in a thermostatically controlled bath, using 125 cc. Erlenmeyer flasks as reaction vessels. The solution was agitated by continuous stirring.

When a half-gram sample of lignin was oxidized at pH 4.1 and 25°C. for 8 hours in 46.5 cc. of solution containing 0.011 mole of chlorine dioxide, the pH dropped to 3.9 in the

blank and to 3.0 in the other flask. The insoluble material was removed on a sintered glass crucible, was washed with water and then with alcohol. Nothing dissolved in these liquids and 27.6% of oxylignin A remained on the filter. The aqueous filtrate was acidified with dilute sulphuric acid to a pH less than 1 and the oxylignin B was isolated in the usual way and in 18.4% yield.

Using similar conditions except that the reaction was conducted in blackened Erlenmeyer flasks, 25.3% of oxylignin A was obtained and 19.2% of B. Light therefore had little effect on the oxidations.

In another case, 1 gm. of lignin was treated at 25°C. and pH 4.1 with 0.0134 mole of chlorine dioxide in 43 cc. of solution. A considerable amount of solid remained undissolved after 21.5 hours and the pH dropped to 2.8 whereas in the blank the final value was 3.4. The solid was removed on a sintered glass crucible and put back into 41 cc. of solution containing 0.0147 mole of chlorine dioxide buffered to pH 4.15 and also at 25°C. After 4.5 hours only a trace of solid remained undissolved, but the reaction was allowed to continue for 25 hours, when the pH of the sample and blank solutions were 3.58 and 3.68 respectively. Acidification of the aqueous solution from the first oxidation yielded no trace of a precipitate of oxylignin B but 0.4135 gm. was isolated after the second oxidation. The aqueous solution from the first oxidation was continuously extracted with ether for 48 hours. On evaporating the extract

to dryness a light brown gum (oxylignin C) remained in 36% yield based on the weight of the original lignin. The second aqueous fraction was similarly treated and yielded a very viscous, clear orange coloured oil in 68.5% yield. Thus the initial oxidation yielded an undetermined amount of oxylignin A, no oxylignin B and 36% of oxylignin C, whereas after the second oxidation no A remained but 41.4% of B and 68.5% of C were obtained.

(b) One-half gram of lignin was treated for 8 hours at 30°C. with 35.5 cc. of unbuffered solution containing 0.0185 mole of chlorine dioxide. The pH, without a buffer to control it, dropped from about 4 to 1.4. To the solid left after centrifuging, alcohol was added to dissolve the oxylignin B. A 4.8% yield of oxylignin A remained, while 34.2% of B was obtained from the extract. Acidification of the aqueous solution gave no more precipitate.

A 9.5-hour treatment of 0.5 gm. of periodate lignin with 34 cc. of solution containing 0.021 mole of chlorine dioxide produced no oxylignin A and a 45.2% yield of oxylignin B. A similar experiment for 2 hours furnished yields of 36.2% of A and 26.2% of B, and from a 4-hour oxidation 8.1% of A and 35.2% of B was obtained.

Larger-Scale Oxidations with Chlorine Dioxide

(a) Four grams of periodate lignin was stirred for 8 hours in 200 cc. of solution containing 0.1 mole of chlorine dioxide at room temperature (24 to 28°C.). Within 3 hours,

the light brown solid became yellow, and the pH of the solution dropped from 3 to 0.8. At the end of the oxidation, the solid was removed by centrifuge and washed with 100 and then 50 cc. of distilled water. On addition of 50 cc. of alcohol it readily dissolved, leaving a turbid solution. Centrifuging once more removed the suspension, leaving a clear orange coloured supernatant liquor. The solid at the bottom of the centrifuge tube dried to a hard grey film which was then powdered in ether and filtered through a sintered glass crucible. Found: Yield 1.5%; OCH_3 3.84, 3.95%.

The addition of ether to the alcoholic solution precipitated a cream coloured powder which was removed from the liquid phase by centrifuging, was suspended in ether and recovered on a sintered glass funnel. The solid, before solution in alcohol, sometimes appeared as a gel with a great deal of water entrapped in it. In such cases the addition of ether to the alcoholic solution resulted in the precipitation of an oil or greasy solid. This material had to be redissolved in alcohol and again precipitated by the addition of 3 volumes of ether. Sometimes it was even necessary to repeat this process in order to attain the usual type of cream coloured oxylignin B. It was also noticed that a cleaner, less greasy solid appeared if the alcoholic solution was added with stirring to a large excess of ether. In order to obtain more oxylignin B, the alcohol-ether solution could be concentrated to a very small volume and then again diluted with ether. However, if

this volume was concentrated too far, the addition of ether caused the re-appearance of an oily material. Owing to the presence of residual traces of sulphuric or hydrochloric acid, the alcoholic solution was concentrated under vacuum at room temperature to lessen the danger of formation of ethyl (or methyl) esters with the acids as catalysts, and for the same reason the oxylignin B was isolated immediately after its precipitation. With these precautions the yield of oxylignin B was raised to 49.5%. Found: Methoxyl, 6.85, 7.00%; Chlorine, 7.8, 7.9, 8.1%.

Evaporation of the alcohol-ether mother liquors at room temperature under reduced pressure left a dark brown granular solid in 13.7% yield. Found: Methoxyl, 12 to 13%; Chlorine, 8.9%. This raised the total recovery of solid to 64.7%.

After blowing out the chlorine dioxide from the aqueous solution by bubbling nitrogen through it for 20 minutes, 1 cc. liberated only enough iodine from an acidified potassium iodide solution to react with 0.5 cc. of 0.05 N sodium thiosulphate. The bulk of the solution was then continuously extracted with ether for 22.5 hours and the extract washed four times in a separatory funnel with a dilute solution of ferrous ammonium sulphate until the aqueous portion showed only a light red colour with a potassium thiocyanate solution. In the presence of ferric ions a deep red colour appears. The ethereal fraction was then dried over anhydrous sodium sulphate for 2.5 hours and

finally evaporated to dryness leaving a 2.1% yield of brown gum. Another 25-hour extraction yielded 3.1% of a similar gum. A 140-hour extraction which followed, removed a clear faintly yellow gum in 21.3% yield. A similar product in 15.9% yield, was obtained by a 3-day ether extraction of the ferrous ammonium sulphate solution. Both of the latter extracts exploded violently when heated on a spatula and readily liberated iodine from an acidified solution of potassium iodide. They presumably contained peroxides. Like the powdered products, these oils and gums were dried for several days *in vacuo* at room temperature.

(b) In another experiment the products were isolated in a slightly different manner.

Twelve grams of lignin was stirred for 10 hours in 835 cc. of solution containing 0.3 mole of chlorine dioxide. The remaining solid was separated by centrifuge and then washed twice with a total of 150 cc. of dilute hydrochloric acid. An aliquot of the solid was dried directly in a desiccator under vacuum giving 1.226 gm. of cream coloured powder. Found: Methoxyl, 4.80, 4.87%.

The remaining aliquot was dissolved in methyl alcohol and then centrifuged to remove the grey residue left (0.319 gm.) Found: Methoxyl, 3.84, 3.96%.

When the methanolic solution was diluted with ether a brown oil precipitated. This oil was redissolved in methanol and then again precipitated with ether, but this time the usual

oxylignin B powder appeared. On standing for 16 hours, more solid came out of solution and was combined with the first portion. Found: Methoxyl, 6.90, 6.88, 6.84%. The 4.180 gm. recovered here raised the total recovery of solid from this experiment to 45.3%.

(c) The aqueous solution from another oxidation, after removal of the oxylignin B, was aerated with nitrogen for one hour and then extracted with ether for 11 hours. The ether was dried over anhydrous sodium sulphate and then evaporated to leave a gum in 7.8% yield. Successive ether extractions of the aqueous residue for 21 hours, 24 hours, and 73 hours removed respectively 2.4, 3.6 and 1.8% yields of similar products. The remaining aqueous portion was extracted three times in a separatory funnel with butanol (250 cc.); 15.6% of a brown glassy solid was obtained on evaporation of the butanol. Evaporation of the aqueous solution left a brown solid in 19.9% yield. Since these yields were based on the weight of periodate lignin used in that particular oxidation, the total recovery from the original aqueous liquor was 51.1% (15.6 + 15.6 + 19.9).

(d) Another aqueous solution of 800 cc. after a chlorine dioxide oxidation of 16 gm. of periodate lignin was flushed with nitrogen for 4.5 hours, and then extracted in a separatory funnel with a total of 500 cc. of butanol (5 extractions of 100 cc. each). The butanol was removed under vacuum at 60°C., leaving a dark red-brown, very viscous oil. To complete the removal, a little water was added and butanol, b.p. 117°C., was

removed as the 38:62 water-butanol azeotrope which boiled at 92.4°C. (86). An attempt was made to remove the last traces of solvent by keeping the oil evacuated for 2 weeks at 0.2 mm. pressure and at room temperature, but even after all this time the material continued to lose weight at the rate of approximately 0.3% per day with no sign of decrease in the rate. From 16 gm. of lignin, 5.63 gm. (35.2%) of this product remained at the end of 2 weeks. The isolated substance was very slowly soluble in water but dissolved very readily in a potassium carbonate solution. Found: Methoxyl, 14.70, 14.84%.

Evaporation of the butanol-extracted aqueous solution left a brown gum which was dissolved in ethanol; the solution was filtered and evaporated to dryness under reduced pressure. As in the previous case, there was a continual loss of weight, but at the end of 2 weeks 3.15 gm. (19.7%) still remained.

Several other larger-scale oxidations were carried out at room temperature (25 to 28°C.) and with no buffer. The initial pH values of 2.5 to 3.0 dropped to pH 0.8 to 0.9. (Table XIX)

Re-oxidations of Oxylignins A and B

Oxylignin B (0.2994 gm.) was treated for 12 hours with 0.011 mole of chlorine dioxide in 23 cc. of solution. The pH dropped from 2.2 to 1.45 and 0.001 mole of the dioxide was consumed. The solid material was recovered on the centrifuge, dissolved in alcohol and reprecipitated with ether to yield

0.1364 gm. (45.6%) of solid. The remainder had probably been transformed to oxylignin C.

Another sample (0.3018 gm.) of oxylignin B was oxidized for 15 hours with 0.013 mole of sodium chlorite in 24 cc. of solution buffered to pH 4.1. The solid dissolved completely and the pH rose to 4.6. Acidification yielded a precipitate which dissolved in alcohol. A total of 0.1726 gm. (57.3%) was recovered by precipitation by ether.

Oxylignin A (0.0774 gm.) was treated for 12 hours with 0.003 mole of chlorine dioxide in 6.1 cc. of solution. The 49.5% of solid left was then completely soluble in alcohol.

Behaviour of Oxylignin B with Sulphuric Acid

Oxylignin B (0.3302 gm.) was heated under reflux for 90 minutes in 10 cc. of a 5% solution of sulphuric acid. The solution was then clear and red-brown in colour, but contained a brown insoluble residue A. This solid was removed by filtration on a sintered glass crucible and washed several times with distilled water. Yield, 0.1718 gm. (52%). The washings were combined with the original solution and the whole was neutralized with barium carbonate. After removal of the precipitated barium sulphate, the volume was reduced under vacuum to 5 cc. of brown turbid liquid. In an attempt to make a phenylhydrazone or an osazone, one cc. of a solution of 50% acetic acid saturated with sodium acetate, and 2 drops of freshly distilled phenylhydrazine were then added; the mixture was heated

TABLE XIX

Oxidations of Periodate Lignin with Unbuffered Aqueous Chlorine Dioxide at 25 to 28°C.

Weight of Lignin (gm.)	Moles of ClO_2	Volume of Solution (cc.)	Duration (hr.)	% Oxylignin B (Precipitated)(a)	% Oxylignin B (Residue)(b)	Aqueous Residue		
						% Ether Extract	% Butanol Extract	% Residue(c)
4	0.101	200	8	49.5	13.7	42.4		
4	0.106	250	8	43.6	14.6	15.6	15.6	19.9
16	0.415	770	9	53.3	8.8		35.2(d)	19.7(d)
8	0.177	600	9	39.6				
12	0.295	835	10	45.0				
31	0.772	1575	9	39.0				
25	0.670	1350	10	34.5				

(a) Precipitated by ether from alcoholic solution.
(b) Obtained by evaporation of the alcohol-ether mother liquor.
(c) Obtained by evaporation of the aqueous solution.
(d) The material very slowly distilled while the last traces of solvent were being removed at room temperature.

on a steam bath for one hour and then allowed to stand at room temperature for 8 hours. The solid left at the end of that time was removed by filtration on sintered glass and washed with alcohol. The alcoholic solution was evaporated and yielded 0.0206 gm. (6.2%) of a brown gum. The solid left on the filter was inorganic.

The product A, unlike the original oxylignin B was not soluble in dilute sodium bicarbonate, but was slowly soluble in 1% sodium hydroxide.

Fractional Precipitation of Oxylignin B

A four gram sample of periodate lignin was oxidized with unbuffered chlorine dioxide in the usual way, and the remaining solid dissolved in alcohol. The small amount (1%) of grey material remaining undissolved was removed by centrifuging and the oxylignin B in the clear solution (90 cc.) was fractionally precipitated by first adding 250 cc. of ether. The light cream precipitate was isolated by the centrifuge, was washed well with ether on a sintered glass filter, and was carefully dried for 48 hours at room temperature and 0.1 mm. pressure. Yield, 27.1% based on the weight of periodate lignin used. Chlorine, 7.9, 8.0, 7.6%.

The clear supernatant liquor was diluted with another 160 cc. of ether and the precipitated material isolated as above. Yield, 12.2%. Chlorine, 8.3, 8.2, 7.8%.

This time the supernatant liquor was concentrated at

atmospheric pressure to approximately 30 cc. and 180 cc. of ether was added. The precipitated oxylignin was isolated by the same technique as before. Yield, 10.2%. Chlorine, 8.15, 8.10%.

The alcohol-ether solution was then evaporated to dryness and the resulting solid again dried *in vacuo*. Yield, 13.7%. Chlorine, 8.9, 8.7%.

Fractionation of an Isolated Oxylignin B

(a) The oxylignin (0.2688 gm.; methoxyl 5.66% and chlorine 7.8%) was dissolved in 5 cc. of cold methanol. The very small amount of insoluble material was removed, and the cream coloured precipitate thrown down by the addition of 3 volumes of ether, was isolated in the usual way. Yield, 0.2102 gm. or 78.4%. Methoxyl, 5.82, 5.67%. Chlorine, 7.8%.

(b) An oxylignin B sample (0.2441 gm.; methoxyl 6.45% and chlorine 7.9%) was dissolved with heating in 5 cc. of dioxane. The small amount of fine material remaining insoluble was removed by the centrifuge and the clear solution was poured into three times its volume of ether. The resulting cream coloured flocculent precipitate was removed by filtration, washed with ether and dried *in vacuo* at room temperature. The dry powder had a slightly darker brown colour than the usual light coloured products. Yield, 0.1925 gm. or 78.9%. Methoxyl, 6.84, 6.87%. Chlorine, 8.07%.

(c) An 0.2164 gm. sample of oxylignin B (chlorine, 7.85%) was heated under reflux in 15 cc. of distilled water for 2 hours. The brown residue was washed on a sintered glass funnel with cold distilled water and dried in vacuo in the usual way. Yield, 0.1120 gm. or 51.8%. Chlorine, 7.1, 7.2%.

The aqueous portion was then taken to dryness in vacuo at 60°C. leaving a brown solid. Yield, 0.0892 gm. or 41.2%. Chlorine, 7.0%.

(d) The oxylignin B (0.1191 gm.) was dissolved in 20 cc. of ethyl alcohol which was then diluted with 20 cc. of distilled water. The addition of 9.0 cc. of 0.1250 N sodium hydroxide brought the pH to 11.96 and the red-brown solution was then extracted 4 times with a total of 50 cc. of butanol. The latter remained colourless, and on evaporation to dryness left no organic material. However, after the aqueous portion was acidified, a butanol extraction in a separatory funnel removed 89% of the original material. As in other cases the butanol was removed first under vacuum, and finally as the butanol-water azeotrope. The residue, 0.1060 gm., had 6.6, 6.7% chlorine.

(e) An 0.1738 gm. sample of oxylignin B was dissolved in 15 cc. of butanol and then extracted 3 times with a total of 25 cc. of a 25% solution of sodium bisulphite. This extraction readily removed all the colour from the butanol portion and on evaporation of the latter, only a small amount of white inorganic salt remained. The bisulphite solution was acidified with dilute

hydrochloric acid, the sulphur dioxide was blown out with nitrogen for 6 hours, and on extraction with butanol only a faintly yellow colour remained in the aqueous portion. The brown butanol solution was concentrated under reduced pressure and the inorganic salts which precipitated were removed by filtration. The remaining clear filtrate was concentrated to dryness. In this case the carbon tetrachloride - butanol azeotrope (b.p. 76.5°C.) consisting of 97.5% carbon tetrachloride (86) was used in an attempt to remove the last traces of butanol. The brown solid residue was then dissolved in methyl alcohol, filtered, and diluted with ether. The resulting brown precipitate was filtered and dried. Yield, 29%; Chlorine, 6.7%.

Dialyses of Oxylignin B in Caustic Soda

(a) One-half gram of oxylignin B (chlorine content 7.8%) was dissolved in 60 cc. of 0.5% sodium hydroxide solution. The resulting red-brown liquid was poured into a cellophane dialysing bag and suspended in 180 cc. of 0.5% caustic soda contained in a 250 cc. graduated cylinder. Within 30 minutes the outer solution began to turn yellow-brown in colour. After 20 hours the solution within the membrane had increased by 10 cc. and the dialysate was a distinct brown colour. The latter was removed and a fresh solution (180 cc.) of 0.5% sodium hydroxide put into the cylinder. Again in 24 hours fresh alkali replaced the dialysate, and this procedure was repeated three more times. Finally the material within the membrane (90 cc.) was removed to a beaker and titrated with 1.87 N sulphuric acid, this

strength being used so that the solution would not become too dilute. To attain a pH of 7.0, 5.85 cc. of sulphuric acid was required whereas the blank required 6.12 cc. of acid. Originally dark red-brown, the solution turned lighter around the neutral point and gradually became a faint straw colour. At a pH of 1.7 turbidity appeared and after reaching pH 1.0 a precipitate was recovered on a sintered glass funnel. The film of brown solid was washed and dried in vacuo, giving 0.0156 gm. with a chlorine content of 7.3%. The filtrate was further acidified to a pH of 0.4, whereupon another precipitate (0.07 gm.) appeared and was recovered as before. Found: 5.4% chlorine. Concentration of the filtrate under vacuum at 50°C. yielded another 0.07 gm. of material, this time with 6.2% chlorine. The differences in chlorine content were probably due to traces of residual sulphuric acid.

(b) Another 0.5 gm. of oxylignin B (8.0% chlorine) was weighed into a 5 cc. volumetric flask and diluted to the mark with 0.127 N sodium hydroxide solution. The mixture was poured into a small cellophane dialysing sac, 15 mm. in diameter, and the flask was washed out with 5 cc. of the alkali. The sac was then suspended in 20 cc. of the sodium hydroxide solution contained in a large test-tube, and the whole covered with a glass plate to prevent evaporation. Within 5 minutes the outer solution became yellow, then gradually darker, and finally in 8 hours it was red-brown in colour. After 24 hours the 8.2 cc. of dialysate left was removed and titrated against 0.348 N sul-

phuric acid, the red-brown colour of the solution becoming yellow as the pH approached neutrality. At pH 1.7 a faint turbidity appeared in the solution and at pH 1.5 there was a definite precipitate. The acid was added until a pH of 0.8 was attained and the resulting precipitate was isolated on a sintered glass filter as a light brown powder (0.108 gm.) with a chlorine content of 6.5%.

A fresh 20 cc. of solution was placed in the test-tube and at the end of 24 hours 9.4 cc. remained. No precipitate appeared in this case even when the pH was lowered as far as 0.8. The procedure was repeated for another 24 hours with 20 cc. of fresh sodium hydroxide solution. This time 11.8 cc. remained, and again no precipitate appeared when the pH was lowered below 1. However, after several days, fine precipitates appeared in this and the preceding dialysates. The combined solutions were extracted with butanol, and the extract washed with water until the filtrate gave a negative test for sulphate with barium chloride solution. However, the washings assumed a yellow colour and a considerable amount of the oxylignin was removed from the butanol before the latter was entirely freed of sulphuric acid. On evaporation of the solvent 0.03 gm. (6%) of a brown film remained.

From within the dialysis sac a 19 cc. aliquot of solution was removed and placed in a fresh membrane immersed in 20 cc. of alkali. After 5 days the dialysate amounted to 10.6 cc. and the colours inside and outside the sac appeared identical.

After acidifying to pH 0.5 the solution was extracted with butanol and the latter washed with barium chloride solution. The resulting precipitate was removed by filtration, the butanol concentrated, again filtered to remove more solid, and finally taken to dryness. Only a very small amount of brown solid remained, most of the oxylignin having been removed as an insoluble barium salt with the barium sulphate.

The dialysing sac was again immersed in a fresh sodium hydroxide solution (14 cc.) and allowed to stand for 2 days. The 10 cc. left at the end of this time was as highly coloured as in the previous cases. When the 33 cc. of solution left within the sac was acidified to around pH 1.7 a precipitate began to appear. This was separated at pH 0.5. Found: 7.15% chlorine.

From the aliquot left in the original dialysing sac a brown powder was isolated when the solution was highly acidified. Found: 6.5% chlorine.

Dialysis of Oxylignin B in Dilute Alcohol

An 0.5143 gm. sample of oxylignin B (7.8% chlorine) was dissolved in 8 cc. of ethyl alcohol to which was then added 16 cc. of distilled water. The clear solution was placed in a cellophane dialysing membrane and then suspended in a 100 cc. graduated cylinder containing 25 cc. of an alcohol solution of similar dilution. After 24 hours a very faint colour appeared in the outer solution and the volume had decreased by 1 cc. Af-

ter 6 days, evaporation of this dialysate (19.5 cc.) left 0.0529 gm. of brown solid with a chlorine content 7.65%. Another 0.0566 gm. of a similar material (7.25% Cl) was isolated after another 8 days. For the next 6 days distilled water was kept on the outside of the membrane. The original volume of 25 cc. decreased to 19 cc. and 0.0311 gm. of solid was isolated. Chlorine content, 6.65%. Another 0.0211 gm. was removed in 5 more days. The solution left within the sac (35 cc.) yielded, on evaporation to dryness, 0.2349 gm. with chlorine content of 7.33%.

Oxidation of Oxylignin B with Nitrobenzene

The method used by Ritchie (59) to obtain vanillin from periodate lignin was used on oxylignin B. A 3.061 gm. sample of oxylignin B was heated in an autoclave at 160 to 175°C. for 3.5 hours in 225 cc. of 8% sodium hydroxide and 7.5 cc. of nitrobenzene. After cooling, the solution was continuously extracted with ether for 36 hours to remove azobenzene, excess nitrobenzene and any other nitrogenous products. The aqueous portion was then acidified to pH 3 with 50% (by volume) sulphuric acid and extracted continuously for 72 hours with ether. This ethereal solution in turn was extracted 5 times with 50 cc. volumes of saturated sodium bisulphite until no more colour was removed. Acidification of the bisulphite solution to pH 1 with sulphuric acid liberated sulphur dioxide which was removed by bubbling air through the solution. The latter was again made alkaline to pH 10 with sodium hydroxide and ex-

tracted with ether to remove non-acidic carbonyl compounds. The residual aqueous solution was dark red-brown in colour, but when it was once more acidified it became yellow again. From the resulting 665 cc., an aliquot of 150 cc. was removed, and to it was added 25 cc. of 2,4-dinitrophenylhydrazine. After heating for one hour on a steam bath, the precipitate which appeared was isolated on a sintered glass filter, but was found to be the original 2,4-dinitrophenylhydrazine, m.p. 190-195°C., mixed m.p. 193-195°C. No traces of the derivatives of vanillin or chlorovanillin were found.

The remaining aqueous solution was again extracted with ether for 72 hours, the latter dried over anhydrous sodium sulphate and then evaporated, leaving 0.9812 gm. of brown gum. A small aliquot of this gum was extracted with hot water, but no crystals appeared on cooling the water extract. The remaining aliquot was then redissolved in ether and 0.0280 gm. of insoluble material removed. The latter charred on being heated, but would not melt. The ethereal solution was evaporated over a steam bath and the residue distilled at a bath temperature of 75 to 85°C. and under 0.1 mm. pressure. A faintly yellow oil which partially crystallized was collected in the receiver. The solid portion was white, but its quantity was small and it was not readily separated from the oil. A solid white material (0.08 gm.) m.p. 164-167°C., was collected on the bulb of the thermometer. Vanillin melts at 81-82°C. The distilled oil had a sharp odour very much unlike that of vanillin, and gave

a negative ferric chloride test for a phenol. The residue in the distilling flask was a brown solid which charred but did not melt.

Molecular Weight Determinations on Oxylignin B

Before examining a sample of oxylignin B, the molecular weight of reagent resorcinol was checked against that of recrystallized azobenzene by the method of Blank and Willard (65). Instead of using the rubber connections described in the original method, ground glass joints were used in the apparatus. The azobenzene (0.0264 gm.) was weighed into a 25 cc. Erlenmeyer flask fitted with a standard taper joint, and 5.2086 gm. of methanol was added. Another similar flask contained 0.0196 gm. of resorcinol along with 6.4106 gm. of methanol. The two flasks were then connected to an inverted Y - tube and immersed in a water bath kept at 65 to 66°C. (See Fig. 12) After 5 hours the flasks were removed, stoppered with glass stoppers, wiped dry and allowed to cool for 15 to 20 minutes. The weight of the solvent in the azobenzene solution was then found to be 4.6784 gm.; while the weight of solvent in the other flask was 5.7012 gm. From these data the molecular weight of the resorcinol was found to be 110.9 as shown from the following calculation. If x is the molecular weight of resorcinol and 182 the molecular weight of the azobenzene, then:

$$\frac{0.0264}{182} \times \frac{1000}{4.6784} = \frac{0.0196}{x} \times \frac{1000}{5.7012}$$

$$x = 110.9$$

After another 5 hours the weight of solvent in the flasks decreased to 3.9706 gm. and 4.8698 gm., giving a calculated value of 110.0. A further 3.5 hours' heating decreased the solvent weights to 3.4745 gm. and 4.2402 gm. respectively, which gave a molecular weight of 110.5.

In another similar trial 0.0248 gm. of azobenzene was placed with 7.7054 gm. of methanol, while 0.0198 gm. of resorcinol was dissolved in 9.7432 gm. of the same solvent. Within 3.5 hours the weights of methanol decreased to 7.0526 gm. and 9.2868 gm. respectively. From these values the molecular weight of resorcinol was calculated as 109.2.

In an attempt to determine the molecular weight of the oxylignin B using azobenzene as standard, 0.0226 gm. of the latter was dissolved in 13.7568 gm. of methanol, and 0.0223 gm. of the former was placed with 8.1458 gm. of solvent. After 3.5 hours the solvent weights decreased to 13.6602 gm. and 7.9296 gm. respectively, giving a calculated molecular weight of 309. During the next six days the value gradually rose to 487. Since considerable solvent was lost from both flasks in this time, more methanol was added, and the final weights of solvent with the azobenzene and oxylignin B were 16.3530 gm. and 6.0199 gm. These data gave the molecular weight 487.

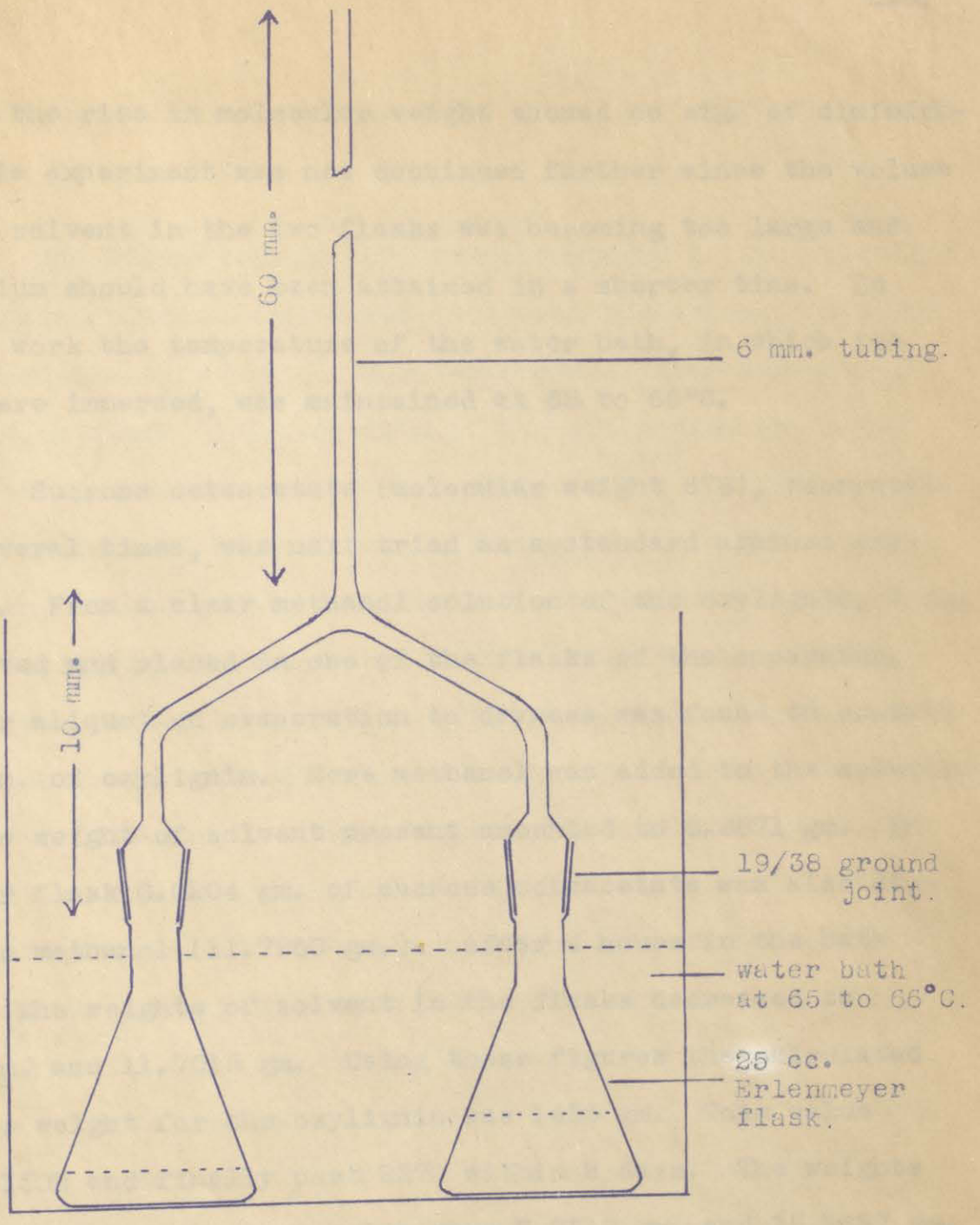


Fig. 12. Apparatus For Molecular Weight Determination.

However, the rise in molecular weight showed no sign of diminishing. This experiment was not continued further since the volume ratio of solvent in the two flasks was becoming too large and equilibrium should have been attained in a shorter time. In all this work the temperature of the water bath, in which the flasks were immersed, was maintained at 65 to 66°C.

Sucrose octaacetate (molecular weight 678), recrystallized several times, was next tried as a standard against oxylignin B. From a clear methanol solution of the oxylignin, 5 cc. was removed and placed in one of the flasks of the apparatus. A similar aliquot on evaporation to dryness was found to contain 0.0323 gm. of oxylignin. More methanol was added to the solution until the weight of solvent present amounted to 8.8871 gm. In the other flask 0.0204 gm. of sucrose octaacetate was also dissolved in methanol (11.7860 gm.). After 4 hours in the bath at 66°C. the weights of solvent in the flasks decreased to 8.6396 gm. and 11.7015 gm. Using these figures the calculated molecular weight for the oxylignin was 1455 gm. This value rose to 1502 and finally past 2270 within 2 days. The weights of solvent giving the last value were 7.2519 gm. and 15.3637 gm.

Using 10 cc. of methanol solution containing 0.0646 gm. of oxylignin B diluted to give a total of 9.8312 gm. of solvent, another molecular weight determination was attempted, 0.0132 gm. of sucrose octaacetate in 13.1298 gm. of methanol being used as the standard. The calculated molecular weights decreased from 4440 to 4000 within 2 days. However, in another

experiment the values rose from 4700, thus indicating all these figures to be unreliable.

The Signer method (67) of determining molecular weights also depends on isothermal distillation of the solvent, but under reduced pressure and at room temperature. The apparatus, described by Clark (68), consists of two bulbs with graduated extensions, joined by a horse-shoe shaped tube. In this tube directly above each bulb is an arm through which the sample and solvent can be added. A clear methanol solution (0.5380 gm.) of oxylignin B calculated to contain 0.00446 gm. of dry material was placed in one side of the apparatus. To this was added another small amount of pure solvent. Sucrose octa-acetate (0.00219 gm.) was then washed, with methanol, into the other bulb of the apparatus, and both bulbs were cooled in a dry ice bath to prevent evaporation while one arm was sealed. The solutions were again cooled thoroughly and the apparatus was evacuated by a Hy-vac pump. The vacuum was retained by closing a stopcock attached by a rubber tube to the apparatus, and the system was then allowed to warm to room temperature. Cooling and evacuation were repeated three times, the final vacuum being 0.35 mm. This procedure removed most of the air from the apparatus, and the second arm was then sealed. After warming to room temperature the volume of solution containing the oxylignin amounted to 1.318 cc. while the other side contained 1.040 cc. After 4 days in a cupboard away from draughts, the volume of the oxylignin solution had changed to 1.378 cc.,

and that of the standard to 0.975 cc. These volumes, which indicated a molecular weight of 980, remained constant for the next four days.

In another determination by this method, 0.9002 gm. of alcohol solution containing 0.00747 gm. of oxylignin, was placed in one bulb, and 0.00415 gm. of sucrose octaacetate in the other. Methanol was added to both sides and the system sealed as described above. The volume of oxylignin solution was 1.140 cc., and of the other 1.395 cc. Within 2 days these volumes changed to 1.190 cc. and 1.340 cc. respectively. From these figures a molecular weight of 1375 was calculated. After a total of 9 days this value dropped to 1290. The volumes of the solutions were then changed by placing one bulb in warm water and the other in cold water. Within about 60 seconds a considerable amount of solvent distilled from one side to the other. When the temperatures of the bulbs had equalized, the volume of the oxylignin solution was found to be 1.480 cc., and that of the other 1.040 cc. In 3 days, the values changed to 1.460 and 1.070 cc. respectively, giving a molecular weight of 895. This value rose to 900 and then dropped again to 895. Even after a week the volumes did not change further.

Acid Equivalents

(a) An 0.3352 gm. sample, completely dissolved in 20 cc. of 0.1258 N sodium hydroxide solution, and potentiometrically titrated against 0.0966 N hydrochloric acid solution, needed

6.30 cc. of acid to attain a pH of 7.0. However, from the plot (Fig. 13) the end-point seems to be closer to pH 7.5 and 5.6 cc. of acid. The first value gave an acid equivalent of 177, the latter 172. A value of approximately 163 was calculated for pH 9.0.

In another run, 0.0604 gm. of oxylignin dissolved in dilute alkali was titrated with 0.03 N hydrochloric acid. A blank treated similarly required 30.80 cc. of acid to reach a pH of 7.0, whereas the sample needed 19.65 cc. The calculated acid equivalent was 180.5. From Fig. 14 the end-point appears to be at pH 7.5 giving an acid equivalent of 176. At pH 9.0 the value was approximately 168.

(b) Oxylignin (0.1976 gm.) was dissolved in 10 cc. of 50% alcohol and titrated with 0.0375 N alkali. Nitrogen was bubbled through the solution as the titration proceeded. This served not only to stir the solution, but also to drive out any carbon dioxide which would interfere with the titration. However, in spite of this precaution, after pH 7.9 the addition of alkali caused an immediate pH increase followed by a slow drifting to a value about 0.2 unit lower within a minute or two (Fig. 11, plot 1). Using pH 7.9 as the end-point the acid equivalent was calculated as 432; at pH 9.0 it was 376. After reaching a pH of 11.5 with 35.0 cc. of alkali, the solution was back titrated with 0.03N hydrochloric acid. To reach a pH of 8.5 which, from plot 2 Fig. 11, was considered to be the end-point, 12.5 cc. of acid was required. Using this value the acid equivalent was 212,

whereas 208 resulted from the assumption that the end-point was at pH 9.0.

(c) An 0.0354 gm. sample of the oxylignin was dissolved in 5 cc. of dilute alcohol and titrated with 0.0108 N barium hydroxide. Nitrogen was continuously bubbled through the solution, and no precipitate of carbonate formed. However, the usual drifting began at pH 7.1. Using this as the end-point the acid equivalent was calculated as 448. However, from Fig. 8 the end-point appears to be closer to pH 8.0 and 8.0 cc. of barium hydroxide. From this value an acid equivalent of 409 was calculated. At pH 9.0 the equivalent was approximately 364.

(d) The calcium acetate method of Yackel and Kenyon (70) was used also. To 0.2534 gm. of the oxylignin in a 125 cc. Erlenmeyer flask was added 60 cc. of approximately 0.5 N calcium acetate solution. The latter was made up of 44 gm. in 500 cc. of solution, boiled, cooled, and filtered, and had a pH of 7.6. The sample was allowed to react for 24 hours, after which time the pH had dropped to 6.28. The residue was removed on a sintered glass crucible and the filtrate together with 100 cc. of washings, was diluted to 200 cc. in a volumetric flask. An aliquot of 50 cc. was removed for titration with 0.0375 N sodium hydroxide, and needed 7.35 cc. to reach a pH of 8.30. A blank treated similarly needed only 0.45 cc. Thus 6.90 cc. of alkali was needed for 0.0634 gm. of oxylignin and acid equivalent was calculated to be 245.

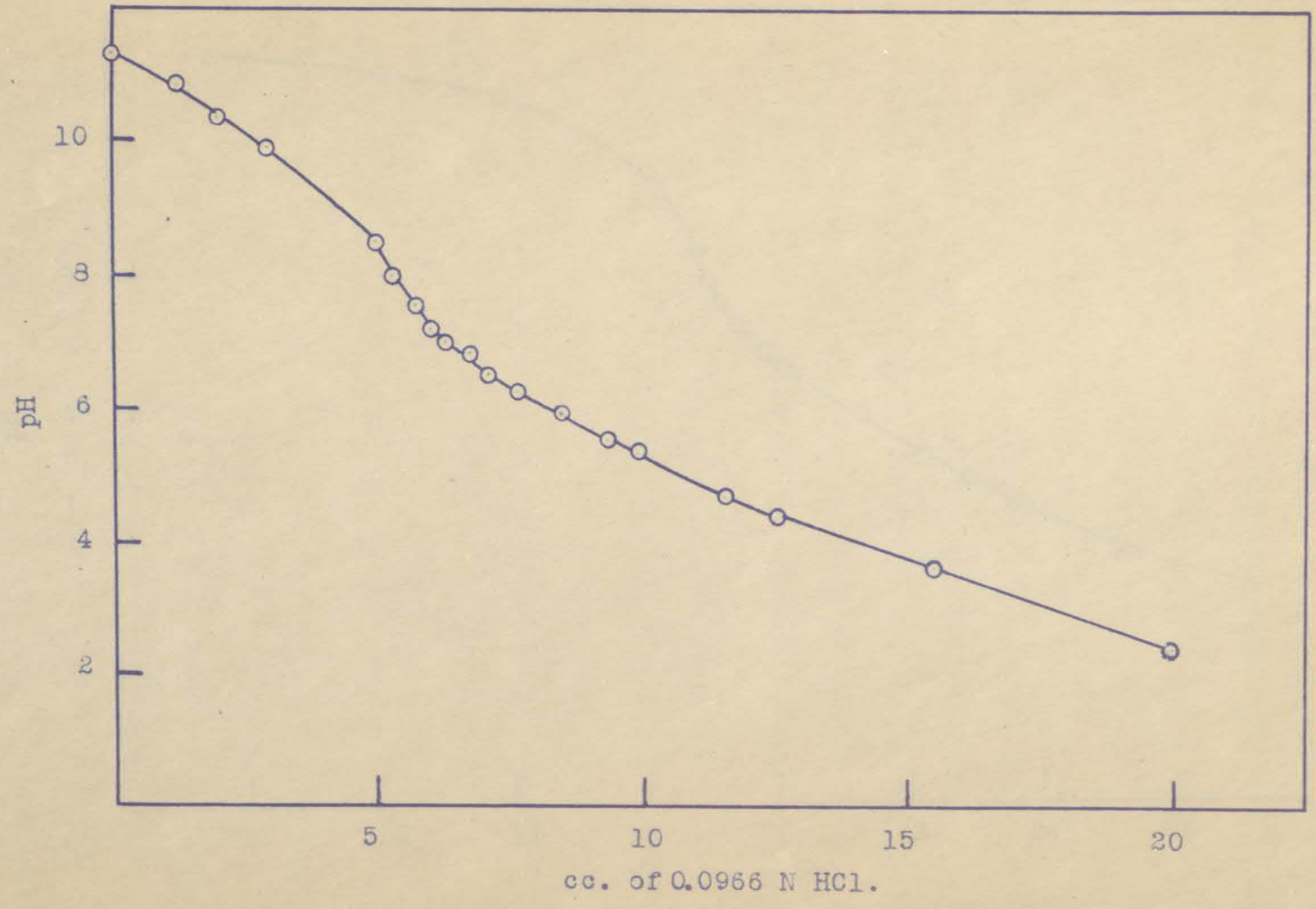


Fig. 13. Potentiometric titration of 0.3352 gm. of oxylignin B and 20 cc. of 0.1258 N alkali with 0.0966 N hydrochloric acid.

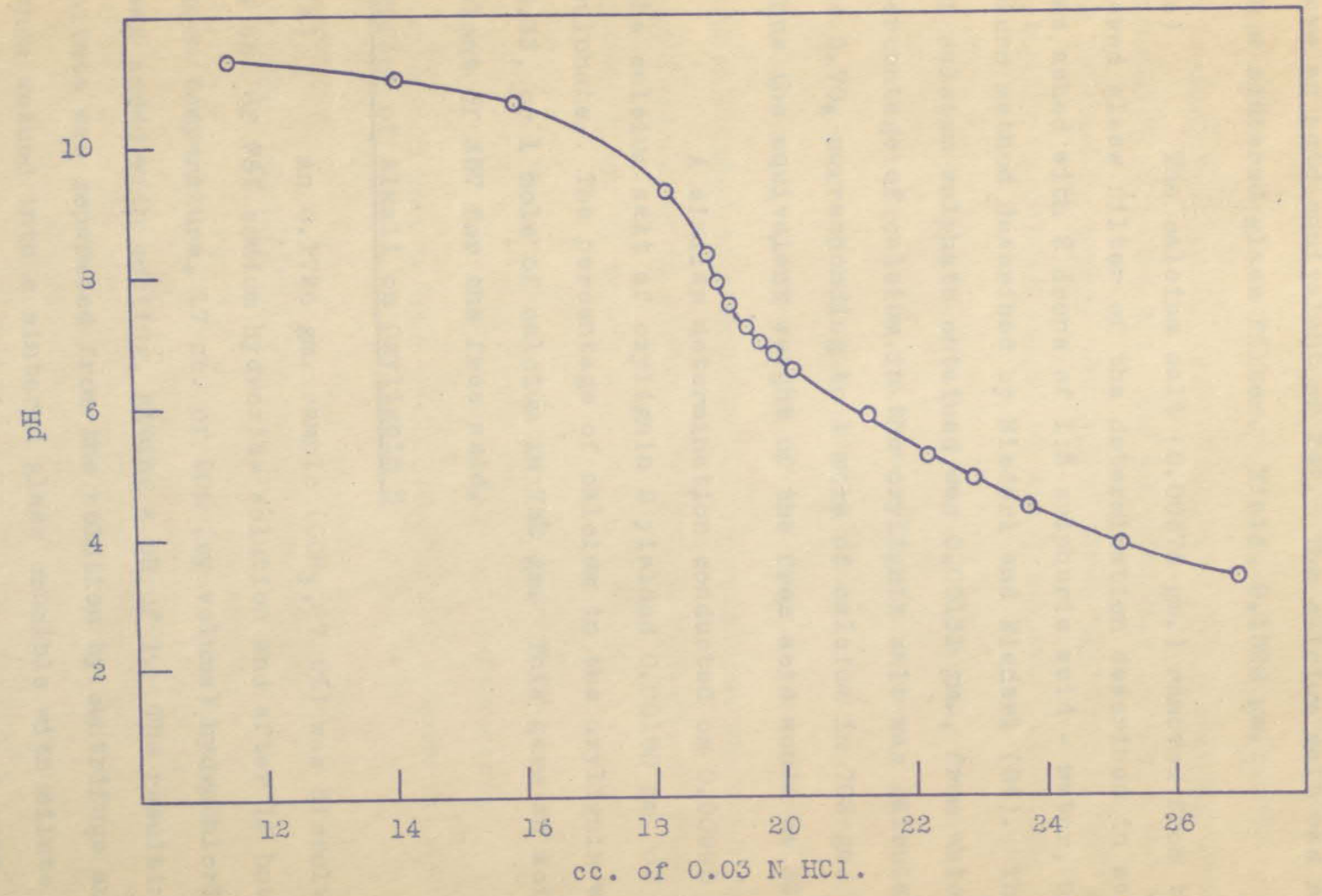


Fig. 14. Potentiometric titration of 0.0604 gm. of oxylignin B and 20 cc. of 0.046 N alkali with 0.03 N hydrochloric acid.

Another sample of 0.2476 gm. was treated similarly and gave an acid equivalent of 241. The calcium salt was recovered on a sintered glass filter. Yield, 0.1528 gm.

(e) The calcium salt (0.00676 gm.) removed from the sintered glass filter of the determination described in section (d) was ashed with 2 drops of 1:5 sulphuric acid - water, using the micro method described by Niederl and Niederl (84). The weight of calcium sulphate obtained was 0.00131 gm., from which the percentage of calcium in the oxylignin salt was calculated to be 5.70, corresponding to 1 mole of calcium in 703 gm. of salt. Thus the equivalent weight of the free acid would be 332.

A similar determination conducted on 0.00995 gm. of the calcium salt of oxylignin B yielded 0.00180 gm. of calcium sulphate. The percentage of calcium in the oxylignin was thus 5.33, or 1 mole of calcium in 752 gm. This gave an acid equivalent of 357 for the free acid.

Effect of Alkali on Oxylignin B

(a) An 0.1726 gm. sample (OCH_3, 7.0%) was dissolved in 4 cc. of 25% sodium hydroxide solution and after 12 hours at room temperature, 17 cc. of 20% (by volume) hydrochloric acid was added with cooling, giving a pH of 1. The resulting precipitate was separated from the solution by centrifuge and was then washed into a sintered glass crucible with dilute hydrochloric acid. After careful drying in vacuum, 0.0338 gm. or 19.6% remained. Methoxyl, 4.70%.

The aqueous solution was extracted three times with 7 cc. of butanol and this solvent was then removed by vacuum distillation. Inorganic salts precipitating during the concentration were removed by filtration, and the remaining brown gum was dissolved in methanol. The solution was filtered and the methanol removed in vacuo. Weight of gummy residue, 0.1307 gm. Ash, 19.4%. Therefore weight of ash-free gum, 0.1050 gm.

(b) The oxylignin (0.9972 gm.) was dissolved in 7 cc. of 4% sodium hydroxide and allowed to stand for 24 hours at room temperature. Dilute hydrochloric acid (3.5 cc.) was then added with cooling, to give a pH of 1. The cream coloured precipitate was collected on a sintered glass crucible, washed with dilute acid and dried in vacuum. Weight, 0.4400 gm. or 44.2%. Ash,0%. Methoxyl, 4.77, 4.84%.

The aqueous solution was extracted with 25 cc. of butanol and the organic material removed as above. The brown solid residue, 0.2164 gm. or 21.7%, had a methoxyl content of 8.98, 9.08%.

Carbonyl Determinations

(a) Fifty cc. of hydroxylamine hydrochloride solution, made up as directed by Gladding and Purves (71), was added to 0.1660 gm. of the calcium salt of oxylignin B left after an acid number determination by the calcium acetate method. This solution had a pH of 5.23 whereas the blank had a pH of 5.25. After allowing both solutions to stand at room temperature for

100 minutes they were titrated to pH 3.2 with 0.1188 N hydrochloric acid. The blank needed 0.00576 mole of acid and the other solution 0.00588 mole. Hence the latter solution had liberated no hydrochloric acid and the calcium salt had absorbed no hydroxylamine hydrochloride.

(b) The sodium cyanide solution for this determination consisted of 0.2068 gm. of the salt dissolved in 100 cc. of water. Of this, 30 cc. (pH 10.4) was added to 0.4025 gm. of oxylignin B in a 250 cc. round-bottom flask. Within one hour the oxylignin dissolved, forming a red-brown solution which was allowed to stand at room temperature for 3 days. A blank was also run. The two flasks were then attached to distilling apparatuses which contained addition tubes through which was added 70 cc. of water containing 5 cc. of 2 N sodium hydroxide. The flasks were equally heated by Glascol heaters connected to a single Variac resistance and the ammonia evolved was absorbed in 40 cc. of 0.03 N hydrochloric acid contained in 100 cc. volumetric flasks. Within one hour both volumetric flasks were filled to the mark. The solutions were then quantitatively washed into 250 cc. Erlenmeyer flasks and titrated with 0.0375 N sodium hydroxide to the end-point shown by a methyl red indicator. The blank required 28.20 cc. of alkali and the sample 23.70 cc. The difference, equivalent to 1.69×10^{-4} mole of ammonia, was presumably evolved from the same number of moles of carbonyl. Thus 1 mole of carbonyl, from these values, was present in 2380 gm. of oxylignin.

Another sample of 0.4053 gm. was first dissolved in 25 cc. of 1% sodium bisulphite and then after 8 hours 5 cc. of 1.25% sodium cyanide solution was added. On this addition the yellow solution turned orange and the pH rose to 6.8. A further 1.5 cc. of alkali raised the pH to 9.7 and changed the colour of the solution to red. A blank was treated in an identical manner. After 3 days at room temperature the distillation and titration were completed as described above. The blank required 28.25 cc. of the 0.0375 N alkali and the sample 24.50 cc. The results indicated one mole of carbonyl in 2880 gm. of the oxylignin B.

Hydrogenation of Oxylignin B

(a) The oxylignin (0.1115 gm.) was partially dissolved in 5 cc. of absolute methanol, and the solution was washed with another 2 cc. into a small glass hydrogenator containing 200 mgm. of reduced Adams' platinum oxide catalyst (87) in 20 cc. of glacial acetic acid. The apparatus was rapidly flushed three times with hydrogen and the shaking motor started. Within a short time the oxylignin completely dissolved. After 16 hours, the sample had consumed 3.0 cc. of hydrogen at 26.5°C. and 756.5 mm. pressure. From these data it was calculated that one mole of hydrogen would be used by 925 gm. of oxylignin.

(b) A palladium oxide catalyst (0.2497 gm.) was prepared for a second hydrogenation by fusion of palladium chloride with sodium nitrate as described by Shriner and Adams (88).

When shaken in the hydrogenator with 15 cc. of absolute methanol approximately 70 cc. of hydrogen was consumed within the first hour, but no more in the following 4 hours. To this reduced mixture was added 0.1866 gm. of oxylignin B, the system again being flushed with hydrogen, and the shaking started. Within 14 hours the sample consumed 4.5 cc. of hydrogen, and no further change occurred in the next 5 hours. After correcting for temperature (26°C.) and pressure (763.2 mm.) it was calculated that 1020 gm. of oxylignin would need one mole of hydrogen.

Another blank containing solvent and catalyst was shaken for 16 hours and consumed no more hydrogen after the 70 cc. uptake during the first hour.

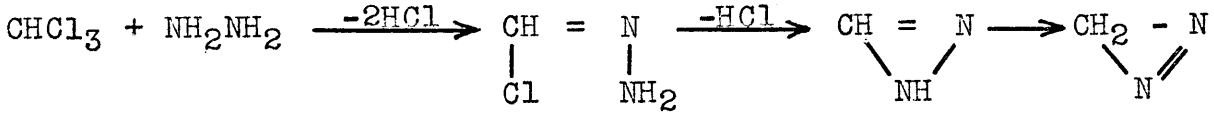
Methylation with Diazomethane

Since the oxylignin B could be dissolved in dioxane only with heating, and since this was not wanted, it was first dissolved in methanol, diluted with dioxane and the methanol removed under vacuum. The removal of all methanol was necessary since alcohol, as well as water can act as a catalyst in causing diazomethane to methylate aliphatic hydroxyl groups (89).

To prevent any danger of peroxides from the dioxane affecting the oxylignin in any way, the solvent was carefully purified as described in Weissberger and Proskauer (90). After the purification, a small amount failed to liberate any iodine from an acidified potassium iodide solution.

The diazomethane was prepared as described by Fieser

(89) using alcoholic potassium hydroxide, hydrazine hydrate and chloroform.



This reaction was very inefficient, giving only about 20% of the theoretical yield, but it proved quite suitable for bubbling the generated gas directly into the dioxane solution of the oxylignin B.

To recover the methylated solid, the solvent was removed at 30 to 40°C. under reduced pressure. Both here and in removal of the methanol, precautions were taken to exclude moisture and excess oxygen. In one preliminary attempt to remove the solvent under reduced pressure, dried air was drawn through the bubbler. This procedure caused the formation of a thick orange syrup which could not be removed even under vacuum, and which very readily liberated iodine from an acidified potassium iodide solution, thus showing it to contain a considerable amount of peroxides. Not only was there danger of damaging the product, but it was also impossible to obtain the methylated material in pure form and high yield, when mixed with this syrup. In later experiments the solvent, in every case, was distilled under reduced pressure with dried nitrogen being drawn through the bubbler. The last traces of solvent, when tested with acidified potassium iodide solution did not liberate any iodine, indicating the absence of peroxides. No syrup appeared and

only an orange coloured brittle solid remained in the flask.

The sample of oxylignin B used in the following methylation studies was specially prepared and had the following analysis: C, 46.6, 47.0, 46.7, 46.6, 46.8; H, 4.45, 4.28, 4.34, 4.32 (3.92); Cl, 9.05, 9.03, 9.05, 9.00; OCH_3, 6.85, 7.00%. Calcd. for $C_{32.8}H_{32.9}O_{20.2}Cl_{2.28}(OCH_3)_2$: C, 46.9; H, 4.35; Cl, 9.07; OCH_3, 6.95%. Mol. wt. 893. Niederl and Niederl's semi-micro method (84) was used for the carbon and hydrogen, and the micro Carius technique for the chlorine analyses.

(a) A 4.1062 gm. sample of oxylignin B was dissolved in 50 cc. of methanol and 20 cc. of purified dioxane. After standing for 16 hours at room temperature, 0.0985 gm. of insoluble material was removed by the centrifuge and the clear orange solution was concentrated at 2.5 cm. pressure and 35 to 40°C., with dry nitrogen being drawn through the liquid. More dioxane was added and the distillation continued until a refractive index taken on the distillate showed that no further methanol was being removed. Diazomethane prepared from 10 gm. of hydrazine hydrate was then bubbled through the clear dioxane solution and the latter allowed to stand at room temperature for 8 hours. A calcium chloride drying tube in the mouth of the flask allowed the nitrogen generated to escape and yet kept the solution dry. The treatment with diazomethane was repeated and 16 hours later was repeated again. Finally, after another 6 hours, the dioxane was removed under vacuum at less than 40°C. with

nitrogen bubbling through the solution. No trace of peroxides appeared. The remaining brittle yellow-orange solid was scraped out and washed thoroughly with ether, in which it was insoluble, on a sintered glass crucible. The resulting light fawn powder was dried for 18 hours at 0.1 mm. pressure and room temperature. Yield, 4.7476 gm. The substance was then extracted with n-pentane for 24 hours to remove any residual ether or dioxane, dried as described above, and analyzed. Found: methoxyl, 16.25, 16.07%; nitrogen, 1.56%.

The remaining 4.2908 gm. was remethylated three times, as described, with diazomethane. Yield, 4.3491 gm. Found: C, 52.4, 52.6; H, 5.56, 5.67; Cl, 6.28, 6.25, 6.34, 6.30; N, 2.02, 2.12; OCH_3, 17.00, 17.05%. Calcd. for $C_{34.7}H_{35.8}O_{14.0}N_{1.36}Cl_{1.60}(OCH_3)_5$: C, 52.4; H, 5.60; N, 2.14; Cl, 6.26; OCH_3, 17.1% Mol. wt. 907. Nitrogen was determined by the micro Kjeldahl method (91), following the directions given by Harte (92) for the reduction of azo and nitro compounds.

Two further methylations gave a product with a slightly lower methoxyl. Found: methoxyl, 16.82, 16.90%.

(b) Oxylignin B (0.1927 gm.; methoxyl, 6.49, 6.37%) was placed in a small Erlenmeyer flask with 10 cc. of dioxane. No heat was applied and much of the sample remained undissolved. To this was added 25 cc. of an ether solution containing diazomethane, and the mixture was allowed to stand at room temperature for 5 hours. A considerable amount of solid precipitated

on addition of the ether, and bubbles of gas, probably nitrogen, appeared in the solution. The clear supernatant liquor was decanted and another 20 cc. of diazomethane solution was added. This addition was repeated after 16 hours and finally 12 hours later the solid was removed on a sintered glass filter, washed with fresh ether and dried at room temperature and 0.1 mm. pressure. Yield of cream coloured solid, 0.1852 gm. Methoxyl 12.88, 12.75%.

(c) An 0.3007 gm. sample of the solid precipitated by acid after treatment of oxylignin B with 4% alkali was dissolved in 8 cc. of methanol. Dioxane (75 cc.) was then added, and the methanol distilled off under vacuum. This operation was followed by three methylations with diazomethane and the product was isolated by careful evaporation. Yield of light orange coloured powder, 0.3464 gm. Methoxyl, 17.80, 17.74%. Further methylations caused the methoxyl values to drop somewhat. Found: methoxyl, 17.32, 17.40%; nitrogen, 3.64%. The sample was extracted thoroughly with n-pentane to remove traces of alkoxyl solvents prior to analysis.

Methylation with Methanolic Hydrogen Chloride

Seven cc. of 7% methanolic hydrogen chloride was added to 0.6420 gm. of oxylignin dissolved in 7 cc. of dry methanol. This solution was heated under reflux on a steam bath for 6.5 hours and the slight residue removed by filtration. Water was then added, and the heavy grey precipitate was removed on a

sintered glass crucible, was dried for 48 hours at 0.2 mm. pressure, was extracted in a soxhlet with n-pentane and finally dried in vacuo. Yield, 0.3826 gm. or 59.6%. Found: methoxyl, 16.30, 16.20%.

To the clear aqueous-alcohol solution, 1.5 gm. of silver oxide was added. The resulting silver chloride and excess silver oxide wereremoved by filtration and the solution concentrated. More inorganic salt was removed, and finally the residue was redissolved in methanol, filtered once more and then taken to dryness. After several days in a vacuum desiccator at 0.1 mm. pressure, 0.1468 gm. of brown solid remained. Found: methoxyl, 16.20, 16.12%. The total recovery was 0.5294 gm.

Methylation with Dimethyl Sulphate

(a) Oxylignin B (0.9997 gm.) dissolved in 10 cc. of 10% sodium hydroxide, was stirred under nitrogen for 24 hours with 10 cc. of dimethyl sulphate and 8 cc. of 30% sodium hydroxide solution. The alkaline solution was then acidified to pH 1 with dilute sulphuric acid, the precipitated solid was removed on a sintered glass funnel, and washed with dilute acid. Yield, 0.6314 gm. or 63%. Found: methoxyl, 10.28, 10.38%.

When the above methylation was repeated once more on the isolated product the yield was only 0.1774 gm. and the methoxyl found was 7.35, 7.28%.

(b) An 0.5059 gm. sample of oxylignin was dissolved in 1.8 cc. of 30% sodium hydroxide and 2 cc. of water. After the

addition of 0.3 cc. of dimethyl sulphate, the mixture was stirred under nitrogen for 12 hours, during which time the pH remained above 11. Dilute hydrochloric acid was then added to pH 1 and the resulting solid removed by filtration. Yield, 0.2238 gm. or 44.2%. Found: methoxyl, 9.60, 9.45%

(c) A sample (0.5052 gm.) fully methylated with diazomethane (17.0% methoxyl) was stirred under nitrogen with 5 cc. of 0.43N sodium hydroxide. After 3 hours an orange coloured solution resulted and 1.2 cc. of dimethyl sulphate was then added. Within 10 minutes the pH was 9 and in 2 hours the mixture had become quite acidic, causing a heavy precipitate to appear. More alkali and 3 cc. of water were added until a pH of 11 was attained and all the solid had redissolved. An additional 1 cc. of dimethyl sulphate was then added and when the pH dropped to 7 more sodium hydroxide was added to keep it between 8 and 12. Dimethyl sulphate was twice more added in 1 cc. portions. After 2 days of this treatment, the homogeneous solution was acidified to pH 1 with dilute hydrochloric acid and the resulting precipitate recovered on a sintered glass crucible. Weight of ash-free solid, 0.4510 gm. or 89.5%. Found: methoxyl, 14.50, 14.58%.

A portion (0.3047 gm.) of this product was dissolved in a small amount of methanol and diluted with a larger amount of dioxane. The methanol was carefully removed by distillation and the solution filtered to remove the small amount of ash. Diazomethane was passed in three times and the methylated product isolated in the usual way. Weight of solid, 0.3036 gm. or 99.5%.

Found: methoxyl 16.90, 16.84%.

Saponification of Methylated Oxylignin B

(a) An 0.0633 gm. sample of oxylignin B, fully methylated with diazomethane (17.0% methoxyl), was allowed to stand for 21 hours at room temperature in 3 cc. of 0.1118 N sodium hydroxide. When the sample finally did dissolve after 5 hours, the resulting solution remained light yellow in colour instead of becoming red-brown as when oxylignin B itself dissolved in alkali. After 21 hours at room temperature, the solution was quantitatively transferred to a 50 cc. beaker and potentiometrically titrated to pH 7 with 0.1191 N hydrochloric acid. A blank was treated similarly. Found: 2.75 cc. for the blank, 1.13 cc. for the sample. The difference of 1.62 cc. corresponded to a saponification equivalent of 328.

Acid was then added until a pH of 0.5 was attained and the resulting precipitate was isolated by centrifuge, and then washed onto a sintered glass crucible with dilute acid. Yield, 0.0287 gm. or 45.4%. Found: methoxyl, 10.1%.

(b) Another sample (0.0675 gm.) of the diazomethane methylated oxylignin was saponified as above with 3 cc. of 0.433 N alkali at room temperature for 22 hours. Titration to pH 7 required 8.94 cc. of 0.1191 N hydrochloric acid, whereas a blank required 10.95 cc. The difference of 2.01 cc. corresponded to a saponification equivalent of 282.

(c) The diazomethane methylated oxylignin B (0.5724 gm.) was dissolved as before in 25 cc. of 0.1118 N sodium hydroxide and then the solution was heated on a steam bath for 8 hours. One hour after starting the heating, the solution turned to the red-brown colour noted whenever unmethylated oxylignin B was dissolved in alkali, and within 4 hours the pH had dropped to 8. At this point another 5 cc. of the alkali was added to the sample and to the blank. After standing at room temperature overnight (10 hr.) the two solutions were, as before, potentiometrically titrated with 0.1191 N hydrochloric acid to pH 7. Found: 27.50 cc. for the blank, 6.00 cc. for the sample. The difference of 21.50 cc. corresponded to a saponification equivalent of 223.5.

Further acidification to pH 0.5 precipitated a cream coloured material which was isolated by centrifuge and filtration, as described in (a). Yield, 0.2535 gm. or 44.3%. Found: methoxyl, 9.04, 9.04%.

The aqueous filtrate was concentrated to dryness and the residue dissolved in methanol. Silver oxide was at once added to remove any hydrogen chloride and after filtering out the inorganic salts, the solvent was evaporated leaving a brown solid. Yield, 0.1497 gm. or 26.2%, the total recovery being 70.5%. Found: methoxyl, 4.34, 4.28%.

(d) The methylated sample (0.2160 gm.), dissolved in 15.0 cc. of 0.1118 N sodium hydroxide solution, was heated un-

der reflux for 5 hours and titrated to pH 7 with 0.1185 N hydrochloric acid. The sample required 4.00 cc. and the blank 14.03 cc., the difference of 10.03 cc. corresponding to a saponification equivalent of 181.5.

The solid isolated at pH 0.7 was dissolved in methanol, reprecipitated with ether, extracted with n-pentane and dried *in vacuo*. Yield, 0.1 gm. or 46.4%. Found: methoxyl, 7.70, 7.65%.

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. The oxidation of isolated periodate lignin (OCH_3, about 11.4%) with aqueous sodium chlorite at a pH of about 4, and with aqueous chlorine dioxide at pH values from 4 to 1, was studied for the first time. Even at 30°C., the oxidations with sodium chlorite required many hours and their rate was greatly decreased when by-product chlorine dioxide was continuously removed by a stream of nitrogen gas. Both oxidants gave products, called oxylignins A, B and C, that were similar in solubilities and in other qualitative behaviour. Oxylignin A differed from the original periodate lignin in dissolving freely in cold dilute caustic soda but not in aqueous sodium bicarbonate. Oxylignin B was an acidic substance freely soluble, when freshly prepared, in alcohol, in sodium bicarbonate, and in aqueous buffers at pH 4, but precipitated from solution at pH 1. Oxylignin C was a red-brown gum soluble in water at pH 1 but capable of being extensively extracted by butanol. The sequence of oxidation was periodate lignin ⟶ oxylignin A ⟶ B ⟶ C and conditions were found to produce either A, B or C in good yield.

2. Later work was concentrated on the oxylignin B fraction, which was recovered in 35 to 55% yield by oxidizing periodate lignin for about 8 hours at 30°C. with approximately 0.4 M aqueous chlorine dioxide. The product, an amorphous cream coloured powder, difficultly soluble after isolation in water, alcohol, and dioxane, but insoluble in ether, seemed homogeneous as to its

chlorine content of 7 to 9% when attempts were made to fractionate it by dialysis or by precipitation from solution. The methoxyl content of about 7% also appeared to remain constant after reprecipitation from solution. Tests for phenolic groups were negative and the substance failed to yield significant quantities of vanillin when oxidized with nitrobenzene and caustic soda under pressure. No evidence of carbonyl or quinone groups was found and acid hydrolysis revealed the absence of reducing sugar residues in the molecule. Low pressure hydrogenation, however, caused a consumption of hydrogen corresponding to about one mole of the gas for 1000 gm. of the oxylignin.

3. The molecular weight of the oxylignin B was determined as 895 to 980 by equilibrating the vapour pressure of a methanol solution against that of a solution of sucrose octaacetate according to Signer's method. Attempts to determine the acid equivalent by direct titration with alkali gave values of 350 to 450 but the potentiometric end-points were uncertain. Reproducible values of 170 to 180 were obtained by adding an excess of standard alkali and back titrating with standard acid. This behaviour was doubtless connected with a marked instability of oxylignin B toward alkali which cleaved it into fractions of 4.8% and 9.0% methoxyl content. The capacity of oxylignin B to reduce hot Fehling's solution was perhaps connected with the same instability.

4. Repeated methylation with diazomethane of oxylignin B, with an apparent molecular formula $C_{32.8}H_{32.9}O_{20.2}Cl_{2.28}$

$(OCH_3)_2$ (mol. wt. 893), raised the methoxyl content to about 17%, introduced about 2% of nitrogen and gave a quantitative yield of a near white amorphous powder analyzing for $C_{34.7}H_{35.8}O_{14.0}N_{1.36}Cl_{1.60}(OCH_3)_5$ (mol. wt. 907), or $C_{41.6}H_{42.9}O_{16.9}N_{1.63}Cl_{1.94}(OCH_3)_6$ (mol. wt. 1091). These analyses discordant with that for the original oxylignin, suggested that the reaction with diazomethane was not entirely confined to the methylation of acidic hydroxyl groups. The methoxyl groups introduced by diazomethane were esters, because a relatively drastic saponification gave an equivalent of 181.5 and a poor yield of a product containing only 7.7% of methoxyl groups.

5. Remethylation of the diazomethane methylated product described in 4 with dimethyl sulphate at pH 11 or less reduced the methoxyl content from 17.0% to 14.5%, but remethylation of this product with diazomethane failed to increase the value to more than 16.9%. The mild alkali of the methylation had therefore saponified some of the methyl ester groups without the dimethyl sulphate methylating any phenolic or aliphatic hydroxyl groups.

6. Direct methylation of oxylignin B with dimethyl sulphate in strong alkali gave very poor yields of a product with little more than the original methoxyl content, presumably because the only hydroxyl groups available were from carboxylic acids, and because the starting material was unstable to alkali. Direct esterification of the oxylignin with methanolic hydrogen chloride, however, gave an 82% yield of a substance with 16.2%

of methoxyl groups.

7. None of the results were inconsistent with the view that chlorine dioxide exercised its characteristic effect on substituted polyhydric phenols, first hydroxylating units of this type in periodate lignin to give the phenolic but non-acidic oxylignin A, then destroying the aromatic rings to give carboxylic acids (oxylignin B) and lower molecular weight debris (oxylignin C). The intermediate oxylignin B had about 2 methoxyl and 3 or 4 carboxyl groups in a molecular weight of about 900, but appeared to be devoid of free phenolic and aliphatic hydroxyl, of ketone and quinone units.

RECOMMENDATIONS FOR FUTURE RESEARCH

The results outlined in the present Thesis amount to little more than a preliminary exploration of the rich field of research on the structure of lignin opened up by the discovery that oxidations with chlorine dioxide can be carried out in a stepwise fashion with good yields. Many of the reactions described for oxylignin B require to be repeated on a scale large enough to permit the products to be studied in detail; in particular the positive results of hydrogenation, the unexpected smoothness of esterification with methanolic hydrogen chloride and the reaction with alkali. Better criteria for the homogeneity or otherwise of oxylignin B and its derivatives are also necessary. Such studies might reveal the way in which the aliphatic

side chains of the lignin molecule are chemically combined together, since chlorine dioxide does not usually affect aliphatic compounds.

It is also obvious that the chemistry of oxylignins A and C deserves intensive study for the same reasons.

BIBLIOGRAPHY

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

2. Willstatter, R., and Zechmeister, L., Ber., 46, 2401 (1913).

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

4. Fredenhagen, K., and Cadenbach, G., Z. angew. Chem., 46, 113 (1933).

5. Paloheimo, L., Cellulosechem., 9, 35 (1928).

6. Hilpert, R.S., and Littmann, E., Ber., 68, 16 (1935).
Hilpert, R.S., and Hellwage, H., Ber., 68, 380 (1935).

7. Wald, W.J., Ritchie, P.F., and Purves, C.B., J. Am. Chem. Soc., 69, 1371 (1947).

8. Read, D.E., Ph.D. Thesis, McGill University (1949).

9. Freudenberg, K., Harder, M., and Market, L., Ber. 61, 1762 (1928).

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

11. Brauns, F.E., and Hibbert, H., Can. J. Research, 13B, 28 (1935).

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

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

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

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

16. Ritchie, P.F., and Purves, C.B., Pulp Paper Mag. Can., 12 74, (1947).

17. Heuser, E., and Samuelsen, S., Paper Trade J., 75, No. 18, 51 (1922).

18. Schwartz, H., McCarthy, J.L., and Hibbert, H., Paper Trade J. 111 No. 18, 30 (1940).

19. Moerke, G.A., J. Org. Chem., 10, 42 (1945).

20. Peill, P.L.D., Nature, 158, 554 (1946).

21. Schmidt, E., and Graumann, E., Ber., 54, 1860 (1921).

22. Giertz, H.W., Cellulosa och Papper, S.P.C.I., 1908-1948, 326-338.

23. N.V. Koninklijke Nederlandsche Zoutindustrie, Brit. 612,615 Nov. 16, 1948; C.A., P 3159h (1949).

24. Taylor, M.C., White, J.F., and Vincent G.P., Tech. Assoc. Papers, 23, 251 (1940).

25. Vincent, G.P., Paper Trade J., 124, No. 26, 53 (1947).

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

27. Heuser, E., and Merlau, O., Cellulosechem., 4, 101 (1923).

28. Schmidt, E., Geisler, E., Arndt, P., and Ihlow, F., Ber., 56, 23 (1923).

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

30. Schmidt, E., Tang, Y.C., and Jandebeur, W., Cellulosechem., 12, 201 (1931).

31. Kurth, E.F., and Ritter, G.J., J. Am. Chem. Soc., 56, 2720 (1934).

32. Ritter, G.J., and Kurth, E.F., Ind. Eng. Chem., 25, 1250 (193

33. Van Beckum, W.G., and Ritter, G.J., Paper Trade J., 105, No. 18, 127 (1937).

34. Wise, L.E., Murphy, M., and D'Addieco, A.A., Paper Trade J., 122, No. 2, 35 (1946).

35. Jayme, G., Cellulosechem., 20, 43 (1942).

36. Larinkari, J., Finnish Paper Trade J., 28, No. 7A, 55 (1946); C.A., 40, 7607 (1946).

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

38. Sohn, A.W., and Reiff, F., Papierfabrikant, 40, 1, 105 (1942).

39. Barton, J.S., Tappi, 33, No. 10, 496 (1950).

40. Wise, L.E., Ind. Eng. Chem., Anal. Ed., 17, 63 (1945).

41. Atchison, J.E., Paper Trade J. 116, No. 21, 23 (1943).

42. Runkel, R.O.H., Holzforschung, 2, 1 (1948).

43. Lovell, E.L., Ind. Eng. Chem., 37, 1034 (1945).

44. Pearl, I.A., J. Am. Chem. Soc., 68, 916 (1946).

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

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

47. Swartz, J.N., Ph.D. Thesis, McGill University (1937).

48. Hann, R.M., and Spencer, G.C., J. Am. Chem. Soc. 49, 535 (1927).

49. Raiford, L.C., and Lichty, J.G., J. Am. Chem. Soc. 52, 4576 (1930).

50. Pschorr, R., Ann., 391, 23 (1912).

51. Raiford, L.C., and Milberry, J.E., J. Am. Chem. Soc., 56, 2727 (1934).

52. Raiford, L.C., and Stoesser, W.C., J. Am. Chem. Soc., 49, 1077 (1927).

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

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

55. Taylor, M.C., White, J.F., Vincent, G.P., and Cunningham, G.L., Ind. Eng. Chem., 32, 899 (1940).

56. White, J.F., Taylor, M.C. and Vincent, G.P., Ind. Eng. Chem., 34, 782 (1942).

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

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

59. Ritchie, P.F., Ph.D. Thesis, McGill University (1947).

60. Jackson, D.T., and Parsons, J.L., Ind. Eng. Chem., Anal. Ed., 9, 14 (1937).

61. Bochvar, D.A., Chernyshev, A.S., and Shemyakin, M.M., J. Gen. Chem. (U.S.S.R.) 15, 844 (1945); C.A. 41, 747 (1947).

62. Handbook of Chemistry, Lange, N.A., Handbook Publishers Inc., Fifth Ed., 1944, p. 1118.

63. Cabott, I., Ph.D. Thesis, McGill University.

64. Rast, K., Ber., 55, 1051 (1922).

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

66. Blackman, P., J. Chem. Soc., 87, 1774 (1905).

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

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

69. Meesook, B., and Purves, C.B., Paper Trade J., 123, No. 18, 35 (1946).

70. Yackel, 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. Brach, H., B.S. Thesis, M.I.T. (1942).

73. Coombs, R.D., B.S. Thesis, M.I.T., (1941).

74. Perlin, A.S., Ph.D. Thesis, McGill University (1949).

75. Brauns, F.E., Paper Trade J., 111, No. 14, 33 (1940).

76. Spencer, E.Y., and Wright, G.F., J. Am. Chem. Soc. 63, 2017 (1941).

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

78. Cramer, A.B., Hunter, M.J., and Hibbert, H., J. Am. Chem. Soc., 61, 509 (1939).

79. Brickman, L., Pyle, J.J., Hawkins, W.L., and Hibbert, H., J. Am. Chem. Soc., 62, 986 (1940).

80. Viebock, F., and Schwappach, A., Ber., 63B, 2818 (1930).

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

82. Technical Assoc. of Pulp and Paper Ind. Standards, T 13 m-45.

83. Palmrose, G.V., Paper Trade J., 100, No. 3, 38 (1935).

84. Niederl, J.B., and Niederl, V., Organic Quantitative Microanalysis, John Wiley and Sons, Inc., New York, (2nd ed.) 1942.

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

86. Horsley, L.H., Anal. Chem., 19, 508 (1947).

87. Gilman, H., and Blatt, A.A., Organic Syntheses, Collective Volume 1, 2nd Ed., 1941, John Wiley and Sons Inc., New York.

88. Shriner, R.L., and Adams, R., J. Am. Chem. Soc., 46, 1683 (1924).

89. Fieser, L.F., Experiments in Organic Chemistry, 2nd ed. 1941, D.C. Heath and Company, New York.

90. Weissberger, A., and Proskauer, E., Organic Solvents, Oxford at the Clarendon Press, London, 1935.

91. Official Methods of Analysis of the Association of Official Agricultural Chemists, 6th Ed., 1945, p. 763. Association of Official Agricultural Chemists, Washington.

92. Harte, R.A., Ind. Eng. Chem., Anal. Ed., 7, 432 (1935).