PREPARATION AND PROPERTIES

OF

PERIODATE LIGNINS

- PAUL F. RITCHIE -

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PERIODATE LIGNINS

A Thesis

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Authorities in the field of lignin chemistry have agreed that the lignin complex is an extremely sensitive material which is chemically changed during isolation by mineral acids or alkalies, by acidulated organic solvents, by hot sulfite solutions or by many other reagents. Since present methods of isolating lignin involve such drastic treatments, the products probably bear only an indirect structural relationship to lignin "in situ". In consequence, very little is known of the nature of lignin as it exists in wood. The problem of isolating unchanged lignin seems to depend upon the development of methods that avoid drastic conditions of acidity and temperature. A successful solution of this problem would obviously facilitate the study of lignin "in situ" since investigations of the mechanism of pulping processes and other reactions would not be complicated by the presence of debris from the carbohydrates.

From the composition of extractive-free spruce wood and its holocellulose component, the composition of spruce lignin "in situ" has been computed by the method of differences. The calculated composition; C, 67.5; H, 6; CH_3O, 14% is independent of the chemical nature or state of combination of lignin in wood. The fact that isolated spruce lignins usually contain from 2 to 5% less carbon than the calculated carbon content was traced, at least in

part, to the customary practice of drying lignins at 105°C prior to analysis, whereupon a slight thermal decomposition results in loss of carbon.

A method of isolating lignin was developed which involved oxidation of the extractive-free wood meal with aqueous solutions of periodate at pH 4.1 and 20°C, solution of the oxidized carbohydrates in boiling water near pH 7 and recovery of the undissolved lignin residue by filtration. The product has been termed "periodate lignin". Although somewhat oxidized, periodate lignin is remarkably similar to lignin in wood with respect to its behavior toward ethanol containing hydrogen chloride gas, high-pressure hydrogenation and alkaline oxidation with nitrobenzene. It has been demonstrated that periodate lignin is completely soluble in sulfite liquor under normal pulping conditions, a particularly impressive observation since other isolated lignins, for obscure reasons, have lost the capacity to dissolve in a standard sulfite cook. Treatment of the periodate lignin with acids, or even water, at elevated temperatures prior to the cook resulted in a greatly decreased solubility of the lignin in sulfite liquor.

HISTORICAL INTRODUCTION

1. General

Early in the development of plant chemistry it was assumed that the tissue of stalks, stems, leaves and trunks of trees was a definite chemical substance. The French chemist Payen was able to obtain from wood a carbohydrate material that he called "cellulose". Payen observed (1) that the carbon content of extractive-free plant material ranged from 47 to 51% and concluded that, since carbohydrates contain only about 45% carbon, there was present some substance or group of substances of non-carbohydrate nature. The plant skeleton became incrusted with these substances during growth. By treating wood in succession with nitric acid and potassium hydroxide he obtained a more or less pure cellulose as a residue and showed that the extracted fraction was richer than cellulose in carbon. Schulze (2) found that the incrustants, which he named "lignin" could be removed by maceration of the plant tissue with a mixture of cold nitric acid and potassium chlorate. The material dissolved by this oxidation included those components now known as lignin and hemicelluloses. In addition to the lines of research just described, the nineteenth century witnessed several developments of great technical importance. The use of strong, hot alkali and of chlorine by Payen for the pulping of woods and grasses led, about 1860, to the kraft and sulfate processes (3) while

the sulfite process was first described in 1867 (4).

Payen's investigations of cellulose convinced him that it was a carbohydrate based on glucose residues. However, acid hydrolyzates of many celluloses obtained from lignified plant tissue were found to contain substantial amounts of galactose, arabinose, mannose or xylose, as well as glucose. Schulze (5) noted that the polysaccharides which yielded these other sugars were much more readily hydrolyzed by dilute acids than cellulose and were extracted from the cell wall by aqueous alkali. These less-resistant carbohydrates, constituted chiefly from sugars other than glucose, were given the name of "hemicelluloses".

For many years there was much speculation as to the manner in which lignin was associated with the carbohydrate constituents of wood. Payen assumed that, in lignified substances, the cellulose was merely surrounded or impregnated with lignin. There were many who opposed this view and maintained the older opinion that plant tissue was essentially an homogeneous chemical entity. The necessity of employing drastic chemical reagents for the separation of cellulose, the hemicellulose and lignin was cited as evidence that these substances were only products resulting from the breakdown of a uniform complex. All attempts to disprove the validity of this "lignocellulose" theory were unavailing until additional information was made available by the development of x-ray and birefringence techniques in

the early years of this century. As a result of this information it became incredible that the regularly organized crystal lattice of cellulose could be in uniform chemical combination with large amorphous molecules of lignin. The existence of a lignin-hemicellulose complex has been a subject of much controversy, however, and remains so until the present day.

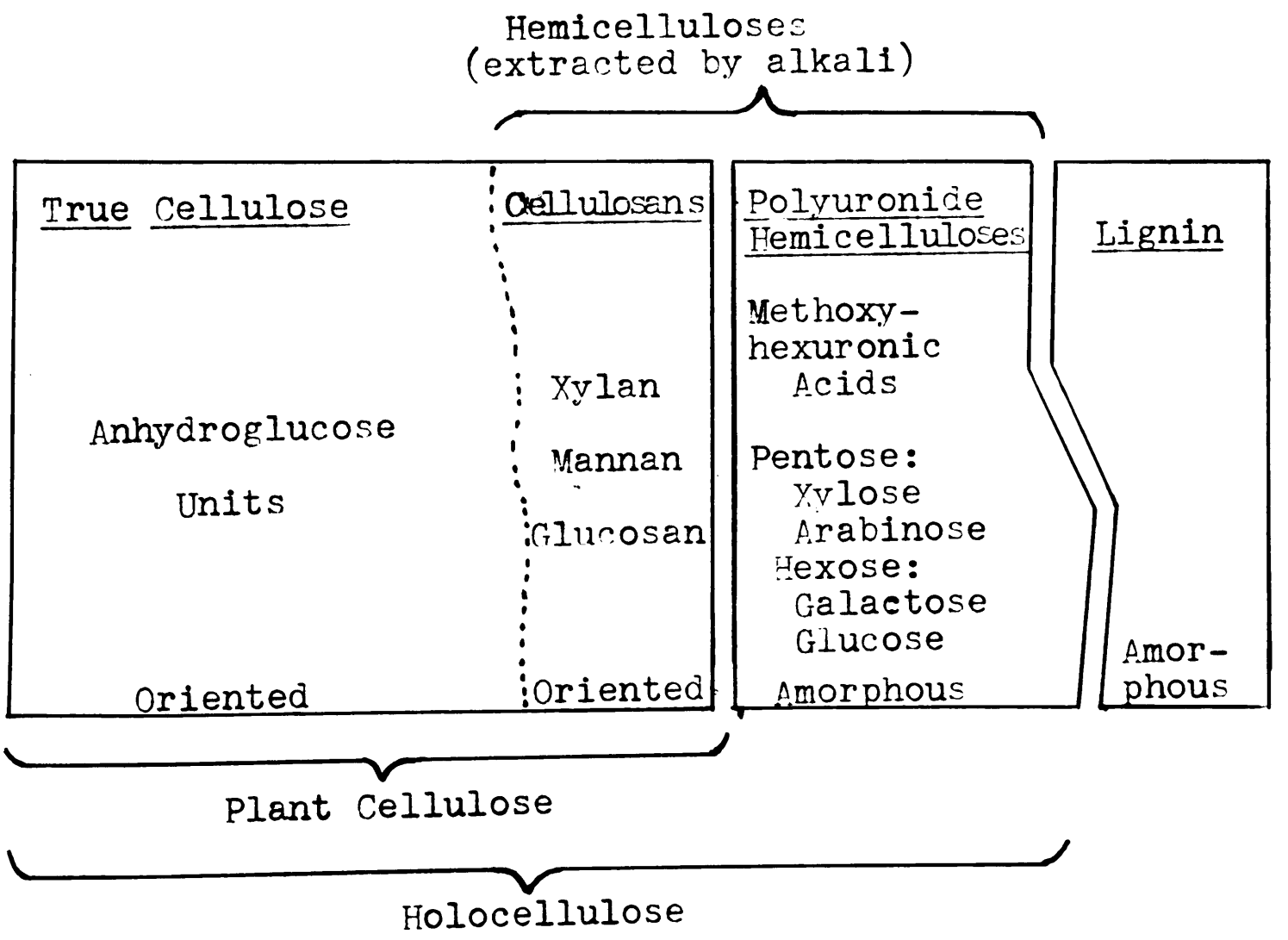
The extreme complexity of plant tissue resulted in the very slow growth of wood chemistry. At the end of the nineteenth century it was known that lignified plant material was composed of cellulose, hemicelluloses and lignin. These components were defined in terms of solubility in various reagents—a property that rarely serves as a basis for a satisfactory definition. The knowledge that had been gained was incomplete and largely qualitative. Cellulose was thought to be a polysaccharide constructed from glucose residues but in an unknown manner. The hemicelluloses were known to furnish galactose, arabinose, mannose and xylose but the actual structures were unknown. Still less was known of the nature of the lignin complex. Quantitative methods for the estimation of these wood constituents had not been developed.

The rapid growth in importance of the pulping industry, combined with the urgent need to utilize forest resources more completely, provided the stimulus for a vast amount of research in wood chemistry. Nevertheless, knowledge of the chemistry of wood is still far from complete. This statement is particularly applicable to the vaguely defined lignin complex. Not only is the nature of the basic units comprising

lignin "in situ" still unsettled but also it is extremely doubtful that native lignin has ever been isolated without chemical change. The chemist Hägglund (6), writing in "Holzchemie", commented as follows on this unsatisfactory state of affairs; "In no field of chemistry is such a lack of agreement of views concerning the constitution of a material likely to exist as in the case of lignin".

In spite of these limitations, however, Norman (7) was able to summarize the present state of knowledge about the composition of wood as shown in the diagram. A portion of the hemicellulose fraction, called "cellulosans" by

FIG. I



Norman, is hard to extract by alkali and is included in the micellar structure of the "true" cellulose. The other por-

tion, which is readily extracted, contains practically all of the "uronic acid" groups and appears to be more closely associated with the lignin than with the cellulose. The term "holocellulose" has been coined for the sum total of the carbohydrate fractions and "lignin" now refers to all constituents of a non-carbohydrate nature.

Schorger (8), and Schwalbe and Becker (9), were the first to develop an analytical scheme for the amount of ash, solvent-extractables, cellulose, pentosans, lignin and methoxyl in wood. The isolation and estimation both of holocellulose and of lignin are of great importance to this discussion and will be considered in detail.

2. Isolation and Estimation of Holocellulose and Lignin:

The need for a method of isolating, as a single fraction, the entire carbohydrate content of wood had been long recognized by workers in the wood chemistry field. The Cross and Bevan method, employing successive treatments of extracted wood with boiling dilute sodium hydroxide solution, cold chlorine water, and hot sodium sulfite solution containing sodium hydroxide, resulted in destruction of the more sensitive hemicelluloses and the isolation of only about 80% of the original carbohydrates(10). In 1921 Schmidt and Graumann (11) claimed the quantitative separation of lignin and the carbohydrates of wood by alternate treatments with 0.2 normal aqueous chlorine dioxide and 2% aqueous sodium sulfite solution at room temperature. The residue, which was entirely free of lignin, was termed "Skelettsubstanz".

Subsequent investigations proved, however, that although the hexoses of wood were unaffected by sodium sulfite solutions part of the pentosans were dissolved (12). Numberous modifications of the method appeared involving, in general, refinements in technique. Schmidt, Tang, and Jandebeur (13) suggested that the chlorine dioxide treatment be carried out in aqueous pyridine solution at pH 6.8. It was claimed that under these conditions the incrustants could be removed in a single treatment and that the hydrolytic effect upon the "Skelettsubstanz" was at a minimum. The method still resulted in loss of carbohydrates, however, and was much too time-consuming for routine work.

Ritter and Kurth (14) employed brief chlorinations of the wood meal alternated by extractions with a solution of 15% pyridine in ethanol. Five or six treatments eliminated the great bulk of the lignin, the residual small fraction of which was later removed by a 30-minute bleach with calcium hypochlorite solution at pH 7.0 to 7.5. They designated the residue as "holocellulose", a term that has become accepted in preference to Schmidt's "Skelettsubstanz". Although the method was very rapid, a slight loss of carbohydrates occurred, particularly with coniferous woods. To overcome this difficulty, Kurth and Ritter (15) increased the concentration of pyridine in the pyridine-ethanol solution to 50% and eliminated the hypochlorite bleach. It was found that the failure of the residue to display the usual orange color characteristic of chlorolignin on chlorination constituted a satisfactory end-point for the preparation of

holocellulose. The procedure gave highly reproducible results which, judging from the lignin estimation, were an accurate measure of the total carbohydrate content of wood. A still more rapid method was reported by Van Beckum and Ritter (16) who recommended replacement of the pyridine-ethanol solution by a mixture of 3% monoethanolamine and ethanol as the extractant. Although satisfactory results were obtained from hardwoods, the data from conifers (17) and straw (18) are reported to be difficult to reproduce.

For some time it has been known that wood can be delignified by treatment with aqueous chlorite solutions at moderately high temperatures (19). More recently Sohn and Reiff (20) made a systematic study on a variety of plant materials and found that lignin-free fibres, having a high hemicellulose content, could be obtained by oxidation with chlorite in a buffered system at 50 to 70° C. About the same time Jayme (21) and others (22) (23) (24) described the preparation of holocellulose from spruce wood shavings with acidified sodium chlorite solutions. In 1945 Lovell (25) reported the isolation of fibrous holocellulose from unextracted pine and hemlock by treatment of the wood, in the form of shavings, with 7.5% aqueous sodium chlorite solutions buffered with acetic acid to pH 5. The removal of lignin was accomplished in four stages at temperatures ranging from 60 to 75° C. After thorough washing of the lignin-free residue, the yield of holocellulose was somewhat lower than that

obtained from the same woods by the chlorine-monoethanolamine method. A similar procedure which resulted in a more nearly quantitative yield of holocellulose was described by Wise (26). Unextracted Redwood and Douglas fir wood meals were subjected to a single, prolonged treatment with 10% aqueous sodium chlorite solutions buffered with acetic acid. After thorough washing of the residue, the hemicelluloses were extracted with aqueous potassium hydroxide, leaving a final residue of α- cellulose. The hemicelluloses were recovered by acidification of the alkaline extract with acetic acid. Analysis of the woods for ash, solvent-extractables and lignin gave results which, when combined with the yields of α- cellulose and hemicelluloses, accounted for very nearly all of the original wood. In a later paper (27) Wise reported that the chlorite method might be used effectively in the summative analysis of coniferous woods but did not appear so satisfactory in the case of hardwoods.

The chlorite method, although showing great promise, is still in the early stages of development. It has the advantage of being applicable to the large-scale preparation of holocellulose, from which the hemicelluloses may be readily extracted in quantity for detailed examination. The chlorine-ethanol-pyridine method is recognized as the most dependable procedure for the accurate and reproducible analysis of any species of wood.

There is at present no entirely satisfactory method known for the quantitative isolation of lignin in a pure and chemically unchanged state. A number of the methods used for estimating lignin may be termed indirect, because they depend upon some property such as methoxyl content, halogen absorption or color reactions with various reagents. Since these procedures do not involve the isolation of lignin and have only an historical value, they will not be discussed further. The remaining methods, referred to as direct, may be divided conveniently into two classes: (a) Those that depend on the removal of the cellulose and other carbohydrates leaving the lignin as an insoluble residue, and (b) those that involve solution of the lignin leaving the polysaccharides undissolved. Many of the direct methods result in only a partial separation of lignin and the carbohydrates with which it is associated and are, therefore, of little interest as analytical procedures. However, they are important in structural studies of lignin and all will be discussed briefly.

(a) Almost all the published methods that involve the solution of the holocellulose are based on hydrolysis of the polysaccharides by treatment of the extractive-free plant material with strong mineral acids. The most important of these procedures employed 72% sulfuric acid although other concentrations varying from 64 to 70% have also been used. This method is generally associated with the name of Klason (28) and, in a modified form, was accepted as a standard

for the estimation of lignin (29). The modified procedure involved digestion of the extracted wood meal with 72% sulfuric acid at room temperature until gelatinization was complete, followed by dilution to 3% acid and boiling under reflux for three hours. The insoluble lignin was collected on a tared filter crucible, dried, and weighed.

Willstätter and Zechmeister (30) were the first to note that cellulose was readily dissolved in hydrochloric acid of 40 to 42% strength, but that the lignin remained undissolved. Willstätter and Kalb's (31) estimation for lignin accordingly used fuming hydrochloric acid at room temperature for four hours, followed by addition of ice and dilution with water. After thorough washing with dilute hydrochloric acid and water, the lignin residue was boiled with water and the acid in the solution was neutralized with soda. The light-brown lignin preparation was collected on a filter, dried, and weighed. Among the many modifications of this procedure that of Urban (32), who employed a mixture of hydrochloric and phosphoric acids for the hydrolysis of the polysaccharides, was most noteworthy.

Fredenhagen and Cadenbach (33) found that anhydrous hydrofluoric acid rapidly converted cellulose and the associated carbohydrates of wood into water-soluble sugar anhydrides at room temperature. The method was superior to those employing sulfuric and fuming hydrochloric acids in that elevated temperatures were unnecessary. Freudenberg (34) alternated treatments of the wood sawdust with boiling

1% sulfuric acid and cold cuprammonium solution. However, the lignin was somewhat soluble in cuprammonium solution and the yield of "cuproxam-lignin" was lower than that obtained by other methods. The procedure was also rather laborious and the product not completely free of cellulose.

An entirely different approach to the problem of isolating lignin, not involving acid hydrolysis of the carbohydrates, was studied by Ploetz (35). The selective dissolution of the polysaccharides by the action of enzymes of the type found in the digestive juices of the Weinberg snail was employed. It was found that the last traces of carbohydrates could not be removed by this treatment and Ploetz used extractions with cupriethylenediamine in order to overcome this difficulty.

(b) Numerous methods depend on the solution of lignin by reagents in which cellulose and the other polysaccharides of plant material are more or less insoluble. In this class may be included the procedures by which holocellulose is isolated in addition to the well-known sulfite, soda and kraft pulping processes. Since the lignin appears to be radically altered during extraction by all of the latter methods the products are of little interest in structural studies of lignin "in situ".

The lignin of straws (36), corn cobs (37), flax shives (38) and similar materials was found to be soluble in hot aqueous solutions containing 2 to 10% of sodium hydroxide. A similar procedure making use of alcoholic

sodium hydroxide was employed by Phillips (39) for the extraction of lignin from corn cobs. The lignin of wood is more resistant to alkalies and Mehta (40) reported that it was necessary to heat wood with 4% sodium hydroxide solution in order to secure partial delignification. Complete removal of the lignin of woods required higher concentrations of alkalies at elevated temperatures (41). In all cases acidification of the alkaline extract resulted in precipitation of the "alkali" lignin.

In 1893 Klason (42) discovered that spruce wood was partially delignified by heating with alcohol containing a trace of hydrochloric acid. It was later noted that anhydrous methanol (43), ethanol (44), butanol (45), amyl alcohol (46), benzyl alcohol (47), ethylene glycol (48) or dioxane (49) containing about 2% of dry hydrogen chloride extracted part of the lignin from wood. The lignin was recovered by neutralization and concentration of the extract followed by the addition of a large excess of water. Bailey (50) reported that the lignin of jack pine and other woods could be removed in part by cooking with butanol and water under pressure at 180 ° C. A more interesting method for the separation of a lignin fraction from wood making use of a neutral solvent without addition of an acid catalyst was discovered by Brauns (51). Extractive-free wood meal was extracted with ethanol in a percolator at room temperature for a prolonged period of time. On evaporation of the alcoholic solution under reduced pressure a light-colored,

amorphous solid was obtained in a yield of 2 to 3% of the weight of the wood. Since the conditions of the extraction were very mild Brauns considered the product to be identical with lignin "in situ" and termed it "isolated native lignin".

Wood was rapidly delignified by treatment with anhydrous phenol containing hydrogen chloride at 80 to 90° C (52). Other methods for isolating lignin fractions include those based upon the use of thioglycollic acid and hydrogen chloride (53), acetyl bromide (54) and formic acid (55).

The various schemes for the proximate analysis of wood are of great practical value in solving problems concerning its possible technological utilization; also in purely scientific studies involving comparison of species, different wood elements and parts of a single tree such as heartwood versus sapwood, bark or roots versus branches. It has already been noted that the most satisfactory method for the quantitative isolation of the total carbohydrate fraction is Ritter's chlorine-ethanol-pyridine (15) or, in some cases, the chlorine-ethanol-monoethanolamine modification (16). The accepted procedure for lignin is Klason's sulfuric acid method (29). The accuracy of these analytical procedures is indicated by the fact that they give yields of holocellulose and lignin that together account for substantially all of the extractive-free wood. Data obtained for several representative species of hard and soft woods (56) are summarized in Table I. The results in the right-hand column show that

99.5 to 100.3% of the original wood was represented by the

Table I

Lignin and Holocellulose Determinations[a]

Wood	Holocellulose[b] %	Lignin by Difference[c] %	Klason Lignin %	Lignin plus Holocellulose[d] %
White Spruce	73.3	26.7	26.6	99.9
Eastern Hemlock	68.5	31.5	31.5	100.0
Balsam Fir	69.9	30.1	30.1	100.0
Willow	78.3	21.7	22.0	100.3
Maple	76.3	23.7	23.5	99.8
White Oak	75.4	24.6	24.1	99.5

(a) Based on oven-dry weight of extractive-free wood.

(b) Chlorine-ethanol-monoethanolamine method.

(c) Extractive-free wood minus holocellulose.

(d) Column 1 plus column 3.

isolated lignin and holocellulose. Freeman and Peterson (57) applied the chlorine-ethanol-pyridine method to six American hardwoods and obtained summative analyses of ash, extractives, Klason lignin and holocellulose which ranged from 99.3 to 101.0% of the weight of the unextracted wood. Very satisfactory concordance between the lignin and holocellulose estimations led some workers to assume that the borderline between lignin and the carbohydrate fractions was quite sharply defined. There is,

however, a whole series of observations indicating that the apparent precision of the summative wood analyses might have been caused by a fortuitous cancellation of errors. In this event, the problem of separating pure, unchanged lignin and holocellulose has not yet been solved.

It has been demonstrated repeatedly that the amount of lignin obtained from wood depends not only on the method of isolation but also is altered by variation of the experimental conditions within any given method. Lignin values ranging from 15 to 28.5%, depending on the isolation procedure, were reported for sprucewood by Hägglund (6). Ploetz (58) varied the concentration of sulfuric acid in the Klason estimation from 62 to 80% and obtained lignin contents from 20.2 to 25.3 % for pine and from 26.8 to 29.5% for elder.

In view of these observations it is not surprising that the elementary composition of the lignin complex varies somewhat with the source and with the method employed for its isolation. Heuser (59) showed that the pine lignins isolated by numerous modifications of the fuming hydrochloric acid method analyzed from 62 to 68.7% carbon while Freudenberg (60) reported that lignins obtained from pine by various methods had carbon contents ranging from 62 to 67%. Schütz and Sarten (61) claimed that the carbon contents of birch and beech lignins varied from 60.7 to 67.1% depending on the method of preparation. A few of the many other similar results are given in Table II and were selected from the compilation by Phillips (62). This dependence of the amount and composition

of lignin upon the exact experimental conditions of the isolation led Schütz and Sarten to the conclusion that the lignins resulted from the breakdown of wood constituents of unknown constitution.

Table II

Carbon, Hydrogen and Methoxyl Contents of Various Isolated Lignins

Source	Method of Isolation	Carbon %	Hydrogen %	Methoxyl %
Spruce	Sulfuric Acid Method	63.9	5.3	14.5
Beech	Sulfuric Acid Method	65.0	5.0	16.6
Spruce	Fuming Hydrochloric Acid Method	64.0	5.3	14.4
Fir	Fuming Hydrochloric Acid Method	62.6	5.2	
Spruce	Urban's Method	63.9	6.0	
Spruce	Aqueous Sodium Hydroxide Method	64.0	5.5	
Spruce	Cuprammonium Method	63.6	5.7	16.0
Spruce	Alcohol Extraction without Acid Catalyst	63.6	6.2	12.1

As such, isolated lignins were mere artifacts unsuitable for further study. Schütz and Sarten demonstrated that extraction of wood with water at 90 to 100°C brought up to 45% into solution. Acidification or heating of the extract at higher temperatures produced a precipitate that corres-

ponded to lignin in its elementary composition, methoxyl content and general chemical behaviour. When the extraction was conducted at 120 to 150°C the total amount of "lignin" recovered from the residual wood and the aqueous extract was 22% greater than the lignin content of the original wood.

Twenty years ago Fuchs (63) attempted to arrive at the elementary composition of lignin "in situ" from the difference between the average composition of the entire wood and the carbohydrate components. The undeveloped state of wood analysis at that time forced him to assume that 71.5% of a wood consisted of hexosans and pentosans in a 2:1 ratio and also to assume reasonable analyses for the wood and for these components. The composition calculated for lignin in the remaining 28.5% of the wood was then corrected by assuming that ash, protein, resin and wax had the average composition C, 50.0%; H, 6.4% and amounted to 4.1%. Fuchs pointed out that the result found for "genuines lignin" C, 63.1%; H, 5.9% was independent of its chemical nature or state of chemical combination. Although similar calculations have been made from time to time by others (64) the underlying analytical data seem to have been assumed rather than determined. This deficiency was avoided by Wald (65) who first eliminated by extraction the resins, waxes and proteins from Northern Pine and then took advantage of the fact that the holocellulose fraction prepared by the method of Kurth and Ritter (15) accounts,

with the Klason lignin, for substantially all of the wood. Direct knowledge of the holocellulose-lignin ratio and of the analyses of the extracted wood and holocellulose then made it possible to calculate the composition of wood lignin without further assumptions. The composition calculated for Northern Pine lignin "in situ" was about C, 67.8; H, 6.0% with a methoxyl content of 16.2%.

The carbon contents found by analysis of isolated lignins (Table II) are of the same order as those calculated by Fuchs but generally are considerably lower than those computed by Wald. The cause of the discrepancy is not obvious but several workers have made observations that may have a bearing on the subject. Norman (66) showed that xylose--a building-stone always present in lignified cell walls--and other sugars formed insoluble residues under the conditions of lignin isolation with strong acids. Schütz and Sarten (61) pointed out that such humic material probably were formed concurrently with the saccharification of the polysaccharides of plant materials and contributed to the residue known as lignin. In a series of experiments, they demonstrated (Table III) that when xylose was added to plant material, up to 50% more "lignin" was present in the mixture than in the original substance. Schütz and Sarten claimed that these experiments explained why the yields and carbon contents of lignins varied according to the saccharification conditions.

Table III

Lignin Content of Mixtures of Xylose and Prehydrolyzed Straw[a]

Parts of Xylose	Lignin %	Increase %
0	22.1	0
20	25.0	13
50	29.0	31
100	34.5	56

(a) Based throughout on 100 parts of straw.

Jayme and Hanke (67) found that most of the lignin was very readily removed from spruce wood by sodium chlorite. When the weight of the residue was only slightly greater than that of the holocellulose of the original wood, as determined by conventional methods, it still contained about 2% of residual lignin and the holocellulose in the residue corresponded almost exactly to the theoretical yield. On purification, the chlorite extract was shown to contain simple sugars which were designated as "überschusskohlenhydrate". The sugars actually identified accounted for 3 to 4% of the weight of the wood and there was evidence of larger quantities, perhaps amounting in all to as much as 8%. The conclusion was that on treatment of wood with

strong acids this part of the carbohydrates was not saccharafied but was built up into "lignin" with a depressed carbon content.

In an unpublished research, Atchison (68) found that the sum of the Klason lignin and holocellulose, the latter isolated by the chlorine-ethanol-monoethanolamine method, amounted to almost exactly 100% of the original sprucewood. Subsequent experiments showed that the holocellulose contained excess nitrogen having an ethanolamine equivalent of 1.8 to 3.5%, based on the weight of the holocellulose. In a similar research, Thomas (69) showed that, despite his ability to account for 99.6% of aspen, 7% of the pentosans of the original wood had been lost. The presence of excess nitrogen in the holocellulose indicated that the loss of pentosans had been compensated for by absorption of ethanolamine. Even after extraction of the holocellulose with hot water, 50% of the excess nitrogen remained. Wise (27), in commenting on these observations, concluded that the apparent precision of the summative wood analyses given in the literature is caused by a neat balancing of slight experimental errors.

The general impression gained from a review of the literature is that present methods fail to accomplish the complete separation of the lignin complex and the total carbohydrate fraction. It is known that the three major constituents of the cell wall, namely, cellulose,

hemicelluloses and lignin, exist in the closest physical association, some of their chemical properties probably overlap and lignin may exist in a state of chemical combination with hemicelluloses (35) (67) (70) (71) (72). These considerations decrease the probability of effecting a sharp separation.

3. Relevant Portions of the Chemistry of Lignin:

When wood or other lignified plant material is heated under pressure with a solution of acid sulfites and sulfurous acid the lignin is dissolved leaving a residue of cellulose and part of the hemicelluloses. Because of the commercial importance of the process and the desirability of utilizing sulfite waste liquors the chemical mechanisms involved have been the subject of extensive investigation. However, lack of definite knowledge concerning the structure of lignin has contributed to the difficulties of determining the nature of the reaction between lignin and the components of the cooking liquor. The many explanations that have been proposed (73) (74) (75) to account for the processes by which lignin is rendered soluble in sulfite liquor are to a considerable extent speculative in character but it is generally concluded that both degradation of the lignin molecule and introduction of lyophilic groups occur (76).

Lindsey and Tollens (77) first showed that the lignin in sulfite waste liquor was present in the form of calcium salts of sulfonic acids. The solid lignosulfonic acid salts have been precipitated from solution by addition of

sulfuric acid, sodium chloride, calcium chloride, lead acetate or β-naphthylamine hydrochloride. Attempts to isolate definite fractions by fractional precipitation indicated that the lignosulfonic acids obtained were mixtures (78) (79). These acids proved to be particularly intractable and unsuited to further study. Treatment of the salts with mineral acids resulted in the formation of free sulfonic acids which rapidly decomposed on exposure to the atmosphere.

The commercial sulfite process consists essentially of the digestion of wood, in the form of chips, at temperatures ranging from 130 to 150°C in an aqueous solution containing alkaline-earth or alkali bisulfites (usually calcium bisulfite) and excess sulfur dioxide. The active constituents of the cooking liquor are Ca^{++}, HSO_3^-, H^+ and SO_2. During digestion lignin combines with SO_2 or HSO_3^- while the less resistant hemicelluloses are hydrolyzed to simpler compounds, a portion of the cellulose is degraded and extraneous components of the wood react with the liquor. The kinetics of sulfite digestion were subjected to critical study by Maass and co-workers (80) and although much valuable information was obtained the results must be accepted subject to the limitations of the laws of chemical kinetics when applied to a system as heterogeneous as that just described.

Early investigators (81)(82) reported that delignification of wood by the sulfite process approximately

obeyed the laws of a first order reaction. Calhoun, Yorston and Maass (80) found significant deviations from the first order reaction rate, particularly in the early stages of the cook. Yorston (83) noted that the rate of peptization of certain colloids yielded plots similar in shape to those corresponding to the rate of pulping of wood with sulfite liquor. He suggested that, in the later stages of the cook, the reaction might well consist of the peptization of solid lignosulfonic acids into a colloidal solution. An investigation of the rate of sulfonation of lignin revealed that uniform sulfonation to the extent of about 5.5% of sulfur in the undissolved lignin was all that was necessary in sulfite pulping (84). Stangeland (82) expressed the view that this minimum sulfur content was acquired early in the cook but the findings of Yorston and Green (84) did not substantiate this opinion.

The importance of temperature in sulfite digestion has long been recognized (85). Numerous workers (86) (87) have supposed that solution of lignin in sulfite liquor occurred only above some critical temperature. Sankey and Hibbert (88) considered 110°C to be this critical point below which the lignosulfonic acids were chemically different from those produced at higher temperatures. However, investigations of the rate of removal of lignin during sulfite pulping have given no support to these opinions and have shown that the process is apparently a continuous function of temperature (89).

Operators of sulfite digestors have noticed that excessive steaming of the charge before liquor was pumped in resulted in some physical or chemical change in the wood that made subsequent delignification difficult. This important observation was followed by the investigation of the effect of various pre-treatments of wood on the rate of sulfite pulping. Corey and Maass (90) showed that heating chips in distilled water at 130°C for lengths of time varying from one to six hours considerably retarded the rate of solution of the lignin in sulfite liquor. During the pre-treatment of wood with water, the latter became acid, owing to the liberation of formic and acetic acids from the wood. This fact suggested that the hydrogen ion concentration of the solution had been responsible for the pre-treating effect and it was subsequently shown that cooking wood in acid or alkaline solutions resulted in greatly increased resistance of the lignin to sulfite pulping (91). Pre-treatment of the wood with solutions of a large number of inorganic salts produced the same effect (92). Corey, Calhoun and Maass (93) found that the pre-treatment effect was increased when the preliminary cook was conducted at elevated temperatures or for prolonged periods of time. A sample of spruce wood meal which had been pre-treated for ninety-six hours at 140°C in a solution of pH 3 underwent no appreciable delignification when subjected to a standard sulfite cook. Similarly Lautsch (94) reported that spruce wood which had

been prehydrolyzed with dilute acids could not be delignified by the sulfite process under the same conditions as untreated wood. During their experiments, Corey, Calhoun and Maass observed that pre-treatment of wood specimens resulted in an increased lignin content of the wood as determined by the method of Ross and Potter (95) or by the Madison sulfuric acid method (96). The amount of "apparent lignin" formed varied from 1 to 5% of the weight of the original wood, depending on the temperature and duration of the pre-treatment. The nature of the changes occurring during pre-treatment was unknown and, although Corey and Maass (90) recognized that chemical changes in the lignin were possible, they considered a physical change to be more probable.

Phillips (97) stated that "isolated lignins, such as acid and alkali lignins and those extracted with an organic solvent in the presence of a mineral acid catalyst, are insoluble in hot bisulfite solutions". Hibbert (98) pointed out that this fact was a strong indication that deep-seated chemical changes occurred during isolation of the lignins from wood since lignin "in situ" was quite readily soluble in hot bisulfite liquor. It was shown that the acetylated oak lignin isolated by Suida and Titsch (99) gave, on deacetylation, and for the first time, an extracted lignin completely soluble in hot bisulfite solutions at 110°C. However, since lignin is normally soluble in sulfite liquor only

at more elevated temperatures, some changes must have occurred in the oak lignin during isolation with the acetylating mixture.

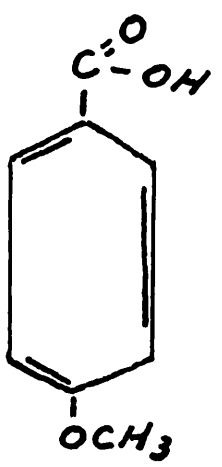
The difficulty of separating lignin from the carbohydrates of plant materials has necessitated the employment, as already noted, of drastic chemical reagents. The products isolated by treatment with strong acids are dark, amorphous, highly-insoluble solids like Klason or Willstätter lignin, while those obtained by extraction with acidulated organic solvents or alkalies are generally light-colored, amorphous, soluble materials like methanol lignin. From the analysis of methanol and phenol lignins and lignin sulfonic acids it is obvious that condensation of lignin with elements derived from the medium of extraction is of frequent occurrence (100) (101) (102). Such drastic treatments of wood probably produce, in addition, obscure and deep-seated structural changes in the lignin itself (103) and these changes are probably greatly increased when elevated temperatures are used as in technical pulping processes. Whatever the nature of these changes may be, isolated lignins differ from their progenitors in wood by remaining largely, or in part, undissolved under the normal conditions of a standard sulfite cook (97) (67).

Many attempts have been made, particularly in the past fifteen years, to acquire information concerning the basic units of which lignin is composed by degradation of isolated lignins. Similar studies have been made on the entire

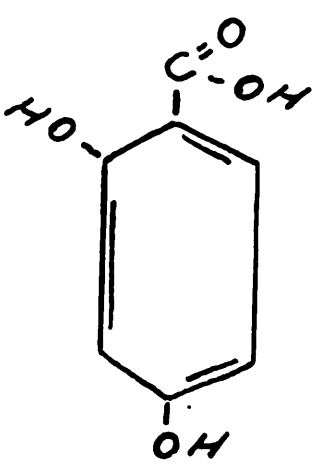
wood without attempting to isolate the lignin. In the former case the unknown alterations which occurred during isolation proved to be a complicating factor while in the latter instance debris from the carbohydrates increased the experimental difficulties. The yields of identifiable fragments have been disappointingly low and the results difficult to interpret.

Oxidation, one of the most important operations used for the degradation of an organic substance, has not afforded results of particular importance insofar as the elucidation of the structure of lignin is concerned. When the extremely sensitive lignin is subjected to oxidation, even under mild conditions, complete disruption of the molecule usually occurs and low-molecular products are obtained. Oxidation of isolated lignins and lignin-containing materials with such common reagents as ozone (104), chromic acid (105), permanganate (106) and activated oxygen (107) were formic, acetic, oxalic, malonic and succinic acids. Pauly and Feuerstein (108) reported that vanillin was produced by the ozonolysis of lignin in glacial acetic acid but their results were not confirmed in later investigations. Anisic acid (I) was obtained by oxidation of methylated alkali lignin from corn cobs with 5 N nitric acid (109). Rassow and Neumann (110) found that solutions of glycol lignin absorbed oxygen from the air in the presence of such catalysts as spongy platinum and the products included p- hydroxybenzoic acid in 6%

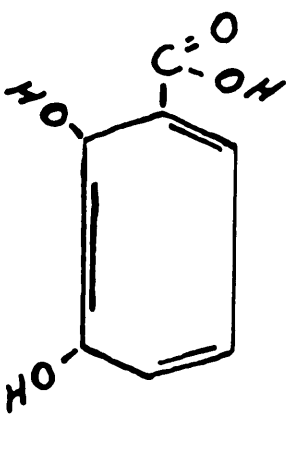
yield. Electrolytic oxidation (111) of butanol lignin at a lead cathode with sodium hydroxide electrolyte produced a high yield of identifiable compounds accounting for approximately 80% of the lignin. Among these products were found β- resorcylic (II), protocatechuic (III), p- hydroxybenzoic and m- toluic acids in a total yield of 23.5% as well as aliphatic acids and ketones.



I

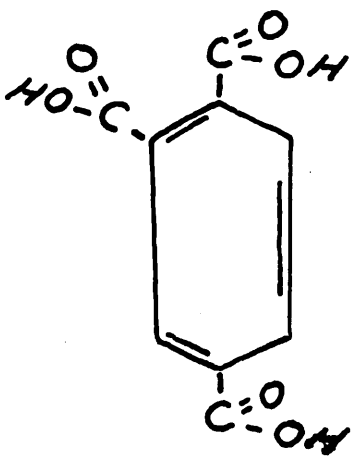


II

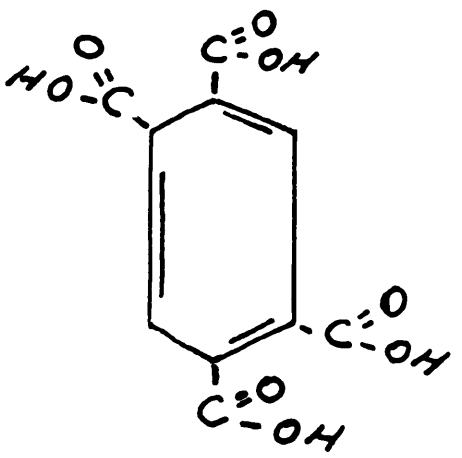


III

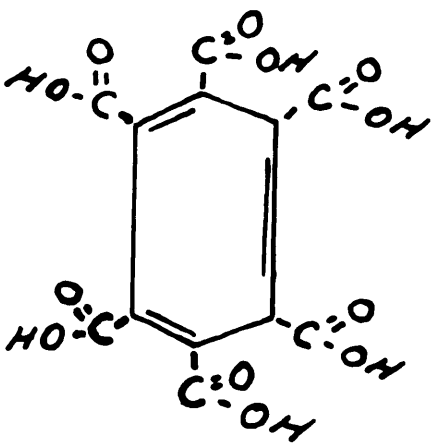
Fischer, Schrader and Treibs (112) subjected isolated lignins to air-oxidation in the presence of alkali under pressure at 200 C and recovered, in addition to simple aliphatic acids, significant amounts of benzoic, phthallic, trimellitic (IV), hemimellitic pyromellitic (V), benzene pentacarboxylic and mellitic (VI) acids. When wood was oxidized in a similar manner the same products were obtained in comparable yields (113). In a parallel series of experiments, cellulose and the humic materials obtained by



IV



V

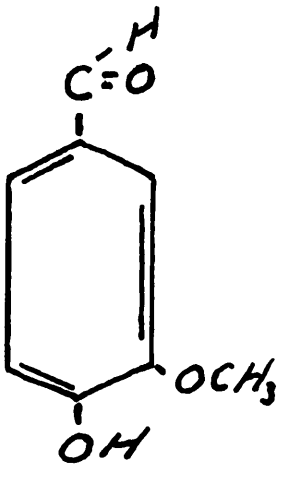


VI

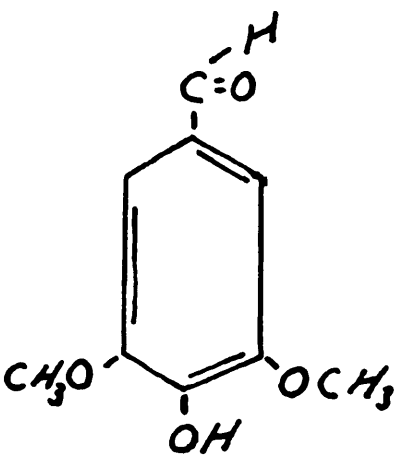
treatment of sucrose with strong acids were oxidized (114) and the products isolated, although in considerably lower yields, were similar to those obtained from lignin. However, in view of the drastic method employed by Fischer and co-workers, it was considered doubtful whether the results could be interpreted as definitely indicating that an aromatic nucleus was present in the lignin molecule or in humified carbohydrates (115). In connection with their studies of various coal components, Bone and co-workers (116) oxidized Willstätter lignin with alkaline permanganate under carefully controlled conditions and isolated from the reaction mixture mellitic and other benzene polycarboxylic acids in a total yield of about 13%. The same workers clearly demonstrated that in the conditions employed only fused ring systems of the polynuclear hydrocarbon type gave rise to benzene poly-carboxylic acids and that rings containing oxygen were completely degraded.

Vanillin (VII) was produced when the dry residue of sulfite waste liquors was heated with lime (117) but no

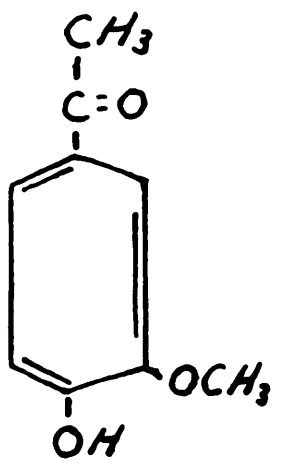
attempt was made to follow up this important observation until recent years. In 1928 Kürschner (118) reported that vanillin was formed when sulfite liquor was heated with sodium or potassium hydroxide while a stream of air was passed through the solution. Subsequently many workers (119) (120) (121) investigated the formation of vanillin from lignin sulfonic acids and concluded that the yield of vanillin depended not only on the conditions of the alkaline hydrolysis but also on the length of the heating period and the temperature prevailing during the delignification of the wood with bisulfite cooking liquor. The lignosulfonic acids obtained from hardwoods yielded syringaldehyde (VIII) as well as vanillin on degradation with hot aqueous alkali (122). In addition to vanillin and syringaldehyde, acetovanillone (IX) and aceto-syringone (X) were identified as degradation products of sulfite waste liquors (123).



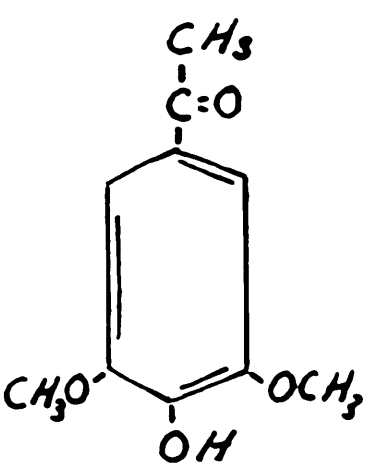
VII



VIII



IX



X

Freudenberg and co-workers (124) reported that the presence of a mild oxidizing agent in the alkaline reaction mixture was necessary for the production of vanillin from isolated lignins or wood. Nitrobenzene was found to be the most satisfactory oxidant and by its use vanillin was obtained from spruce "cuproxam" and Willstätter lignins as well as from the spruce wood itself in yields of 19.3 to 24.3% of the weight of the original lignin.

Pearl (125) showed that cupric hydroxide and other mild inorganic oxidants could be substituted for the nitrobenzene. Hibbert and co-workers (126) (127) applied Freudenberg's alkaline nitrobenzene oxidation to a wide variety of woods and determined the yields of vanillin and syringaldehyde (Table IV) by fractional sublimation of the crude mixtures obtained from the bisulfite-soluble fraction of the reaction mixture. Syringaldehyde and vanillin were recovered from hardwoods in a ratio of about 3:1 while softwoods yielded vanillin only.

Valuable information concerning the nature of lignin has been gained through high-pressure hydrogenation studies within recent years. Among the earliest workers were Fierz-David and Hanning (128), who subjected Willstätter lignin to catalytic hydrogenation under a pressure of 250 atmospheres, nickel being used as the catalyst. In the absence of a catalyst practically no hydrogenation took place even at 300 atmospheres pressure.

Table IV

Nitrobenzene Oxidation of Extractive-free Woods

Species	Vanillin[a] %	Syringaldehyde[a] %
White Spruce	23.5	None
White Pine	18.5	None
Red Cedar	24.0	None
Hemlock	22.1	None
Redwood	23.5	None
Red Maple	10.2	34.7
Silver Maple	12.7	35.2
Yellow Birch	11.0	33.7
Aspen	9.4	32.1
Red Oak	10.5	34.3

(a) Yields calculated on the basis of the Klason lignin content of the woods.

A yield of about 8% of phenols was reported but no attempt was made to identify specific compounds. Boomer and Edwards (129) subjected wood to high-pressure hydrogenation in a tetralin medium at high temperatures and found complete solution in the absence of a catalyst. The products were, however, classified only on the basis of solubility.

The first detailed characterization of the hydrogenation products of lignin was accomplished by Harris, D'Ianni

and Adkins (130). Methanol lignin from extractive-free aspen wood was hydrogenated in dioxane solution at a pressure of 220 to 400 atmospheres and a temperature of 260°C in the presence of a copper-chromium oxide catalyst. After eighteen to twenty hours the absorption of hydrogen ceased and on removing the catalyst a colorless or slightly yellow solution was recovered. The cyclic products positively identified were 4-n-propylcyclohexanol-1 (XI) and 4-n-propylcyclohexanediol-1,2 (XII). The high yield of methanol (Table V) was undoubtedly in part caused by the

Table V

Products from Hydrogenation of Methanol Lignin

Product	Yield[a] (8m.)
Methanol	22
4-n-propylcyclohexanol-1	9
4-n-propylcyclohexanediol-1,2	3
3-(4-hydroxycyclohexyl) propanol-1[b]	20

(a) From 80 grams of lignin.

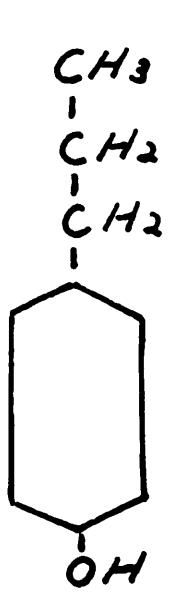
(b) Not positively identified.

presence of methoxyl groups derived from the dioxane during the hydrogenation. These other products accounted for about 50% of the lignin after allowance was made for its methoxyl content. In addition, high-boiling materials

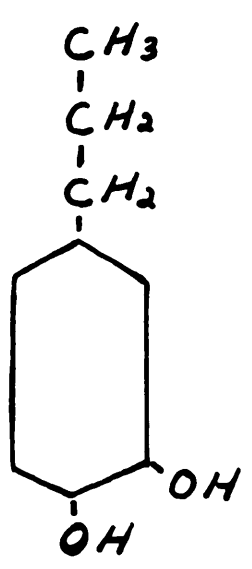
containing more than nine carbon atoms were obtained in a yield of about 30% and with an average composition of $C_{18}H_{32}O_3$ or $C_{24}H_{42}O_4$.

Adkins, Frank, and Bloom (131) hydrogenated "Meadol", isolated by the soda process, under approximately the conditions employed in hydrogenating methanol lignin. It was found that "Meadol" gave a lower yield of 9- carbon compounds and a correspondingly higher yield of high-boiling compounds. Of the high-boiling fraction, a considerable portion contained 35 to 70 carbon atoms per molecule. It was concluded that condensation and cyclization had occurred during isolation of the lignin by the soda process and that the resultant structures were resistant to hydrogenolysis.

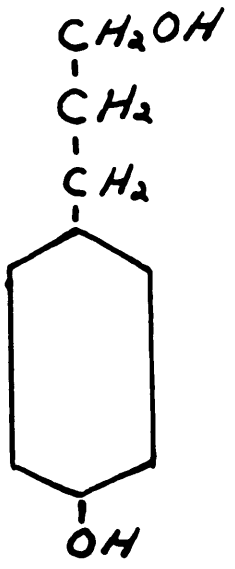
The hydrogenation of maple ethanol lignin by Hibbert



XI



XII



XIII

and co-workers (132) (133) gave the same 9- carbon compounds

as aspen methanol lignin but in a yield that indicated a difference in structure between the two types. The diol reported by Harris and co-workers was positively identified as 3-(4-hydroxycyclohexyl) propanol-1 (XIII). Hydrogenation of maple and spruce woods yielded propylcyclohexane derivatives to the extent of about 36% of the Klason lignin content of the wood. The low-boiling fractions obtained by hydrogenation of butanol lignin (134) contained products that suggested combination of lignin with the solvent during isolation. The presence of tetrahydrofurfuryl alcohol was interpreted as an indication of the existence of furan rings in lignin, although this result might have been explained by assuming that the separation of lignin and pentosans was incomplete.

By proper adjustment of the reaction variables substantial yields of phenols have been obtained by the hydrogenation of lignin. Lautsch (135) found that in an alkaline medium and at lower temperatures it was possible to obtain mainly phenols. In a further investigation of the reaction in an alkaline medium, Freudenberg and coworkers (136) (137) obtained phenols almost exclusively at 260°C when not too active catalysts were employed. The yield of phenols amounted to 40 to 50% of the weight of the lignin. It was concluded that the presence of alkali lowered the hydrogenation velocity and thus repressed ring saturation.

In an unpublished research, Pepper (138) hydrogenated

maple wood in alkaline aqueous dioxane at 165°C over Raney nickel catalyst. The following products were obtained in a total yield of 23.8% based on the Klason lignin content of the wood; 4-hydroxy-3-methoxyphenylethane, 2-(4-hydroxy, 3,5-dimethoxy-phenyl) ethanol, and 4-hydroxy, 3,5-dimethoxy-phenylethane. Rehydrogenation of the high-boiling fractions gave a further yield of 3.5% of ethyl and propyl substituted cyclohexanols. The fact that 8- carbon compounds were obtained was attributed to the drastic cleavage action of Raney nickel in the presence of alkali. The hydrogenation of hydrochloric acid lignin with less active catalysts (139) gave a 50% yield of aromatic oils from which pyrocatechol and dihydroeugenol were isolated.

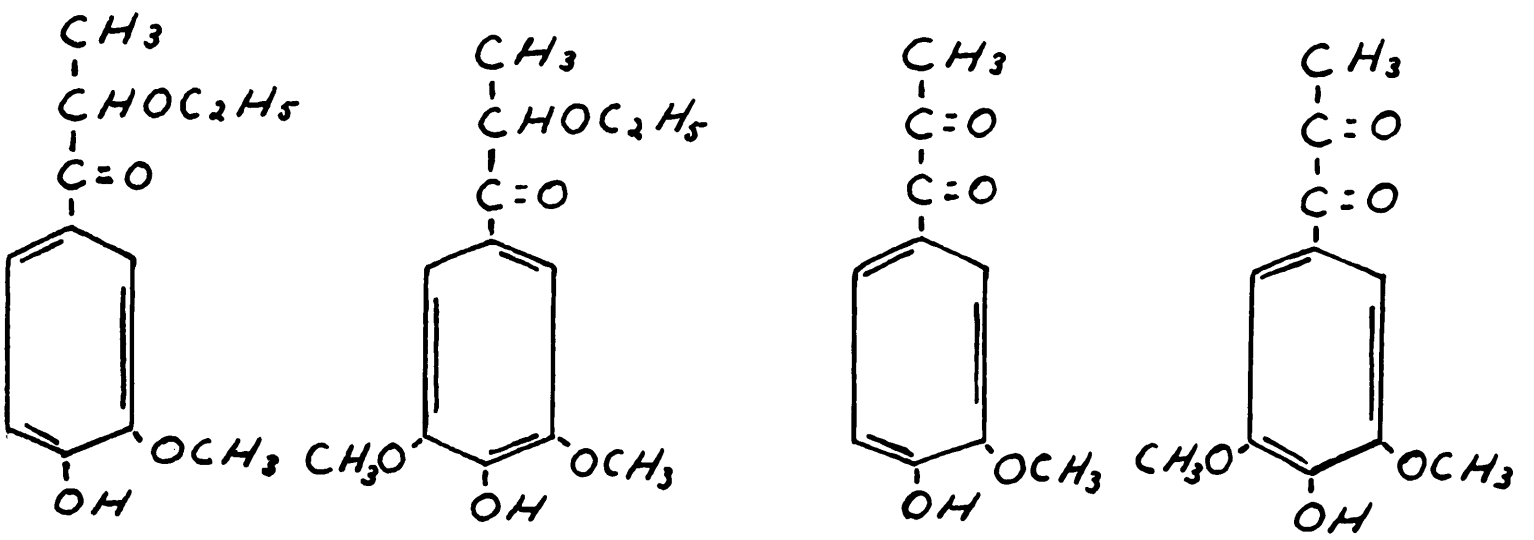
Hibbert demonstrated (140) that cyclic structures were not present among the products obtained by hydrogenation of holocellulose under the conditions employed in lignin studies. In contrast to this work, Fierz-David and Hanning (128) investigated the hydrogenation products of starch and cellulose and claimed that guaiacol and xylenol were found in a total yield of 2.4%. Cyclohexanol and cyclopentanol were also present. However, the yields were much lower than those obtained from lignin and it has been considered highly improbable that the cyclic structures isolated by the hydrogenation of the latter were derived from polysaccharide components of wood.

In the isolation of lignin by heating wood or other plant material with alcohols in the presence of an acid

catalyst, the extract obtained is filtered, neutralized, concentrated, and poured into an excess of water, whereupon the lignin is precipitated. For some time the practice was to discard the filtrate from the lignin but recently Hibbert and co-workers (141) found that this filtrate contained important degradation products of lignin, which could be isolated by extraction with ether or benzene. By differential extraction of this oily extract with sodium bisulfite, sodium bicarbonate, and sodium hydroxide solutions, it was resolved into carbonyl, acidic, phenolic, and neutral components. The proximate composition and yields of the fractions thus obtained were studied (142). The total yield of oils obtained from hardwoods was much greater than that from softwoods. When the ethanolysis was carried out in an inert atmosphere no acidic fraction was obtained (143).

A detailed study of the phenolic fraction of the oil from spruce wood revealed the presence of α- ethoxypropiovanillone (XIV) (141) whereas both α- ethoxypropiovanillone and α- ethoxypropiosyringone (XV) were identified in the similar fraction from maple wood (142). In the bisulfite-soluble oils from maple wood vanillin, syringaldehyde, methyl-4-hydroxy-3-methoxyphenyl diketone (XVI) and methyl-4-hydroxy-3,5-dimethoxyphenyl diketone (XVII) were found (144). However, the similar fraction from spruce wood contained only vanillin and methyl-4-hydroxy-3-methoxyphenyl diketone. As in the case of the alkaline oxidation of

lignins, ethanolysis of softwoods yielded only guaiacyl derivatives whereas both guaiacyl and syringyl types were obtained from hardwoods (145).



XIV XV XVI XVII

The ethylated compounds were undoubtedly formed through interaction of ethanol with the corresponding hydroxy structures.

Hibbert postulated that the action of ethanolic hydrogen chloride on lignin involved both polymerization and depolymerization changes (146). This assumption accounted for the fact that ethanolysis removed only part of the lignin from wood (142) and that the remainder was not readily decomposed in sulfite solutions under the conditions of a standard cook (147) This belief was strengthened by the discovery that re-ethanolysis of ethanol lignins yielded the same units as were obtained

by the customary wood ethanolysis procedure (146) and that the phenylpropane derivatives isolated were very sensitive compounds having a strong tendency, even under the influence of mild reagents, to undergo ortho- and para-nuclear condensations forming polymers (148).

4. Structures Suggested for Lignin:

It has been shown that the nature of the lignin complex varies from one species to another (149) and even with the age of the plant and the locality in which it grew (150). Because the lignins studied by the many workers in the field varied both in source and method of preparation, a satisfactory correlation of results has been made most difficult. The consequent wide divergence of opinion as to the nature of lignin "in situ" was expressed by Bailey (151) as recently as 1940; "Lignin is a compound, either aliphatic, aromatic or heterocyclic in nature, which has a morphological but not crystalline arrangement, which has no fusion point, which has no known inert solvent, whose reactions are obscure, whose derivatives are unexplained, whose molecular weight is decidedly uncertain and whose fundamental chemical structure is definitely unknown." Despite the truth of Bailey's commentary on the status of the lignin problem, several workers have attempted to correlate the scattered evidence in the form of suggested structures for lignin. These formulations, while highly speculatory in nature, may perhaps serve some useful purpose in that they constitute a

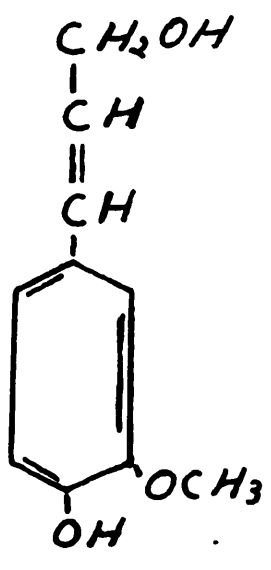
summation of present knowledge from which future investigations may be carried on.

Hilpert and his collaborators (152) assumed the extreme position that isolated lignins did not exist as such in the plant but were nothing more than the resinified debris obtained by interaction of certain labile methylated carbohydrate components of wood with the reagents used for the isolation of lignin. Schütz and Sarten (61), after discussing the indecisive evidence available, concluded that lignin suffered deep-seated transformation during isolation and that no sharp line of chemical demarcation existed between it and the well-known carbohydrate components of wood. These authors pointed to the drastic methods employed for isolation of lignin, to the fact that similar treatments of carbohydrates produced lignin-like materials, to the detection of small quantities of aromatic compounds in the reaction products of humified carbohydrates and to the isolation of "überschusskohlenhydrate" by Jayme as substantiation of their belief.

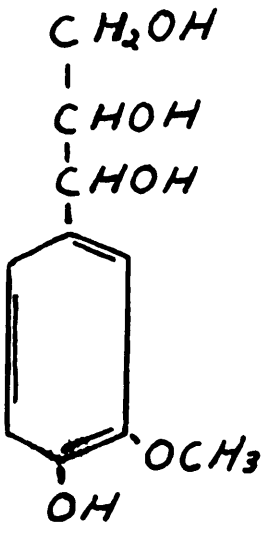
It may be stated, however, that the existence of Hilpert's labile methylated carbohydrates has never been demonstrated. Furthermore, the assumption that lignin "in situ" exists in the form of a carbohydrate is in gross discordance with the carbon values calculated for unchanged lignin by Wald (65) since the carbon content of even a fully methylated hexosan or pentosan falls below

55%. Measurement of the ultraviolet absorption spectra of lignin and lignin derivatives indicated a benzenoid structure (153) (154) in agreement with the evidence obtained from alkaline oxidation, high pressure hydrogenation and ethanolysis. Several authorities, including Phillips (155), Freudenberg (156), Hibbert (157), Percival (158) and Erdtman (159) recently reviewed the chemistry of lignin and all agreed that the substance was a condensation product based upon polyhydroxy phenols with oxygenated side chains three carbon atoms in length.

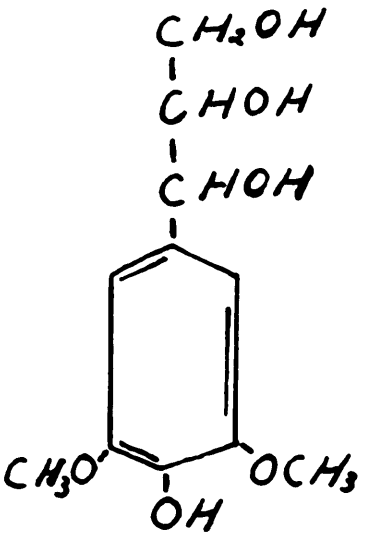
Klason (160) was the first to suggest an aromatic structure for lignin and considered that it was essentially a condensation product of coniferyl alcohol (XVIII) and oxyconiferyl alcohol. On the basis of his experimental results, Freudenberg (161) concluded that lignin was a poly-ether formed by the condensation of phenylpropane units of the type of XIX and XX, or of similar carbonyl compounds.



XVIII



XIX



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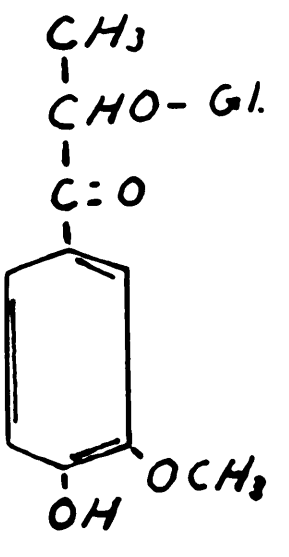
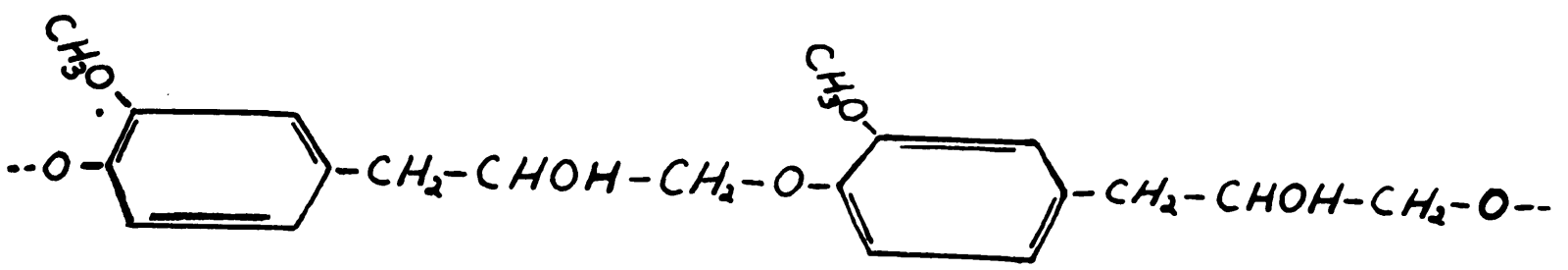


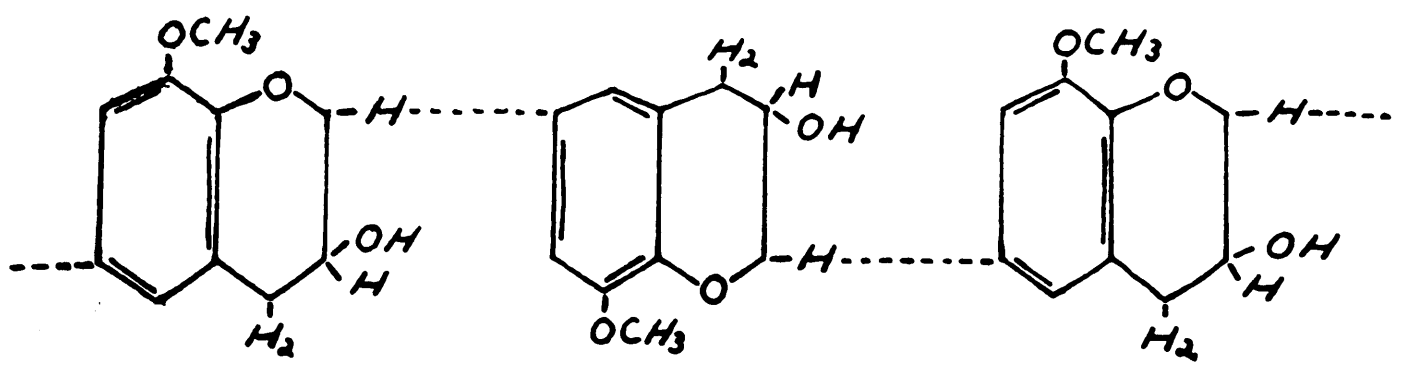
XXI

For a number of years Freudenberg considered spruce lignin to be a linear polymer of structure XXII while hardwood lignins had an analogous structure based on units containing



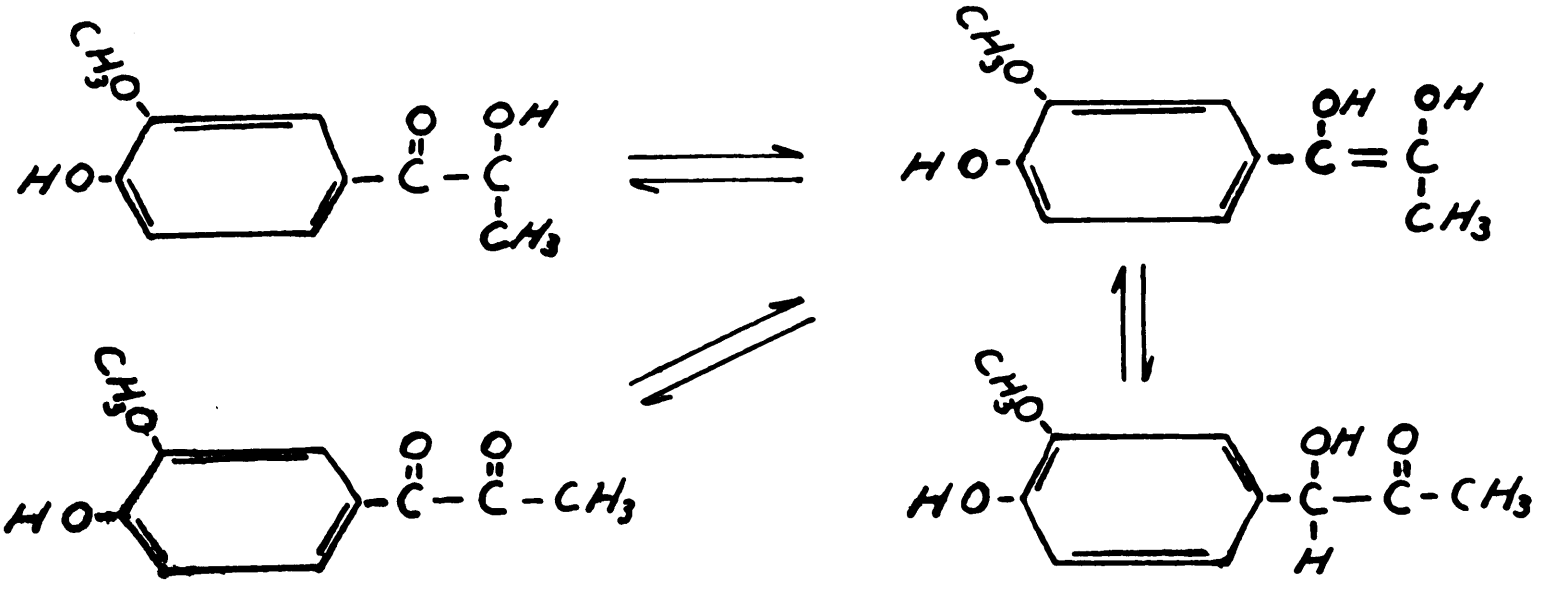
XXII

the syringyl nucleus (XX). Such a simple structure should be easily degraded by hydrolysis or hydrogenolysis giving high yields of phenylpropane derivatives similar to those actually isolated. The fact that lignin does not readily undergo degradation suggests a continuous carbon-carbon chain throughout much of the structure and, consequently, Freudenberg modified his views (162). He then considered that spruce lignin was a polymer formed by the nuclear condensation of etherified, oxygenated phenylpropane units, the etherification taking place through a phenolic and an alcoholic hydroxyl group of the side chain (XXIII).



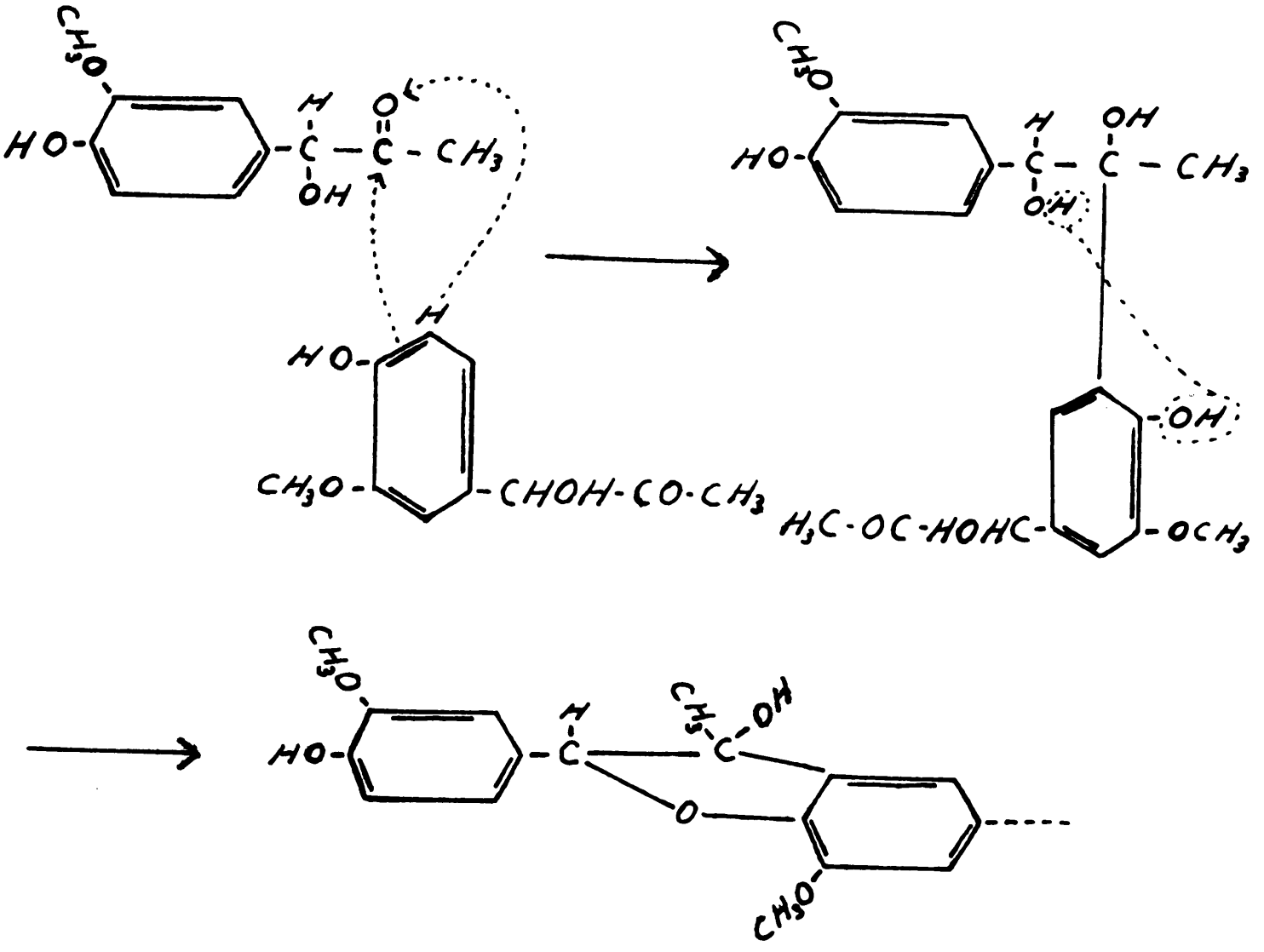
XXIII

Freudenberg expended considerable effort in an attempt to co-ordinate these lignin formulae with a theory of the bio-synthesis of lignin involving continuous condensation of simple phenylpropane building-stones of the type shown (156). Of particular interest to this theory was the discovery by Hibbert (142) that units (XIV, XV, XVI, XVII), similar to those postulated by Freudenberg, actually existed in a variety of plant materials and could be extracted by treatment with ethanolic hydrogen chloride. The fact that these oxygenated phenylpropane building-stones were isolated as soluble ethyl derivatives indicated that they did not exist as such in wood. Erdtman (159) suggested that they probably existed originally in combination with carbohydrates linked through a glycosidic bond as in (XXI). Coniferin, a compound of this type, occurs in the cambial sap of sprucewood (163). Hibbert also showed that α- hydroxypropiovanillone and α-hydroxypropiosyringone readily underwent intramolecular transformations involving hydrogen migrations and oxidation-reduction systems (157). These phenolic derivatives had a



XXIV

strong tendency, even under the influence of mild reagents, to undergo ortho- and para-nuclear condensations with the formation of polymers (XXV).

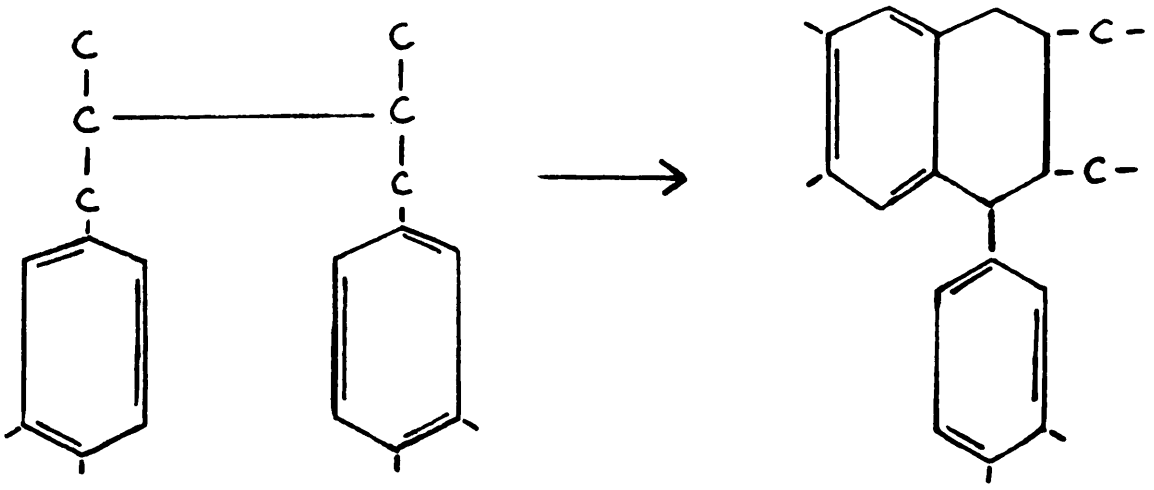


XXV

This lignin polymer contained a furan ring similar to that proposed by Freudenberg (XXIII) and the only essential difference between the two structures was in the mechanisms postulated for their formation.

It is of unusual interest to the Hibbert and

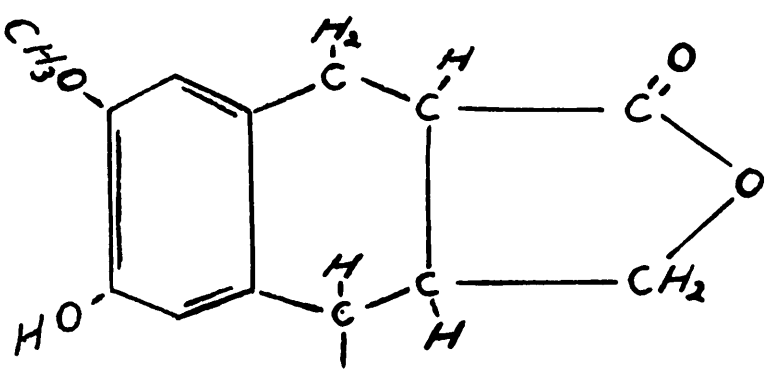
Freudenberg theories of lignin structure that dimeric forms of simple phenylpropane units have been isolated by ether extraction of the heartwood of several species of wood. These crystalline lignans, such as pinoresinol, lariciresinol and matairesinol, have the skeletal structure (XXVI). The "sulfite liquor lactone", which Lindsey and Tollens (165) first isolated from the sulfite waste liquor of spruce, was shown to have the structure (XXVIII). This lactone, conidendrin, was probably derived from the extraneous components of the wood and



XXVI XXVII

connection between it and lignin, or primordial lignin, has not been established. Nittner (166) thoroughly reviewed the chemistry of the lignans and pointed out their importance as probable intermediates in the bio-synthesis of lignins.

The lignin structures visualized by Freudenberg and Hibbert do not account for the formation of benzene polycarboxylic acids during oxidation of isolated lignins with alkaline permanganate, since these structures contain nothing but phenol-type nuclei. Many of the lignans of the type (XXVI) are extremely sensitive and undergo,

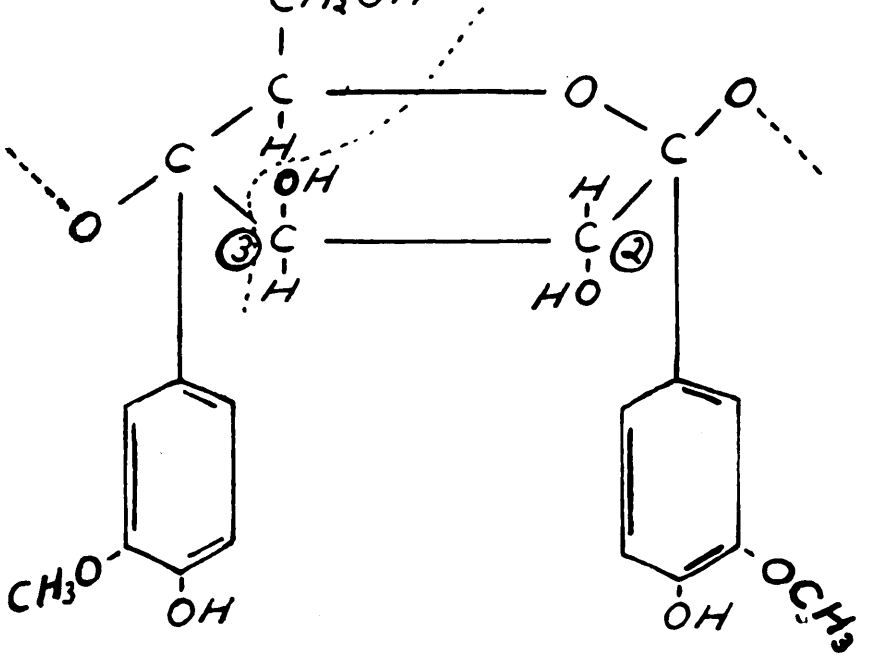


XXVIII

under the influence of a trace of acid or alkali, intramolecular condensations to the tetrahydro-1-phenylnaphthalene form (XXVII). R. D. Haworth (167) pointed out that lignin might be a mixture of polypropylbenzenes resulting from a general scheme of biosynthesis in which the dimeric forms, the lignans, are involved as intermediates. It may be that, during the isolation of lignin with strong acid or alkali,

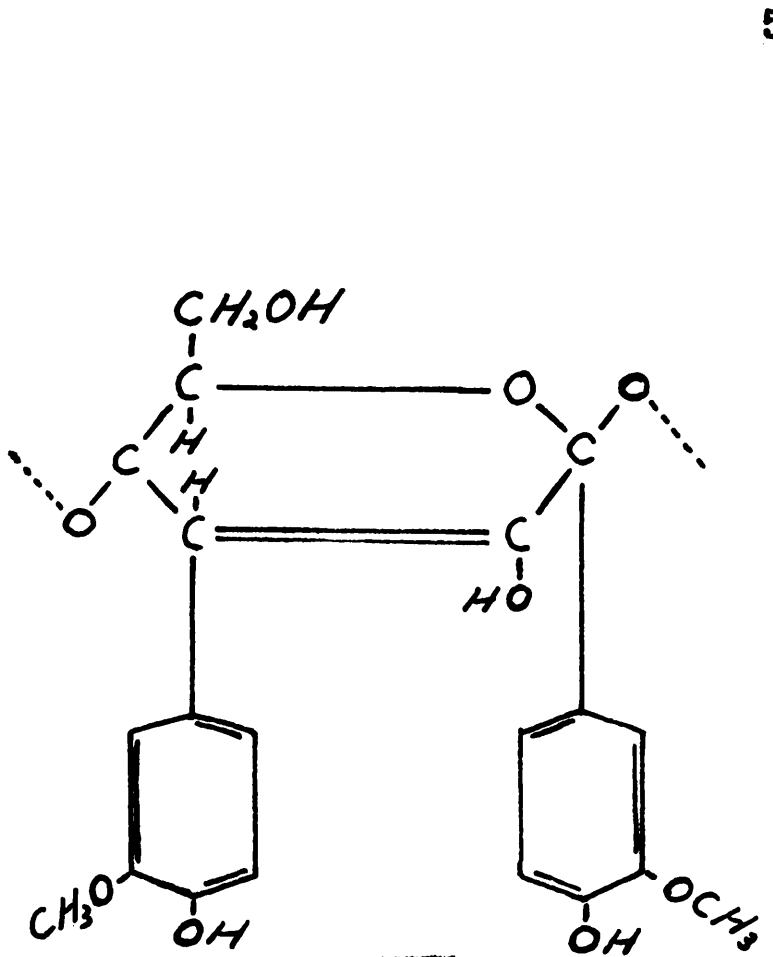
similar intermolecular and intramolecular condensations occur resulting in complicated systems of fused rings and carbon-carbon cross-linkages. On this basis it is possible to account for the formation of the fused ring systems that Bone (116) found to exist in Willstätter lignins.

Jayme and Hanke (67) concluded from their isolation of "überschusskohlenhydrate" from spruce wood that lignin was chemically combined with a portion of the polysaccharides. They suggested that lignin "in situ" was a dehydrated polyhexosan containing two guaiacyl substituents in each glucose unit. This structure (XXIX) was only

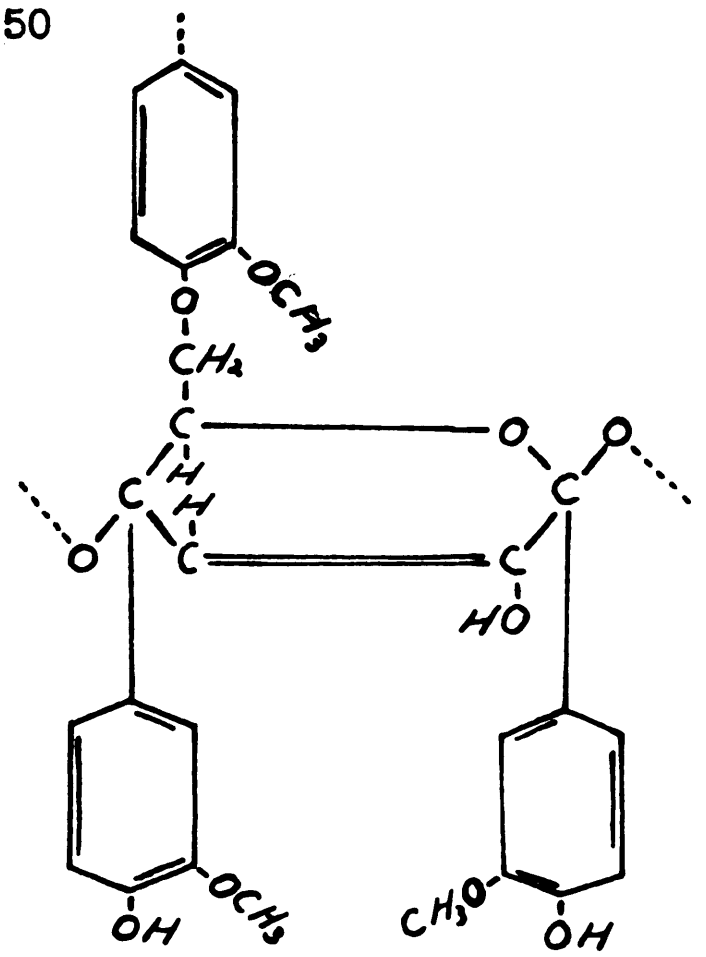


XXIX

one of several forms of lignin "in situ" as other types were supposed to be produced by dehydration of the polysaccharide unit (XXX) or by condensation of phenolic and alcoholic hydroxyl groups of adjacent chains (XXXI).

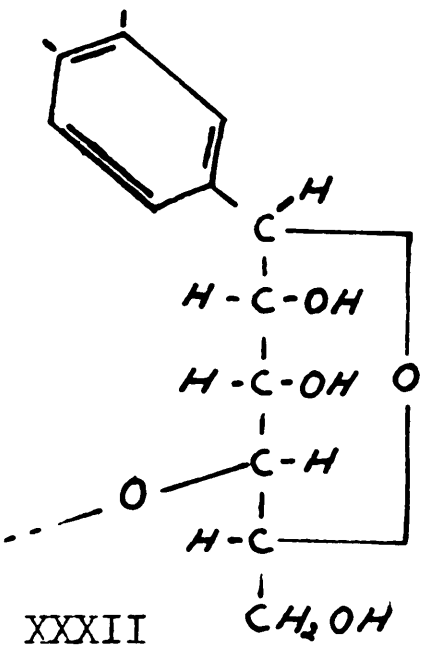


XXX



XXXI

All these types and their modifications presumably existed "in situ" as a mixture. Jayme's attempts to explain many of the known reactions of lignin on the basis of these structures are, in many cases, plausible, but his concept of the formation of phenylpropane derivatives by fission of the bond between carbon atoms two and three of the glucose unit seems open to criticism. The carbon content of less than 60% calculated for these lignin structures is grossly inconsistent with the values computed by Wald. McKinney (168) suggested that at least part of the lignin of wood might exist as phenolic glycosides of polysaccharide components of wood. The question of whether the phenolic residue (XXXII) was a simple unit or complex group of aromatic nuclei was left open.



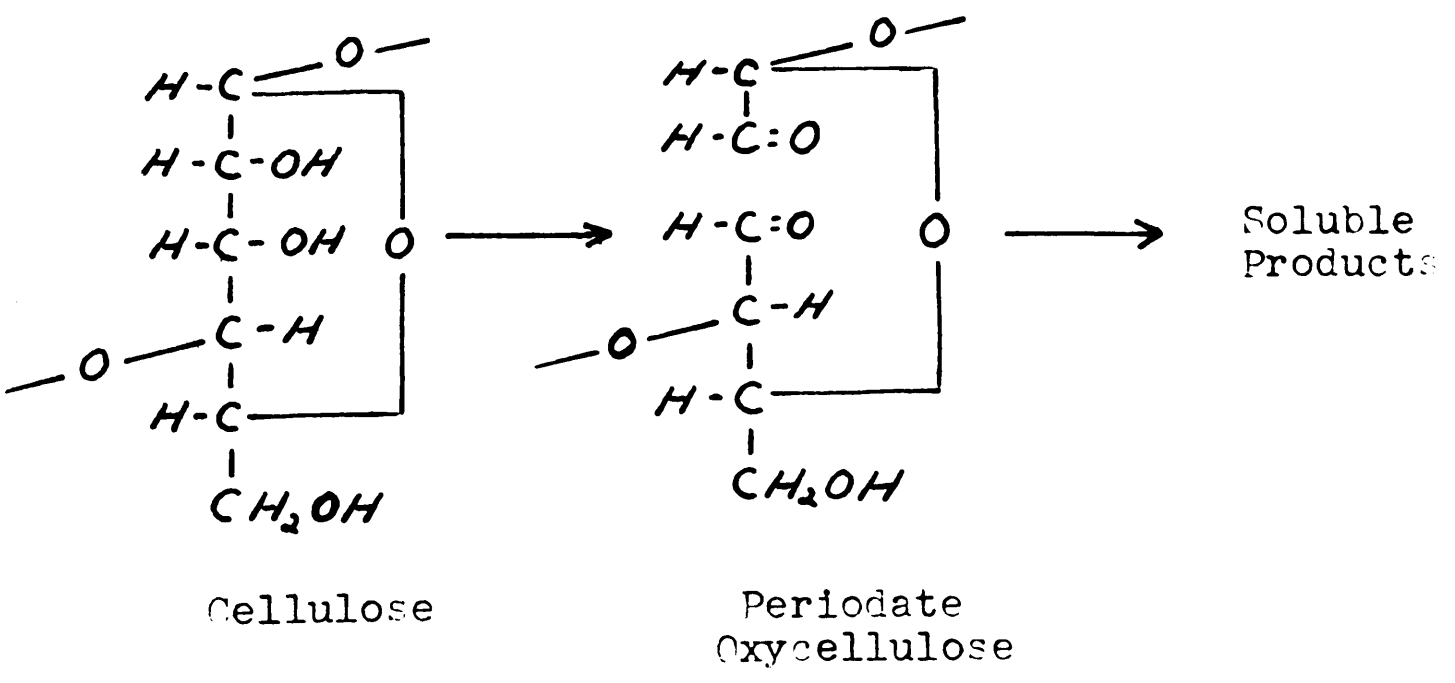
XXXII

The variety of structures that have been proposed for lignin is itself sufficient indication of the state of disagreement among lignin chemists. In 1944 Phillips (169) commented upon this state of affairs in the following words; "Although evidence of a fragmentary nature can be mustered for each of these formulas it must be stated that all of them are speculative in character. It cannot be emphasized too strongly that it is entirely premature to propose any constitutional formula for lignin considering our incomplete knowledge of the chemistry of the substance." Future progress in the field seems to depend, to a great extent, upon the ability of chemists to devise a method for the separation of chemically unchanged lignin from the carbohydrates of plant material.

5. Possible Approaches to the Study of Lignin "In Situ":

The solution of the problem of isolating lignin without change probably requires new methods that avoid high temperatures and employ neutral chemical reagents.

Brauns' (51) extraction of "native lignin" with 96% ethanol at room temperature met these requirements, but the failure of 97% of the lignin to be so extracted suggests that his "native lignin" is not identical with the bulk of the lignin in wood. Ploetz' (35) selective dissolution of the holocellulose by enzymes constitutes another approach but his incidental use of extractions with cupri-ethylene-diamine solutions to remove the last traces of carbohydrates increased the risk of chemical change. A third possible method originated in the discovery by Jackson and Hudson (170) that cellulose oxidized with cold periodic acid became soluble in hot water, owing to the nearly quantitative formation of a readily decomposed periodate oxycellulose.



Cellulose

Periodate
Oxycellulose

Later work by Grangaard, Gladding and Purves (171) showed that side-oxidations of the cellulose were at a minimum at pH 4. Jayme and co-workers (172) (173) (174) extended the reaction to xylans, starch and other carbohydrates found in

plant material. The cleavage products were found to be solubilized by hot water. On the other hand, Freudenberg and his collaborators (175) briefly stated that lignin was apparently unchanged by periodate, although a decrease from 16% to 10% in the methoxyl content of the lignin was observed. Oxidation with dilute aqueous periodate was also employed by Pennington and Ritter (176) to free certain lignin sulfonic acids from traces of polysaccharides. Their research clearly showed that a methoxyl-containing fragment was cleaved from a lignin sulfonic acid during the oxidation.

Such investigations opened up the possibility of oxidizing the holocellulose portion of solvent-extracted wood with periodate at pH 4 and 20°C, removing the oxidized product by solution in hot water at pH 7, and of recovering the undissolved lignin concentrate. Since the procedure involved neither greatly elevated temperatures, mineral acids nor alkali, it would be expected to isolate wood lignin with little or no resinification, although the product might be somewhat oxidized. Preliminary experiments by Wald (65) showed that one gram samples of Northern Pine Klason lignin consumed about 0.5×10^{-3} moles of 0.5% aqueous periodate in three hours at 20°C and pH 4.1 but that further consumption of periodate was minute. In the same conditions, pine holocellulose required about 2×10^{-3} moles, and the pine wood about 1.5×10^{-3} moles of the oxidant. Both wood and holocellulose suffered little or no decrease in weight when oxidized as described, but a sharp decrease occurred when the

products were boiled in water at pH 7. It was found that the undissolved residues were again sensitive to periodate oxidation and that the oxidation-extraction cycle could be repeated until holocellulose was completely dissolved. With Northern Pine wood, thirteen repetitions of the oxidation-extraction cycle increased the Klason lignin content from the initial figure of 28.4 to only 56.9%. At this point the work was discontinued. The present work consists of an extension of Wald's investigations to several species of wood and an examination of the behaviour of the isolated periodate lignins toward standard pulping reagents, hydrogenation, ethanolysis, oxidation and similar procedures. Detailed analyses of spruce wood and holocellulose, leading to a calculated composition for spruce lignin "in situ", are also presented.

DISCUSSION OF RESULTS

A. ELEMENTARY COMPOSITION OF SPRUCE LIGNIN "IN SITU"

The method which Wald (65) developed for computing the elementary composition of lignin "in situ" from those of the wood and the residual holocellulose was applied to spruce lignin. It is important to note that the results of these calculations represent the composition of the lignin actually removed from the original wood, but since only a small fraction of the total lignin remains in the holocellulose, the calculated composition of "lignin removed" very closely approximates that of the entire lignin complex "in situ".

In calculating the composition of "lignin removed" from those of the wood and the holocellulose, the ratio by weight existing between these two constituents of wood must be determined as accurately as possible. The yield of holocellulose (Table VI, Column A) when subtracted from 100 gives the percentage of "lignin removed" as shown in the fifth column. This calculation assumed no error in the estimation of holocellulose and automatically corrects for the small amount of lignin deliberately left in the latter. Alternatively, the Klason lignin determinations on wood and holocellulose can be assumed to be correct and their difference (Column 6) gives a value for "lignin removed". When making this computation, it was necessary to express the amount of lignin in the holocellulose as a percentage of the wood (Column B). The data in columns 5

TABLE VI

Ratio of Holocellulose to Lignin Removed (a)

Black Sprucewood

Analysis	Holocellulose Yield % (A)	Holocellulose Lignin (b) % (B)	Klason Lignin % (C)	Lignin Removed (100-A)	Lignin Removed (B-C)	Lignin Removed Average
1	72.1	0.8	28.8	27.9	28.0	27.9 ± 0.1
2	72.6	1.3	28.8	27.4	27.5	27.4 ± 0.1

Error in ratio (Analysis 1). $\frac{27.9 \pm 0.1}{72.1 \pm 0.1}$ or ± 0.2%

(a) All analyses corrected for moisture and ash.

(b) As percentage of wood.

and 6 are not identical and the probable error noted in the average of each pair corresponds to the amount by which the lignin-free holocellulose plus the Klason lignin failed to account for exactly 100% of the wood. Numerous other workers have obtained material balances of the same order of precision (57). Although this precision probably owed something to a chance cancellation of errors of unknown size (27) consideration shows that precise knowledge of such errors would throw light upon the chemical nature of the "lignin removed" rather than alter its average composition. The divergences noted were ± 0.1% and the ratio of "lignin removed" to holocellulose was accordingly in doubt by ± 0.5%, or by ± 0.2% on the basis of the wood.

The carbon, hydrogen and methoxyl contents determined for the holocellulose and wood, all data being corrected for moisture and ash, are summarized in Table VII. The percentage of carbon in the "lignin removed" was obtained in each case from the relationship:

(% C in holocellulose)(yield of holocellulose)
+(% C in "lignin removed")(yield of "lignin removed")
=% C in wood x100

Similar equations enable the hydrogen and methoxyl contents of the "lignin removed" to be calculated. This method of calculation is illustrated by Table VIII in which line 3 expresses the analyses of the holocellulose (line 1) as fractions of the wood, and the carbon, hydrogen and

TABLE VII

Analysis of Spruce Wood and Holocellulose

Sample	Moisture	Ash	Lignin	Holocellulose	Carbon (a)	Hydrogen (a)	Methoxyl
Spruce Wood	7.43%	0.35±0.03%	28.77±0.03%	70.9±0.1%	51.3±0.1%	5.98±0.01%	4.73%
Holocellulose A	(b)	0.78±0.07	1.17±0.07		44.8±0.1	5.98±0.23	1.06±0.02
Holocellulose B	(b)	0.93±0.10	1.75±0.02		44.9±0.2	6.13±0.17	1.27±0.04

(a) Corrected to the basis of a moisture- and ash-free sample.

(b) Dried for 6 hours at 105°C.

methoxyl contents of the "lignin removed" were obtained by difference. The limits of divergence between the duplicate or triplicate analyses were carried forward additively in the calculations and created an uncertainty of ± 0.7% in the percentage of carbon in the lignin. Fortunately the calculated analyses were not very sensitive to slight changes in the holocellulose-lignin ratio and the additional possible error from this cause was only ± 0.2%. The extreme calculated error in this case was ± 0.8% in the carbon, and also ± 0.7% in the hydrogen and methoxyl analyses, so that the true values are probably not far from C, 67.5; H, 6% (Table IX).

A comparison of the calculated carbon and hydrogen contents of spruce lignin with those obtained for Northern Pine lignin by Wald (Table IX) reveals a very close agreement. The methoxyl value computed for spruce lignin is somewhat lower than either that assigned to pine lignin "in situ" or that found by analysis of several isolated spruce lignins (56). This discrepancy is difficult to explain unless it is assumed that the particular spruce wood used was characterized by a low methoxyl content. In agreement with Wald, it was found that the carbon contents of isolated lignins generally fall 2 to 5% below that calculated for lignin "in situ". This observation may indicate that isolated lignins are not identical with lignin as it exists in the original wood. Lautsch and Piazolo (136), however, quote values as high as C, 67% for certain

TABLE VIII

Calculation of Lignin Composition[a]

(Analysis I)

	Base Weight	Carbon	Hydrogen	Methoxyl
Holocellulose	100	44.8±0.1	6.0±0.2	1.1±0.02
Wood	100	51.3±0.1	6.0±0.0	4.73
Holocellulose[b]	72.1	32.1±0.1	4.3±0.2	0.8±0.01
Lignin Removed	27.9	19.2±0.2	1.7±0.2	3.9±0.01
Lignin	100	67.8±0.7	6.1±0.7	14.0±0.04

Applying possible error of 0.2% in lignin-holocellulose ratio.

Lignin (corrected) C, 67.8±0.8; H, 6.1±0.7; CH_3O, 14.0±0.1%

(a) All analyses corrected for moisture and ash.

(b) Corrected to wood basis by factor $\frac{72.1}{100}$.

lignins isolated from spruce and their results agree well with those calculated for spruce lignin in Table IX.

The accepted method of drying wood or lignin for analytical purposes consists of heating the sample in air at 105°C until the weight becomes approximately constant. A sample of spruce Klason lignin, heated in this way for six hours, decreased in weight by 8.21% (Table X), but when dried to constant weight at room temperature "in vacuo" over phosphorus pentoxide the decrease was only 7.58%. This decrease corresponded almost exactly to the water content as determined by the Karl-Fischer method. It is important to note that the conventional method of drying used in the present research underestimated the true dry weights of wood and Klason lignin by not more than a few tenths of one per cent. In consequence, the carbon and hydrogen percentages, corrected for apparent "moisture" content, were high only by smaller variable amounts. It, therefore, seems that the discrepancies between observed and calculated carbon contents of lignins cannot be entirely explained on the basis of the moisture determination.

Heating at 105°C for six hours had apparently removed about 0.6% of the dry substance of the Klason lignin. More prolonged periods of heating (Table XI) caused an additional loss of 0.2 to 0.5% of the lignin substance. The losses in weight were accompanied by decreases of 2 to 3% in the carbon content of the dry lignin, as compared to

TABLE IX

Theoretical and Observed Analyses of Spruce Lignin

	% C	% H	% CH_3O
Extreme Calculated values			
Spruce Anal. 1	67.3 - 68.3	5.9 - 6.3	13.9 - 14.1
Spruce 2	67.4 - 68.0	5.0 - 6.1	13.6 - 14.0
Probable Values			
Spruce	67.5	6.0	14.0
Pine (a)	67.5	6.0	16.0
Isolated Lignins			
Native Spruce (b)	63.9	6.15	14.9
Spruce Klason (c)	65.7	5.82	15.4
Theoretical Lignin Units			
Oxygenated phenylpropane resins (d)	61 - 70	6.0	15.8
Hydroaromatic derivatives	60 - 68	9.0	15.3
Dimethyl pentosans (e)	52.5	7.5	38.8

(a) Calculated by Wald.
(b) Analyzed by Brauns (51)
(c) After drying in air at 105°C for 6 hours.
(d) Analyzed by Cramer, Hunter and Hibbert (141)
(e) Hilpert and Hellwage (152)

the value obtained by allowing for the "moisture" content of unheated samples. Similar effects were noted when spruce wood was heated in air at 105°C although it has been reported (65) that holocellulose is stable under these conditions. Since heating air-dry spruce Klason lignin in a stream of nitrogen carefully purified from traces of oxygen, produced similar results, the decrease in carbon content could not be attributed to an oxidation. In view of Moore's (177) observation of the evolution of small amounts of carbon dioxide and lower hydrocarbons from coals heated "in vacuo" at 100°C after being degassed at room temperature, the decrease in carbon content of lignin under similar conditions may be tentatively attributed to a slight, but undoubtedly complex, thermal decomposition.

It is possible that the wide spread practice of analyzing lignins after drying at elevated temperatures is responsible for the customary deficiency of 2 to 5% in carbon content. The necessity of using unheated samples for analytical purposes is obvious.

A review (65) of various lignin "building-units" suggested from time to time in the literature shows that the majority require carbon contents of 53 to 65.7%, the exceptions being three proposed by Freudenberg (178)(179) with 67.2 to 67.7% carbon. Although the carbon content of a recent variant of the phenylpropane structures, 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone, and

TABLE X

MOISTURE IN SPRUCE KLASON LIGNIN

Heated in air 6 hr. at 105°C	8.21%
Dried "in vacuo" over P_2O_5	7.58%
Karl-Fischer estimation	7.63%

TABLE XI

ANALYSES[a] OF WOOD AND LIGNIN AFTER HEATING AT 105°C

	Hours at 105°C in air		% C	% H	% Change in weight[b]
Spruce Wood	0	(c)	51.2	6.2	(+ 7.49)
	24		48.8	6.1	- 0.21
	48		48.5	6.3	- 0.40
Spruce Klason lignin	0	(c)	67.4	5.4	(+ 8.2)
	24		64.1	6.1	- 0.23
	48		63.8	6.1	- 0.28
	Hours at 105°C in nitrogen				
Spruce Klason lignin	0	(c)	67.4	5.4	(+8.2)
	24		64.1	6.1	-0.24
	48		64.7	6.1	-0.50

(a) Duplicate combustions were all within 0.18% of the averages quoted.

(b) Based on weight after drying 6 hr. at 105°C

(c) On air-dry samples and analysis corrected to base weight after drying 6 hr. at 105°C.

its dismutation isomers, is only 61%, the substance readily yielded a resin (141) with a carbon content of 70% (Table IX). Less drastic resinification might well leave the composition within the acceptable range. A glance at the composition of methylated pentosans eliminates them from consideration as possibilities for lignin "in situ". If less highly methylated carbohydrates are assumed in order to reduce the methoxyl content to the proper range, the gross discordance in carbon content is increased.

It was previously pointed out that the composition of lignin calculated from those of the entire wood and the holocellulose is independent of its chemical nature or state of chemical combination. The results found for spruce "lignin removed" C, 67.5; H, 6% place it definitely within the range of aromatic compounds and exclude the possibility that lignin "in situ" exists as a carbohydrate. The introduction into theoretical lignin formulae of many hydroaromatic units also appears to be inadmissible, because such units would raise the hydrogen content from the calculated value of 6% to the neighbourhood of 9%.

B. PREPARATION OF PERIODATE LIGNINS

I Preliminary Investigations:

The mean analyses of the extractive-free woods used in this research are summarized in Table XII. Oven-dry wood was not used, since it is well-known that this process impairs the colloidal properties of wood and since it was observed that the carbon content of oven-dry wood was less

TABLE XII

ANALYSES OF EXTRACTIVE-FREE WOODS

Analysis	Birch %	Beech %	Maple %	Spruce %
Moisture	6.21	9.84	7.48	7.49
Ash[(a)]	0.12	0.26	0.15	0.35
Lignin[(b)]	20.0	23.3	21.6	28.8
Holocellulose	-	-	-	70.9
Pentosans	-	-	-	13.2
Methoxyl	-	-	-	4.73
Carbon	-	-	-	51.3
Hydrogen	-	-	-	5.97

(a) Calculated on the basis of a moisture-free sample.

(b) All remaining values are based on moisture- and ash-free samples.

than the value calculated from the combustion of air-dry samples. It will be noted that the sum of the lignin and holocellulose contents of spruce wood amounts to 99.7 ± 0.17% of the wood and constitutes a satisfactory check upon the accuracy of the experimental technique.

The results of preliminary experiments to determine the rate characteristics, and the extent of some periodate oxidations of the woods are indicated by the plots in Figs.II and III. Samples, 0.3 to 1.0 grams, of air-dry spruce wood meal (corrected for moisture content), oven-dry holocellulose and oven-dry Klason lignin, were oxidized with an excess of approximately 1% sodium paraperiodate buffered to pH 4.1 and kept at 20°C. In all cases the molar excess was based on the assumption that the plant materials consisted entirely of anhydroglucose units, but the results are expressed as moles of paraperiodate consumed per gram of sample. At various times 5 ml. aliquots were removed from the suspensions which were continually and mechanically stirred at a slow rate. Analysis of these aliquots showed the amount of periodate remaining at any time and, by difference, account was taken of the periodate withdrawn in the successive aliquots. The uniformity of the results was somewhat marred by experimental difficulties connected with the removal of accurate and representative aliquots from the heterogeneous reaction mixture. The first series of oxidations employed 0.006 moles of periodate per gram of wood, holocellulose, or Klason

lignin (Fig. II, plots II, III, IV) and the second, 0.012 moles per gram of sample respectively (Fig. III, plots I, II, III). The data corresponding to the plots in Figs. III and IV are summarized in Tables XXI and XXII.

In general, this work confirms the results of Wald on Northern Pine. The oxidation of the carbohydrate portion of wood has been shown to proceed somewhat more rapidly than that of wood itself (Fig. II, plots I and II; Fig. III, plots II and III). The lignin complex is much more resistant to the attack of the periodate ion. In all cases a rapid initial reaction is followed by a slower consumption of periodate. After a period of three hours, the oxidation of holocellulose and wood with one molar equivalent of periodate proceeds at a slow, approximately constant rate although less than one third of the oxidant has been consumed. The nature of this slow reaction is difficult to assess. A secondary oxidation under the conditions chosen seems improbable. Nevertheless, if such is the case, holocellulose should show a rapid consumption of one mole of oxidant followed by a slower reaction. Since an increase in the concentration of periodate increased its consumption by wood and holocellulose, the amount of oxidation attained in any experiment probably depended on physical factors. In the case of wood, the slow reaction could be accounted for on the basis of a protective sheath of resistant lignin leaving only a fraction of the carbohydrate components

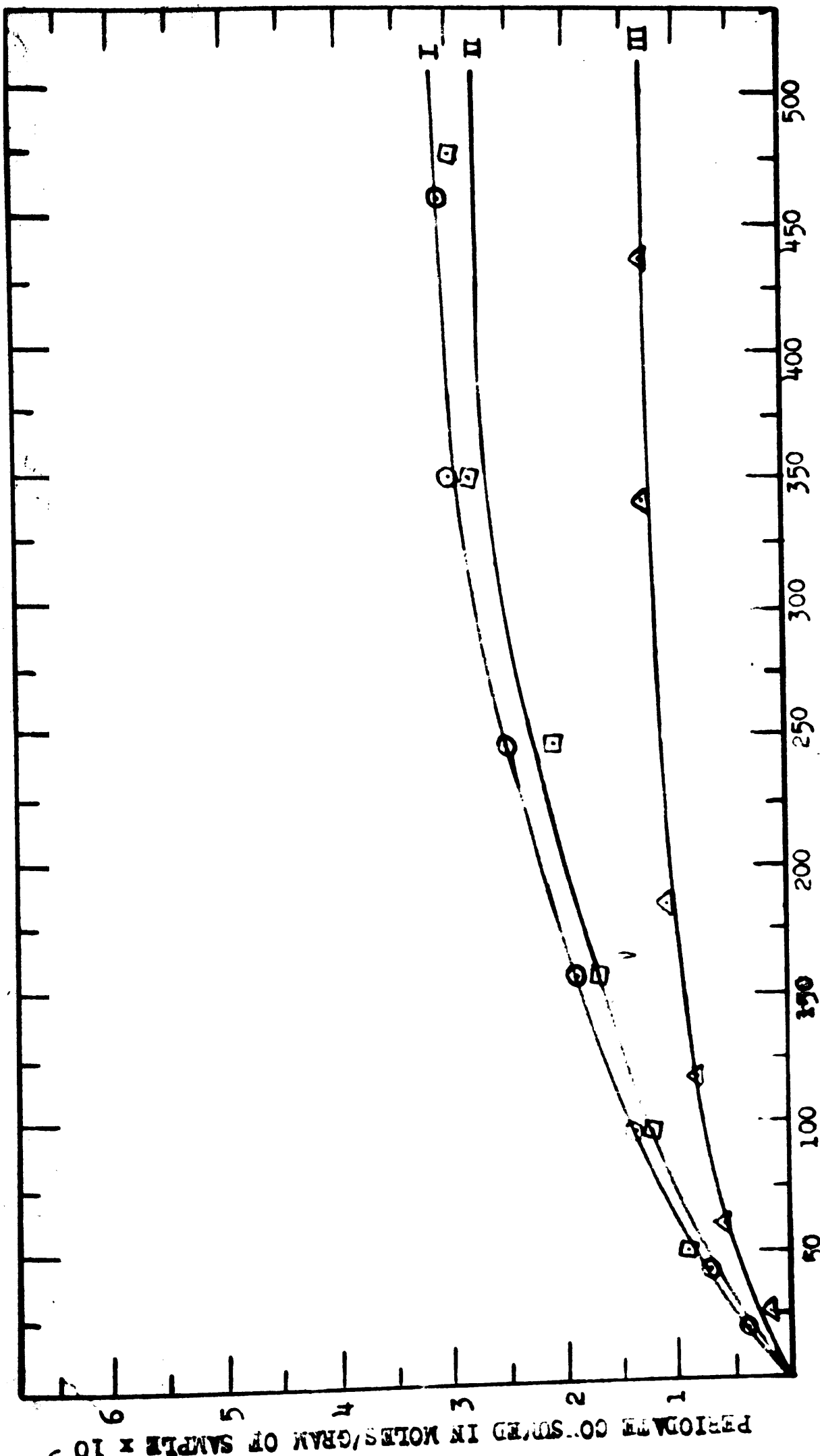


Fig. 11. Moles of 14 $Na_3H_2IO_6$ consumed at pH 4.1 and 20°C per gram sample. Plot I, spruce holocellulose; Plot II, extracted spruce wood; Plot III, spruce Klason lignin

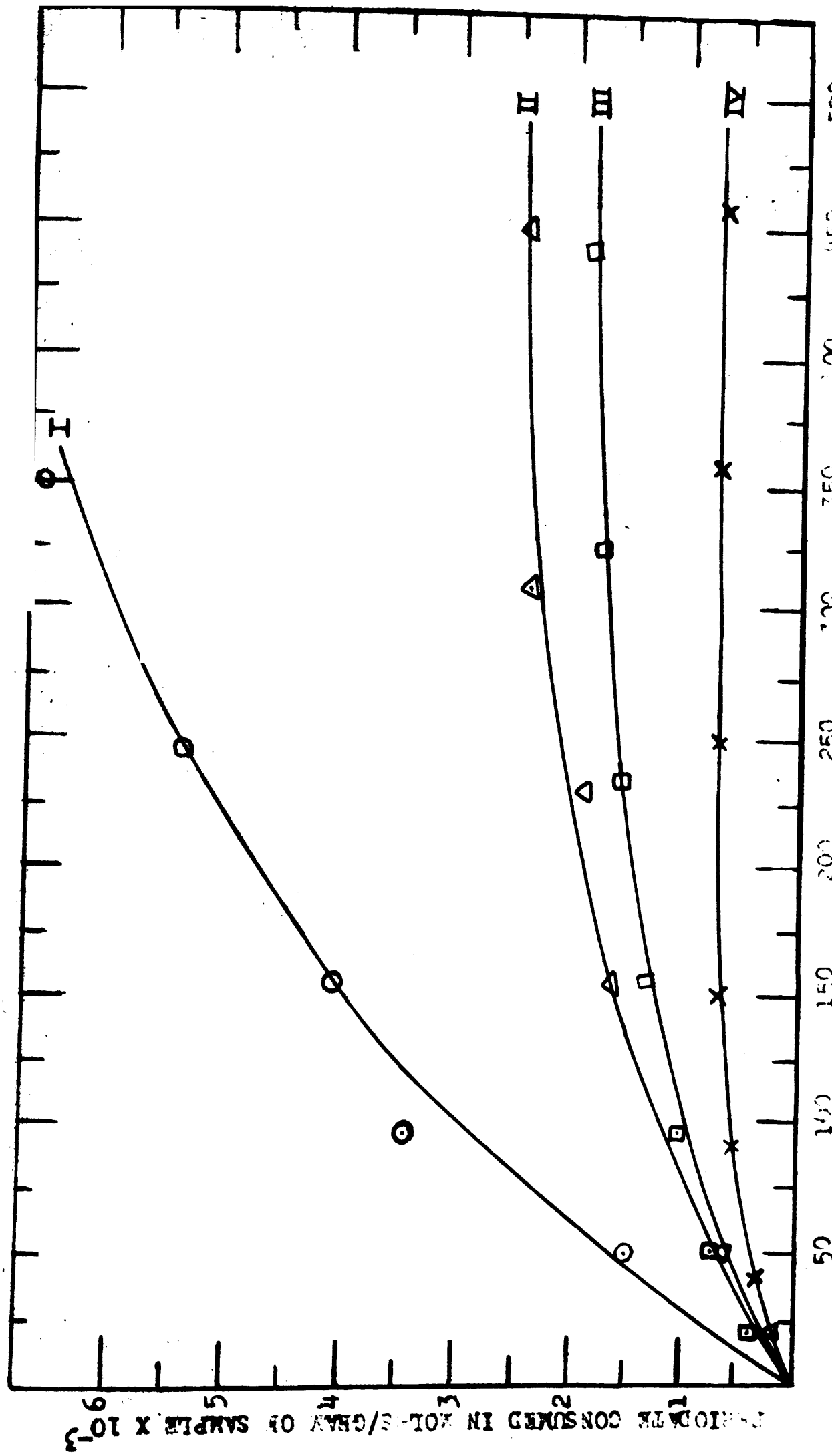


Fig. III.- Moles of $Na_2H_2IO_6$ consumed [illegible] Plot I, spruce [illegible] with H_5IO_6; plot II, [illegible] with .54 M H_5IO_6; Plot III, extracted [illegible] IV, spruce [illegible] with 0.54 M $Na_2H_3IO_6$.

available for oxidation. However, this consideration would not apply to holocellulose itself, which shows only a slightly greater rate than wood. It is more probable that the reduced rate is largely due to difficulty experienced by the reagent in penetrating to the interior of the intricate and complex carbohydrate structure. Increase of the colloidal surface through mechanical means or swelling should result in a more rapid oxidation. Increasing the concentration of the reagent results in an increase of rate for both the initial and secondary reactions, as shown in Figs. II and III.

It was found by Wald that the initial consumption of periodate by Klason lignin corresponded to approximately one mole of oxidant per Brauns-Hibbert building unit (65) or 0.0012 moles per gram. Fig.II, plot III, shows that oxidation of Klason lignin with a molar equivalent of periodate results in an initial consumption considerably below this amount, followed by an exceedingly slow disappearance of the reagent. The oxidation with two molar equivalents of oxidant shows an initial consumption of approximately 0.0012 moles per gram of lignin, in good agreement with Wald's result. The fortuitous nature of this agreement became apparent when the concentration of the periodate was trebled (Fig.III, plot I), all other conditions being unchanged, and a consumption of 0.0066 moles per gram of lignin in 5.5 hours, with no substantial decrease of weight during that time, was noted. It is doubtful if

information as to the equivalent weight of lignin can be obtained from such plots, since there is no information as to the number of units in the lignin molecule susceptible to attack by periodate. It is clear, however, that Klason lignin is not entirely inert toward periodate and that increasing the concentration of the latter increases the rate of oxidation.

A large-scale oxidation of extractive-free spruce wood was carried out. In a similar experiment, Wald had attempted to minimize the oxidative effect of periodate on lignin by using solutions of low concentration and decreasing the amount of periodate added as the oxidation proceeded. After thirteen alternate oxidations and hydrolyses the residue analyzed only 56.9% Klason lignin and contained considerable amounts of carbohydrates. It was felt that the number of treatments necessary might be reduced and the lignin content increased by maintaining the concentration of reagent throughout the procedure.

One hundred grams (corrected for 7.58% moisture 92.4 grams) of wood meal was divided into two equal portions and each placed in a two-litre Erlenmeyer flask fitted with a sealed stirrer. Each portion was oxidized with 700 ml. of a solution containing 18 grams of sodium paraperiodate buffered to pH 4.1 with glacial acetic acid. During the oxidation stirring was carried out and the temperature maintained at 20°C in a thermostatically controlled water bath. On addition of the reagent to the

wood meal, the temperature was observed to rise to 23°C and then, in a few minutes, to fall again to 20°C.

After three hours the oxidized wood meal was washed free of periodate and iodate ions with water and boiled under reflux with 4 litres of distilled water for three hours. After refiltering, the oxidation-hot water cycle was repeated. The liquors from the hydrolysis were bright yellow in color and were saved for future investigation. The pH of these liquors was never below 6.5 and the pH of the periodate solutions remained constant at 4.1 during the oxidations. Fig.IV, a plot of the consumption of periodate in grams in the successive oxidations against the number of the oxidations, shows that the effectiveness of the individual oxidations decreased in an irregular fashion. The analytical data corresponding to Fig.IV are summarized in Table XXIII. At intervals, a small sample of the oxidized, hot-water extracted wood was removed, dried through solvent exchange with anhydrous methanol and benzene, and analyzed for Klason lignin. The Klason lignin content (Table XXIV) is plotted against the number of oxidations in Fig.V. Since it was felt that grinding would open up the wood structure and thus permit the more ready penetration of the reagent, after seven oxidations the residue was disintegrated in the Waring blender for one hour. After ten oxidations the residue was again disintegrated for three hours. The particle size was visibly reduced to almost colloidal dimensions by these treatments and the increases

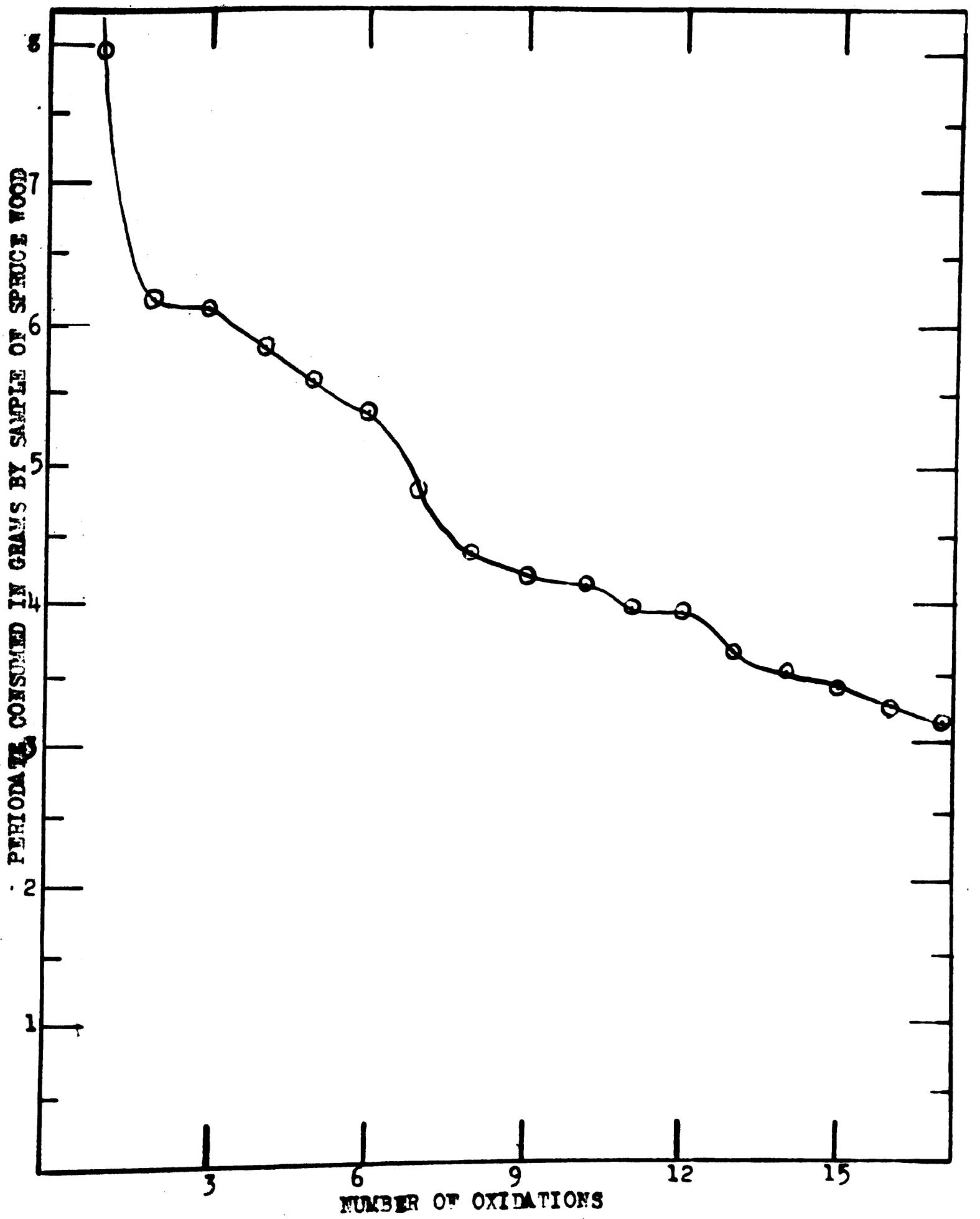


Fig.IV. - Progressive oxidation at pH 4.1 and 20°C of 92.4 gm. of extracted spruce wood with 1.2% $Mn_3H_2O_6$

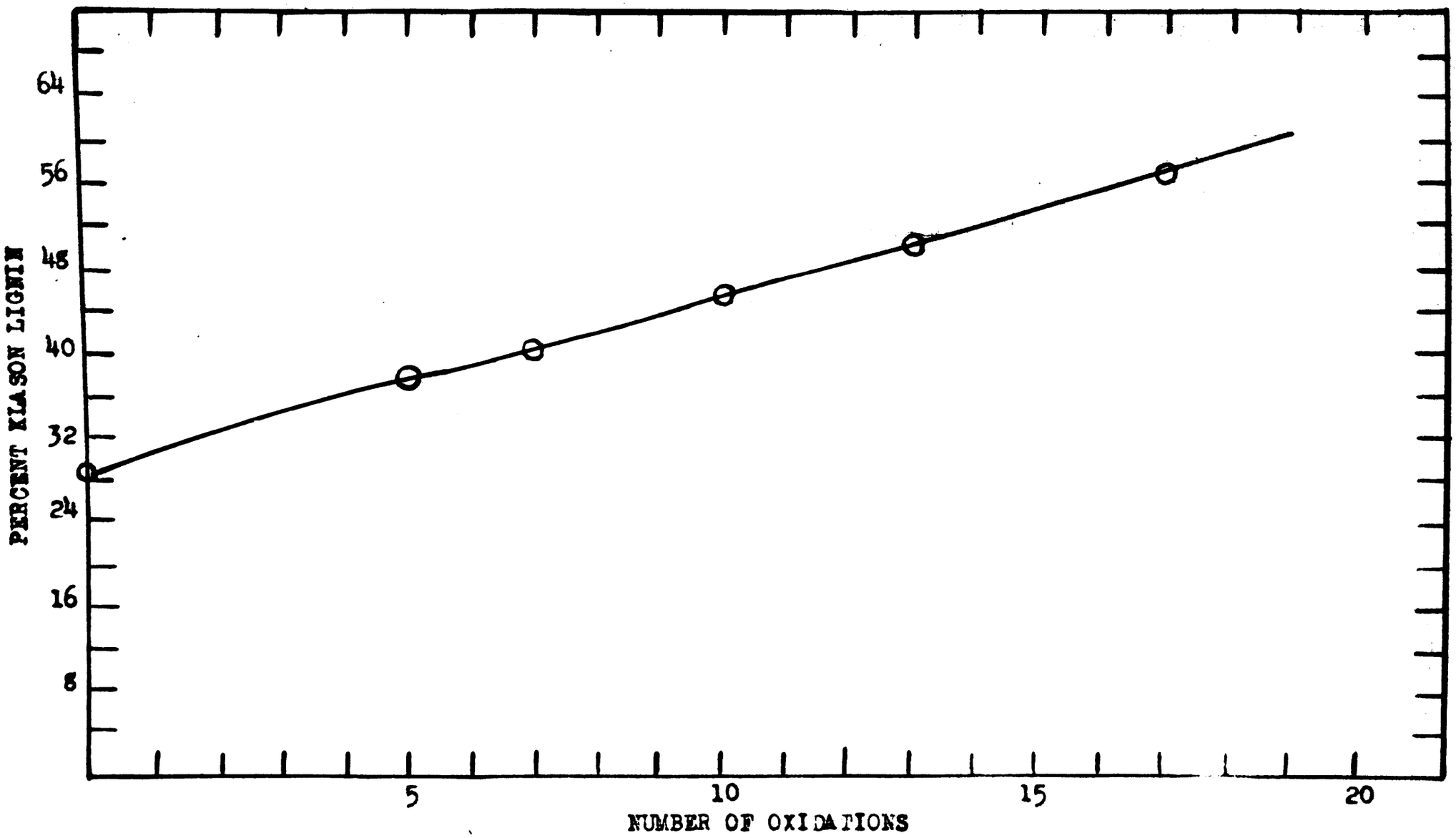


Fig.V. - Increase in Klason lignin content of residue from spruce wood repeatedly oxidized by excess of 2.6% $Na_3H_2IO_6$ at pH 4.1 and 20°C for three hour periods.

in the consumption of reagent and in the Klason lignin content are marked in Fig.IV and noticeable in Fig.V.

After 17 oxidations the material was dried through solvent exchange and under vacuum. The residue weighed 44.9 gm. (corrected for samples withdrawn for lignin analysis) and represented 48.6% of the original wood. The Klason lignin content was now 60.7%. If the lignin structure had been unattacked by the treatment, the lignin content, calculated from that of the wood and the loss of weight, should have been 63.8%. However, small amounts of the material were undoubtedly lost in the Waring blender and no correction could be made for such losses. Thus little or none of the original lignin appears to have been lost as a result of the oxidation.

Although the relatively high lignin content indicated that eventually a lignin concentrate completely free of carbohydrates could be obtained, the procedure is open to criticism because of the length of time required. Since an increase in concentration of the periodate resulted in a higher consumption (Fig.III) the rate and extent of the reaction over a wider range of concentration was investigated. Five samples of spruce wood meal weighing approximately two grams each were separately oxidized with 200 ml. of aqueous periodate of increasing concentration (Table XXV, Columns 2 and 3). The mixtures were kept at pH 4.1 and 20°C and all other conditions, save periodate concentration, were identical. The progress of the

oxidations was followed by the removal of aliquots and the titration of the residual periodate. Fig.VI, in which the consumption of periodate, in moles per gram sample, is plotted against time, shows that the extent of the initial rapid oxidation increases with the concentration of the periodate. The slower, secondary oxidation, which dominates after twenty or thirty hours in all cases, also increases somewhat with periodate concentration. After 120 hours in the first four cases, and after 72 hours in the fifth, the wood residues were washed free of iodate and periodate and were boiled with water at pH 6.5 for 3.5 hours. Drying of the residues was through anhydrous methanol and benzene and "in vacuo" at room temperature. Table XIII, Column 4, gives the weights of these residues as percentages of the original wood. When the Klason lignin content (28.8%) of the wood is divided by the fractional yield of residue, the result (Column 8) represents the Klason lignin content of the residue on the assumption that no lignin was lost during the oxidation. Comparison with the observed Klason lignin percentages (Column 7) suggests that in the first four cases only a small fraction of the lignin was removed by oxidation in a water-soluble form. However, since the reliability of the lignin estimation is questionable, the discrepancies may not be significant. The loss in lignin during run 5 was 68.8/80.5 or 11.1% and is substantial. Nevertheless, 88.9% of the original lignin was recovered in a state of

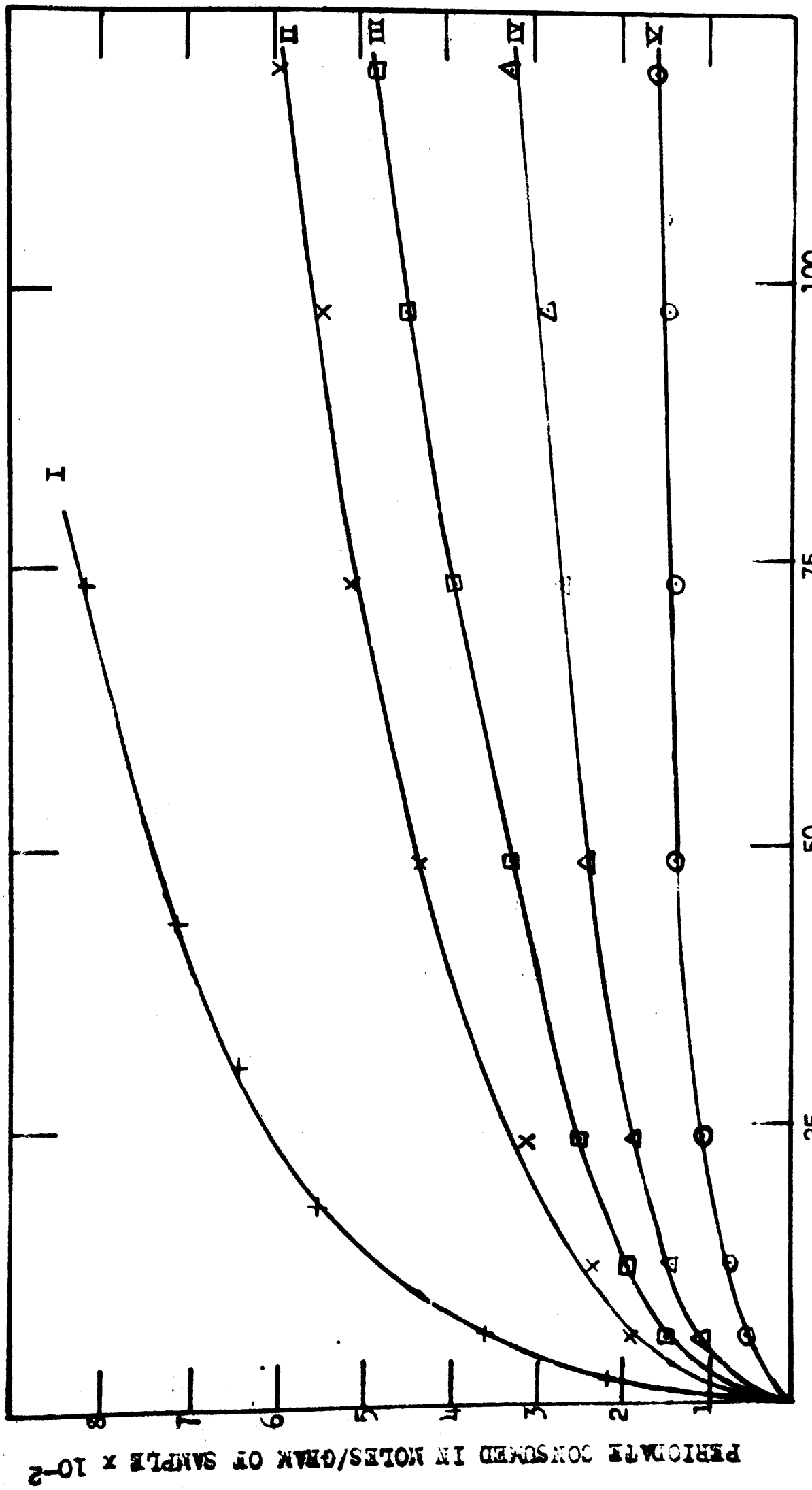


Fig.VI. - Consumption of $Na_3H_2IO_6$ by extracted spruce wood at pH 4.1 and 20°C.
Plot I. 5% $Na_3H_2IO_6$; Plot II. 3.3% $Na_3H_2IO_6$; Plot III. 2.2% $Na_3H_2IO_6$; Plot IV. 1.2% $Na_3H_2IO_6$; Plot V. 0.[illegible] $Na_3H_2IO_6$.

68.8% purity by a single oxidation lasting three days. It is evident that the task of preparing a lignin of still higher purity would be greatly facilitated by employing periodate solutions of higher concentrations for shorter times.

Fig.VII describes successive oxidations of 9.1 gm. of spruce wood meal (corrected for 7.43% moisture) by 350 ml. of 1.3% periodate solution at 20 C and pH 4.1. Each oxidation lasted 72 hours and was followed by the extraction of buffer and salts from the residue with cold water and the dissolution of oxidized carbohydrates in hot water. The upper plot gives the total amount of sodium paraperiodate in grams present at the beginning of each oxidation. Inspection of the lower plot shows that the consumption of oxidant decreased very sharply after the fifth oxidation in spite of the fact that the concentration of periodate available was not greatly changed (Table XXVI). The residue from the seventh oxidation, after drying through solvent exchange, weighed 26.17% of the original wood and analyzed 85.67% Klason lignin. These figures account for 78.2% of the lignin originally present.

II <u>Large-scale Preparation of Periodate Lignins:</u>

Fig.VIII summarizes the large-scale oxidation (Spruce Periodate Lignin A) described in the Experimental Portion and planned from the results of the smaller-scale experiments just described. The weight of sodium paraperiodate initially present in each oxidation was about one-third,

TABLE XIII

Analyses of Periodate-oxidized Spruce Wood (a)

Sample Number	Sample Weight gm.	Weight of Oxidized Residue gm.	Percent Loss in Weight	Weight of Sample for Lignin Analysis gm.	Weight of Lignin Residue gm.	% Klason Lignin	Calculated % Klason Lignin (b)
1	1.9173	0.7697	64.28	0.7510	0.5167	68.80	80.54
2	1.9752	0.8484	57.04	0.8232	0.5429	65.22	66.96
3	2.0515	1.0021	51.15	0.9299	0.5361	57.65	58.89
4	1.9939	1.3710	31.24	1.3054	0.5285	40.49	41.84
5	1.9303	1.7132	11.25	1.6263	0.5193	31.91	32.42

(a) All weights are corrected for moisture.

(b) Calculated lignin values are deduced from the loss in weight and the original lignin content.

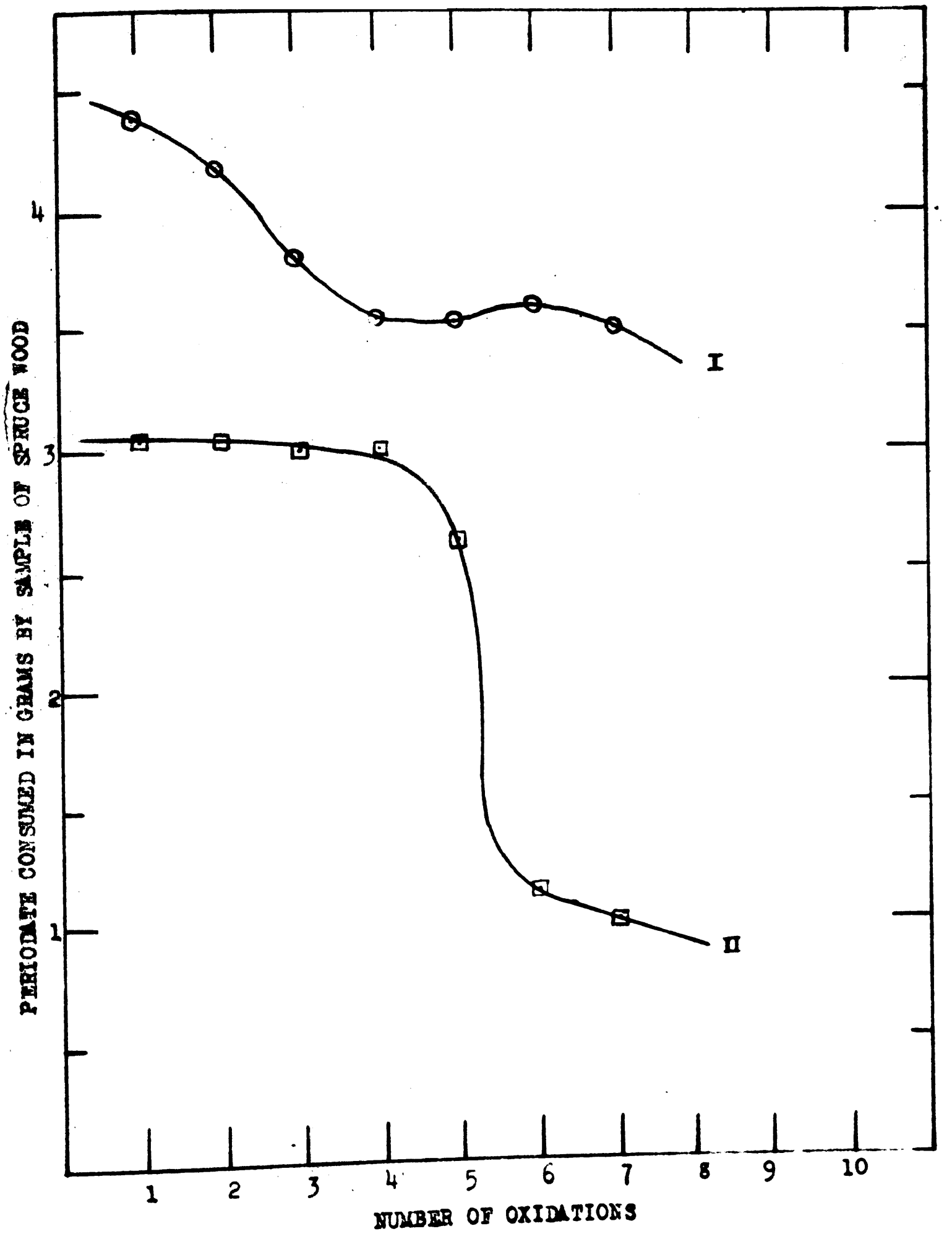


Fig.VII.- Progressive oxidation at pH 4.1 and 20°C of 9.06 gm. of extracted spruce wood with 1.3% $H_3H_2IO_6$. Plot I. periodate added; Plot II. periodate consumed.

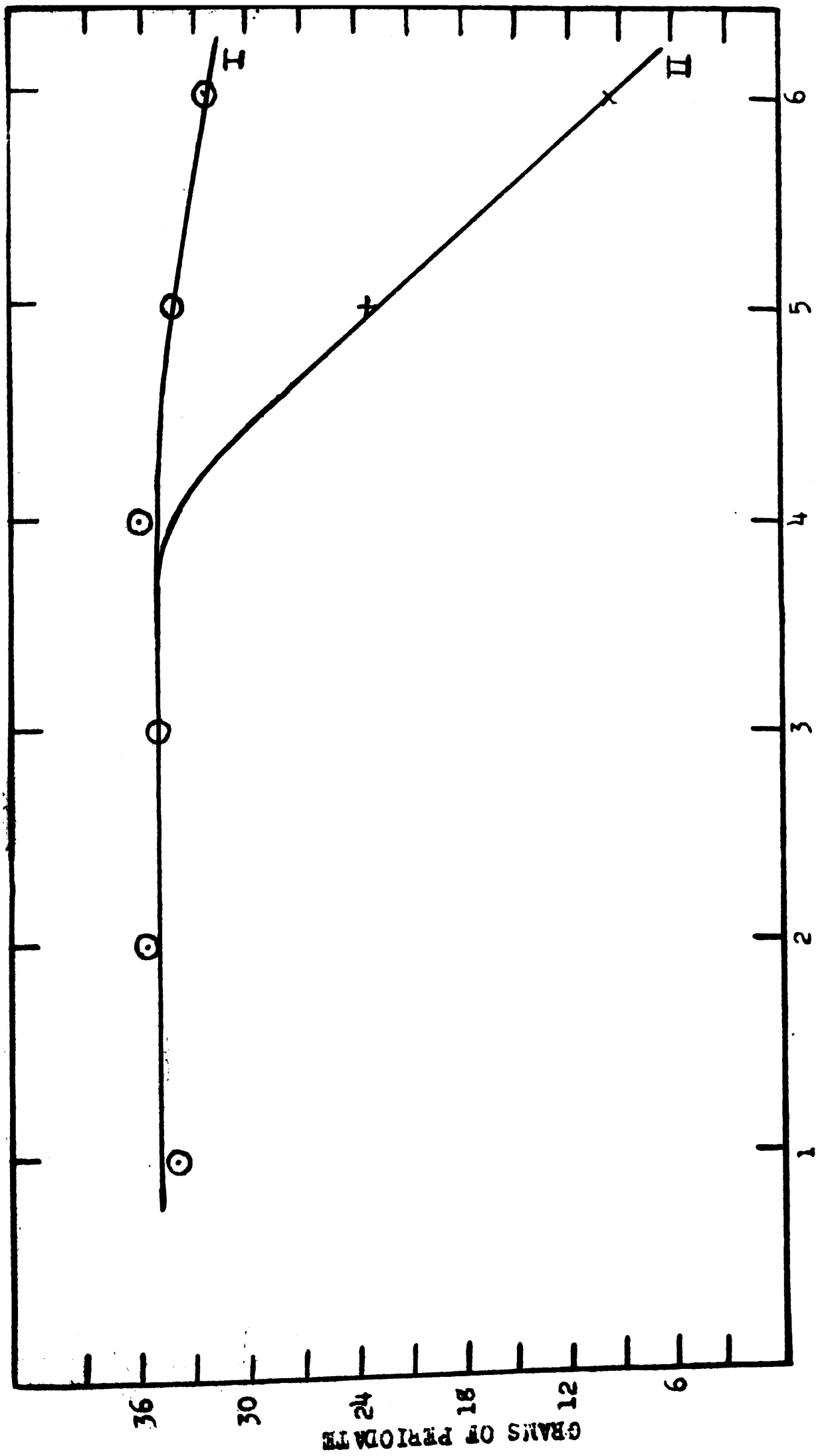


Fig. VIII. - Dry spruce wood 101.7 g., oxidized for sixty hours six times with 2 liters of 1.75% $H_3H_2IO_6$ (35 g.) at pH 4.1 and 20°C. Plot I. g. periodate added; plot II, g. periodate consumed.

instead of about one-half, the weight of the wood meal. With this change, all the added periodate was consumed in the first three oxidations and the time of sixty hours allowed for each oxidation was probably unnecessarily long (Table XXVII). In two subsequent preparations (Spruce Periodate Lignins B and C), the period of oxidation was accordingly reduced to twenty-four hours. The consumption of periodate by the samples is plotted against the number of oxidations in Figs.IX and X (Tables XXVIII and XXIX). Table XIV summarizes the conditions of all three oxidations and the yields of products obtained. Analytical data for the isolated lignins are given in Table XV, all analyses being carried out as described in the Experimental Portion.

The chlorinations in the holocellulose estimation (periodate lignin A) lasted three minutes each and caused the periodate lignin immediately to become orange-yellow in color. After the first extraction with 50% alcohol-pyridine (for 3.5 hours) the residue was a sticky, dark-brown mass that had to be disintegrated with a glass rod prior to re-chlorination. These color changes persisted to the seventh chlorination, the product from which was completely soluble in alcohol-pyridine. The pentosan estimation of periodate lignin A proceeded normally except that the distillate failed to give the usual pink color with aniline hydrochloride test paper and presumably contained no furfural. When phloroglucinol was added, the

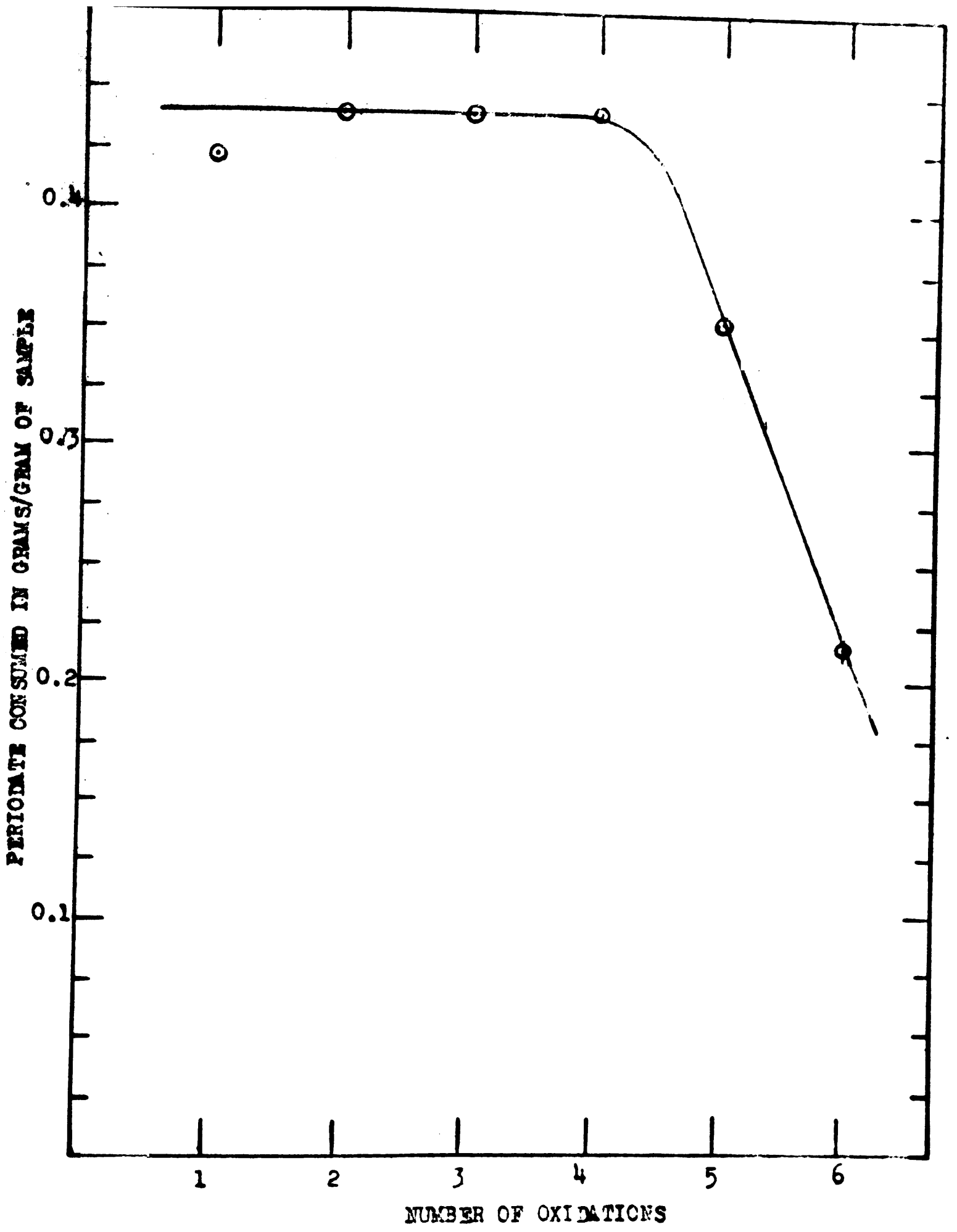


Fig.IX.-Progressive oxidation of 226 gm. of extracted spruce wood at pH 4.1 and 20°C with 5% $Na_3H_2IO_6$

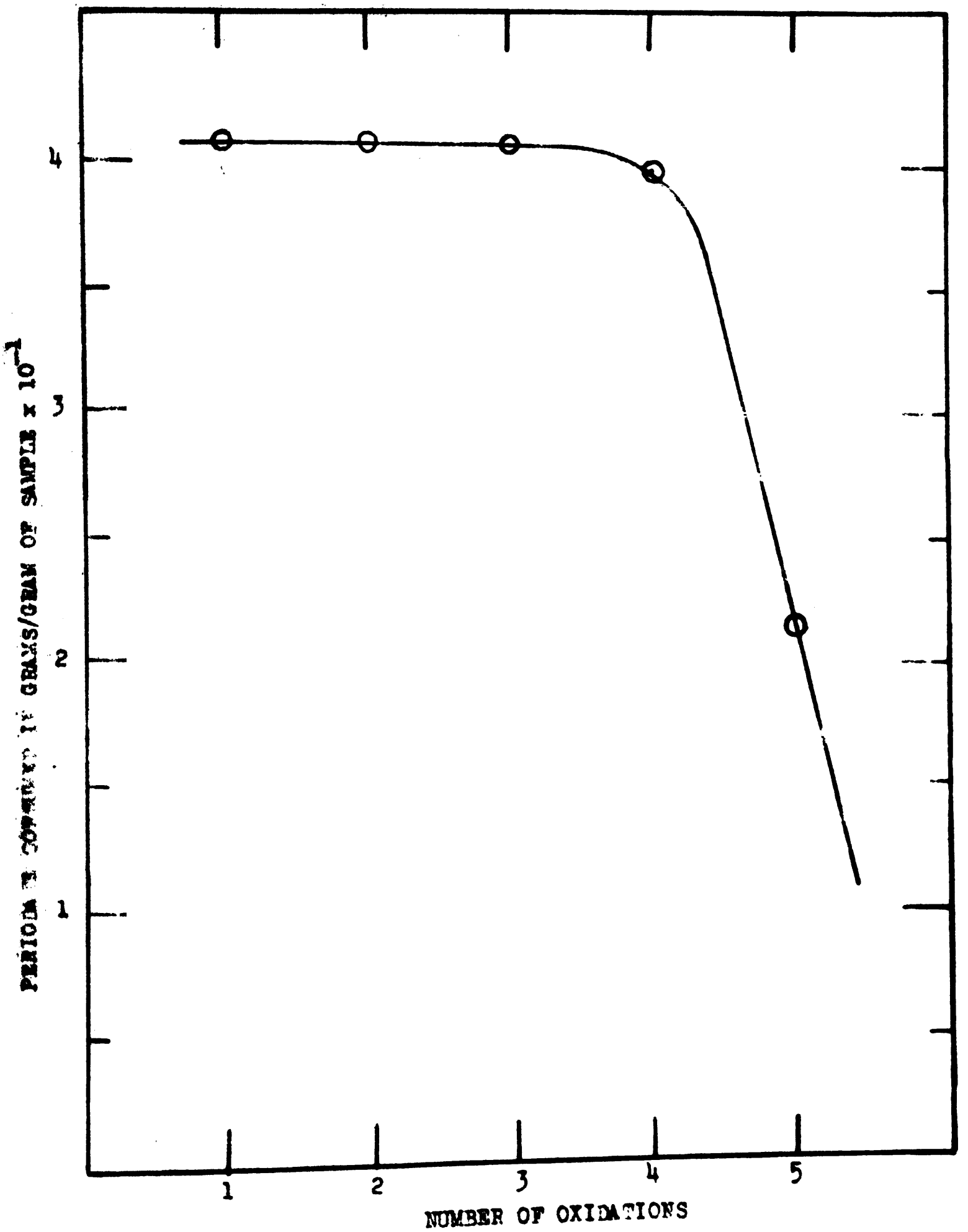


Fig.X. - Progressive oxidation at pH 4.1 and 20°C of 497.6 gm. of spruce wood with 3.4% periodate solutions.

TABLE XIV

Large-scale Preparations of Spruce Periodate Lignins[a]

Preparation	Weight of Spruce Wood (gm.)	Number of Oxidations	Duration of Oxidation (hr.)	Periodate Lignin		
				Yield (gm.)	Klason Lignin %	Yield[e] %
Spruce Periodate Lignin (A)	101.9	6	60[b]	29.6	86.0	86.7
Spruce Periodate Lignin (B)	225.2	6	24[c]	67.4	93.7	97.4
Spruce Periodate Lignin (C)	497.6	5	24[d]	139.5	95.8	93.2

(a) All calculations on the basis of moisture- and ash- free samples.

(b) With 2 litres of 1.7% periodate solution.

(c) With 2 litres of 5.0% periodate solution.

(d) With 6 litres of 3.4% periodate solution.

(e) Calculated from Klason lignin contents of periodate lignins and original wood.

TABLE XV

Analyses[(a)] of Spruce Periodate Lignins

Spruce Periodate Lignin	Moisture %	Ash %	Klason Lignin %	Holocellulose %	Carbon %	Hydrogen %	Methoxyl %
(A)[(b)]	2.02	1.56	86.0	None	60.5	6.0	10.8
(B)	3.13	1.78	93.7	-	61.2	5.9	12.2
(C)	2.36	1.43	95.8	-	61.7	6.0	12.0

(a) Corrected to the basis of moisture- and ash-free samples.

(b) Table XIV.

color of the "furfural" phloroglucide precipitated was brown instead of the usual black. This precipitate was probably the phloroglucide of formaldehyde, since traces of the latter were isolated from similar distillates as the crystalline dimedon derivative, m.p. 185 to 186°C, by the method described in the Experimental Portion. In addition an unidentified material was isolated as the bright red, crystalline, chloroform-soluble 2,4-dinitrophenylhydrazone, m.p. 175 to 184°C. It was concluded that periodate lignin A was free, not only of holocellulose, but even of any degraded pentosans that might have become solubilized and escaped detection in the holocellulose determination.

In a parallel series of oxidations, periodate lignins were prepared from approximately 35 gm. samples of extractive-free spruce, maple, birch and beech woods using 600 ml. of 5% aqueous periodate solution at pH 4.1 and 20°C in each stage of the oxidations. The yields and analytical data for these periodate lignins are summarized in Table XVI. The plots of periodate consumed (Table XXX) against number of oxidations given in Fig.XI clearly show that, from the fourth oxidation onward, the wood residue becomes increasingly resistant to oxidation by periodate. This resistance is characteristic of all species of wood examined, since all the curves have the same general shape. It is difficult to explain the differences in position of the curves of Fig.XI in the light of present knowledge of

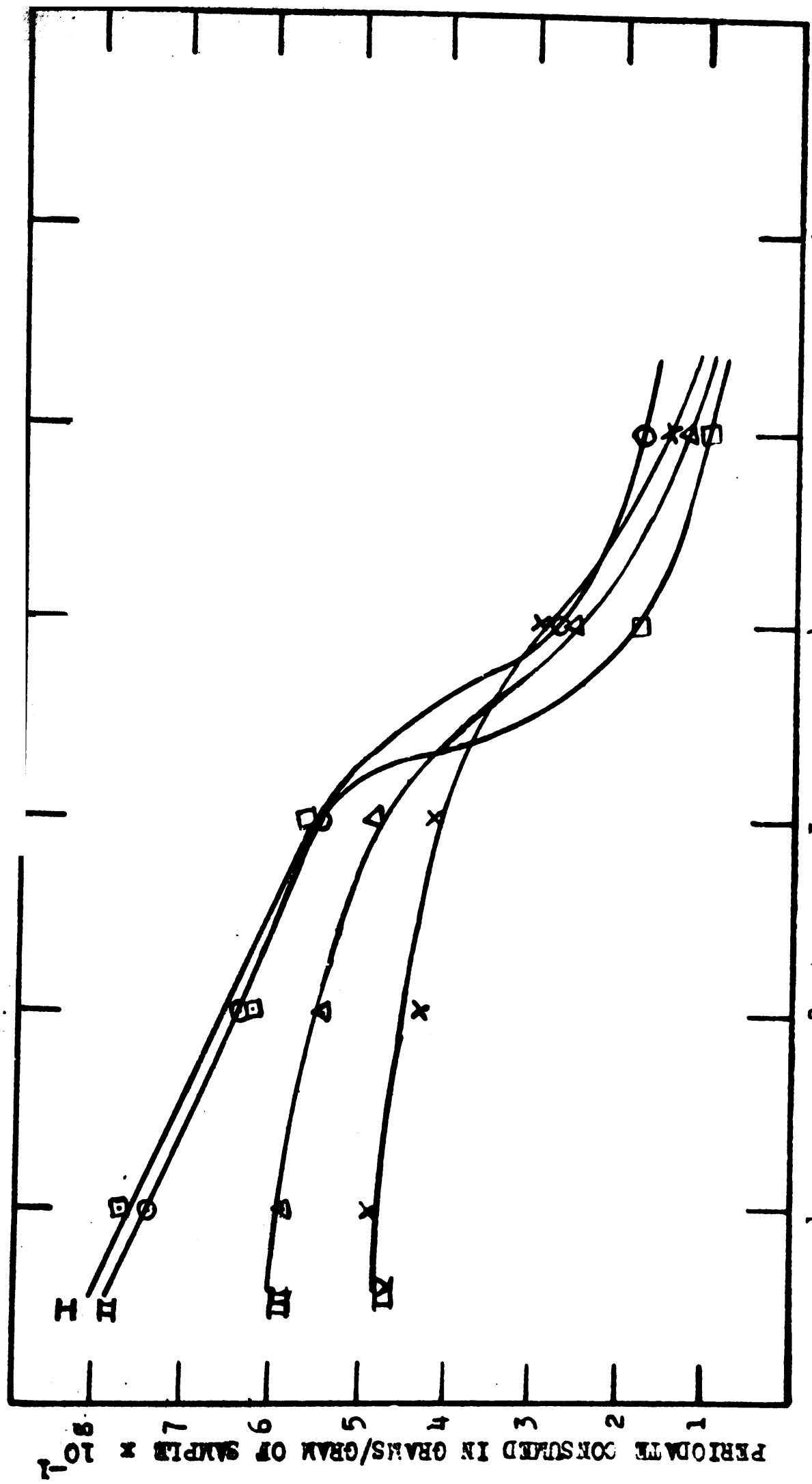


Fig.XI. - Progressive oxidation of extractive-free woods at pH 4.1 and 20°C with 5% NaH_3IO_6. Plot I, Birch; Plot II, Spruce; Plot III, Maple; Plot IV, Beech.

TABLE XVI

(a)
Yields and Analytical Data for Isolated Periodate Lignins

Original Woods			Periodate Lignins						
Species	Klason Lignin %	Ash %	Yield		Klason Lignin %	Ash %	Methoxyl %	Carbon %	Hydrogen %
			Wood Basis %	Klason Lignin Basis (b) %					
Spruce	28.8	0.35	29.8	97.2	93.7(c)	1.97	12.2	61.4	6.0
Maple	21.6	0.15	22.3	77.3	74.9	1.14	20.4	58.4	5.4
Birch	20.0	0.12	21.8	85.4	78.5	1.08	21.4	57.6	5.3
Beech	23.3	0.26	24.4	75.2	72.2	2.17	16.7	54.6	6.1

(a) Average of duplicate values corrected for ash and moisture content of the sample.

(b) Yields calculated on the basis of the Klason lignin contents of the original woods and the periodate lignins.

(c) Periodate lignin B.

the chemistry of wood. Undoubtedly such factors as the ratio of lignin to holocellulose, penetrability and the structure of woods are influential in determining the reaction rate. Table XIV (Column 5) and Table XVI (Column 4) show that, in the case of spruce wood, the yield of periodate lignin was about 1% higher than that of Klason lignin from the same wood. When the periodate lignin was itself submitted to the Klason procedure, the sample from spruce analyzed from 86 to 96% Klason lignin and accounted practically quantitatively for all of the latter present in the original wood. The case was sharply different for the three hardwood lignins, which contained only 70 to 80% of Klason lignin and accounted for only 75 to 85% of the Klason lignin of the wood. In these cases, wood constituents accounting normally for 15 to 25% of the Klason lignin were lost during the periodate oxidations. The ready solution of roughly the same amount of hardwood, as opposed to softwood lignins, has been noted by other workers (182).

In the following experiment an attempt was made to determine if a wood residue stable to periodate could be obtained. A sample of spruce periodate lignin (A) weighing 4.1 gm. was reoxidized with 400 ml. of a solution containing approximately 17 gm. of sodium paraperiodate buffered to pH 4.1. After sixty hours the residue was hydrolyzed in one litre of boiling water for three hours. Five oxidations were carried out in all, the consumption of

periodate being plotted against number of oxidations in Fig.XIII, plot I. Although the material had not ceased to reduce the oxidant, the consumption dropped to a very small amount. The Klason lignin analysis of this material had risen to 96.3% but the elementary analysis was not greatly changed at 62.1% carbon and 5.8% hydrogen. Fig.XII, plot III, records the results of a preliminary experiment of the same nature. In this case, 1.9 gm. of the same spruce periodate lignin was repeatedly oxidized with 3.5 gm. of paraperiodate. The product, yield 85.1% by weight, contained 92.2% of Klason lignin, whereas 101% was the theoretical figure calculated from the yield. In both series of oxidations some of the materials analyzing as Klason lignin had been lost as water-solubles.

In order to determine the behavior of spruce Klason lignin toward periodate more fully, a progressive oxidation was carried out on a sample of this lignin weighing 2.0gm. Approximately 4 gm. of the periodate was dissolved in 200 ml. of water and buffered to pH 4.1 with acetic acid. The oxidation was allowed to continue for 72 hours at 20°C with stirring. The residue was then recovered, washed, and hydrolyzed for three hours in 500 ml. of boiling water. The hydrolysis liquors were definitely colored, indicating that partial solution had occurred. Five oxidations and hydrolyses were carried out. The residue weighed 1.5 gm. after drying for eight hours at 105°C. This weight represents a loss of 28.0%. Consumption of periodate by the

sample is plotted against the number of oxidations in Fig.XII, plot II. Data corresponding to the plots in Fig.XII are summarized in Table XXXI. Although it is true that spruce Klason or periodate lignins could be made to suffer a loss of magnitude similar to that found during the preparation of hardwood lignins, the conditions of the periodate oxidations had to be made much more drastic.

Throughout this work the standard analysis by the 72% sulfuric acid method has been used in following the lignin content of the oxidized wood. The accuracy of this method is questionable as it has been shown (66) that fructose, xylose and other carbohydrates form insoluble residues under the conditions of the Klason lignin analysis. In addition, there is the possibility that aldehydes resulting from the periodate cleavage of carbohydrates may condense with themselves or with phenols of the lignin complex in the presence of sulfuric acid. Such a condensation would result in high results for lignin. Accordingly, attempts were made to determine the result upon the Klason lignin analysis of adding holocellulose and oxidized holocellulose to periodate lignin samples prior to analysis. About 0.5 gm. of the spruce periodate lignin A (analyzing 86.0% Klason lignin) was mixed with about 1.5 gm. of lignin-free holocellulose from the same wood specimen to give a ratio of lignin to holocellulose approximately the same as that in the

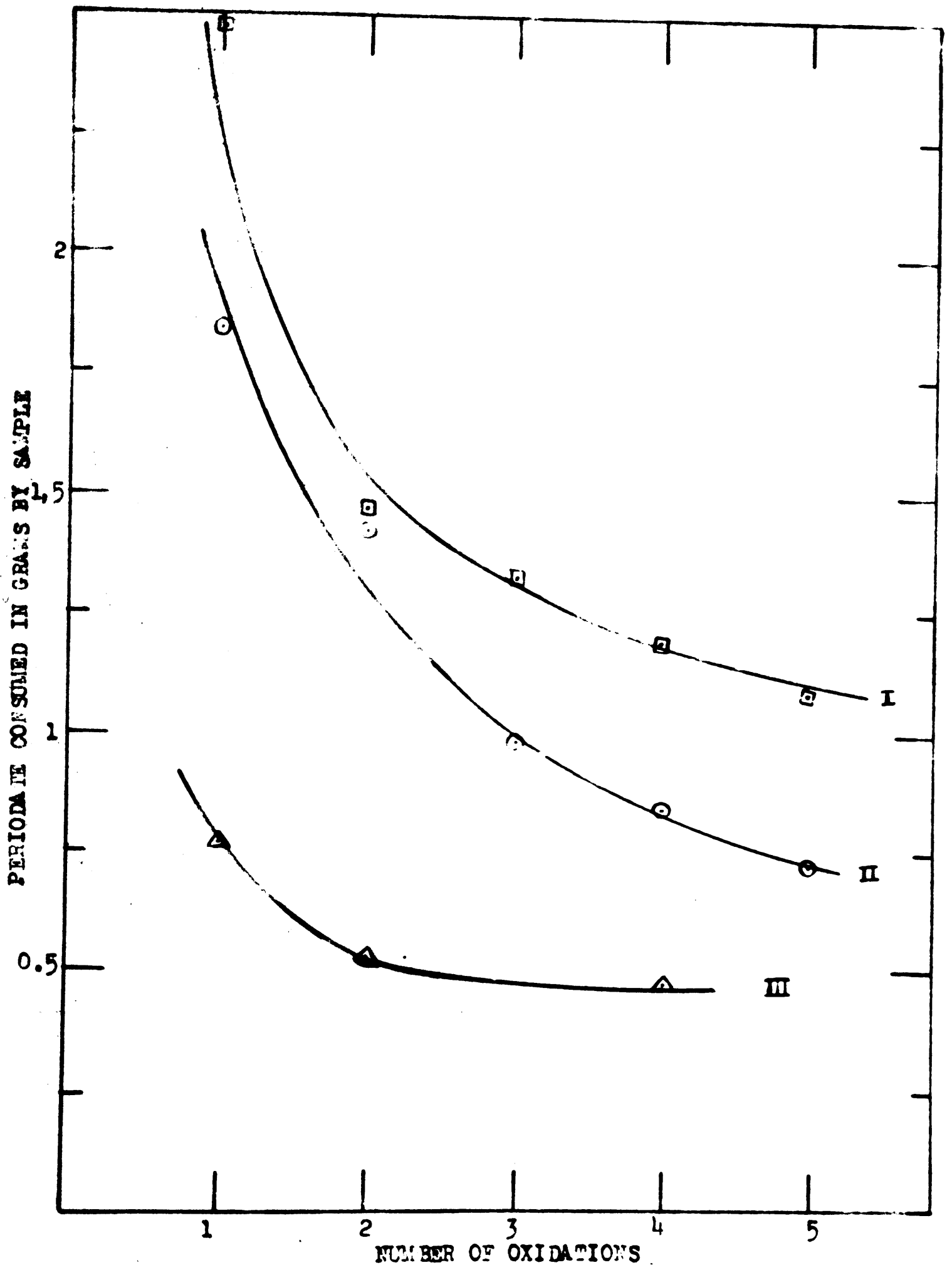


Fig.XII.- Progressive oxidation at pH 4.1 and 20°C. Plot I, 4.07 gm. periodate lignin with 0.25% $Na_3H_2IO_6$; Plot II, 2.05 gm. Klason lignin with 2.2% $Na_3H_2IO_6$; Plot III, 1.90 gm. periodate lignin with 2% $Na_3H_2IO_6$

original wood. Analysis of this mixture in the standard manner with 72% sulfuric acid gave the original value of 86.0%, calculated on the basis of the periodate lignin used. The Klason lignin estimation therefore appears to be indifferent to the carbohydrates present in the holocellulose. Periodate-oxidized holocellulose was then mixed with periodate lignin in approximately the ratio of lignin and holocellulose in wood. The mixture was boiled for three hours with distilled water, filtered and the residue dried. The original Klason lignin content of the periodate lignin had been 86.0% but the values obtained for the mixtures ranged from 90 to 95% calculated on the basis of the periodate lignin originally present in the mixture. These results support the view that the Klason lignin analysis may give only an approximate estimation of the lignin content of periodate oxidized wood.

The periodate lignins prepared in this research were quite free of iodine, were bright brown in color and retained much detailed morphological structure. The lignin was entirely insoluble in cold solutions of bicarbonate or alkali while methanol, ethanol, ether, benzene, chloroform and dioxane completely failed to dissolve any of the lignin even when suspensions in these liquids were boiled for several hours. Since it is felt that the method of isolation used could not have resulted in resinification, the insolubility of periodate lignin suggests that native lignin is also insoluble in all ordinary solvents and is a

substance of high molecular weight. The assumption that lignin "in situ" is a low molecular phenolic body, rendered insoluble by combination with carbohydrates, suggests that the carbohydrate-free periodate product would have exhibited some solubility, which was not the case.

Although the yield of spruce lignin was close to quantitative, the carbon analyses (Table XIV and XVI) were definitely lower than those calculated for lignin "in situ" and are evidence of oxidation. This oxidation had also removed some methoxyl groups but even drastic treatments with periodate failed to depress the methoxyl content by more than one-third, or below 10%. Both the yields of 75 to 85% and the carbon contents below 60% (Table XVI) observed for the hardwood periodate lignins showed the effects of oxidation, but nevertheless the methoxyl contents remained in the range calculated for hardwood lignins.

C. SULFITE PULPING OF PERIODATE LIGNINS

Semi-cooked periodate lignins were invariably recovered as highly swollen materials which readily collapsed on washing with 95% ethanol. Even after these residues had been carefully dried "in vacuo" at 55°C they returned to their swollen state on standing in cold water for a few hours. This observation is of interest in connection with the theory that during the later stages of a sulfite cook solid lignosulfonic acids from the wood are peptized into solution (183).

The data for the rate of pulping of spruce periodate lignin (Fig.XIII) are uncorrected for the time required for the bomb to assume the temperature of the bath. This omission should not change the shape or slope of the curve but merely alter its position relative to the horizontal time axis. For purposes of comparison a curve from data obtained on spruce wood meal by Calhoun under conditions comparable to those employed in this research is included in Fig. XIII. The reaction rates corresponding to the slopes of the semi-log plots are in quite close agreement, although if the rates are determined as the reciprocal of the time to 90% delignification, a significantly lower rate pertains to the pulping of periodate lignin. Calhoun's data were corrected for temperature-lag and the residual lignin values were incidentally corrected for ash, since they were obtained after an acid treatment which undoubtedly removed combined calcium. The periodate lignin residues were too small for reliable ash determinations. Similar corrections, however, would cause the plot more closely to approach that for the pulping of spruce wood (Fig.XIII). Although investigators of the rate of removal of lignin from wood during sulfite pulping found that the process is a continuous function of temperature (185) the plot of residual spruce periodate lignin against temperature (Fig.XIV) shows a discontinuity between 118 and 127°C. Further evidence of the occurrence of at least two

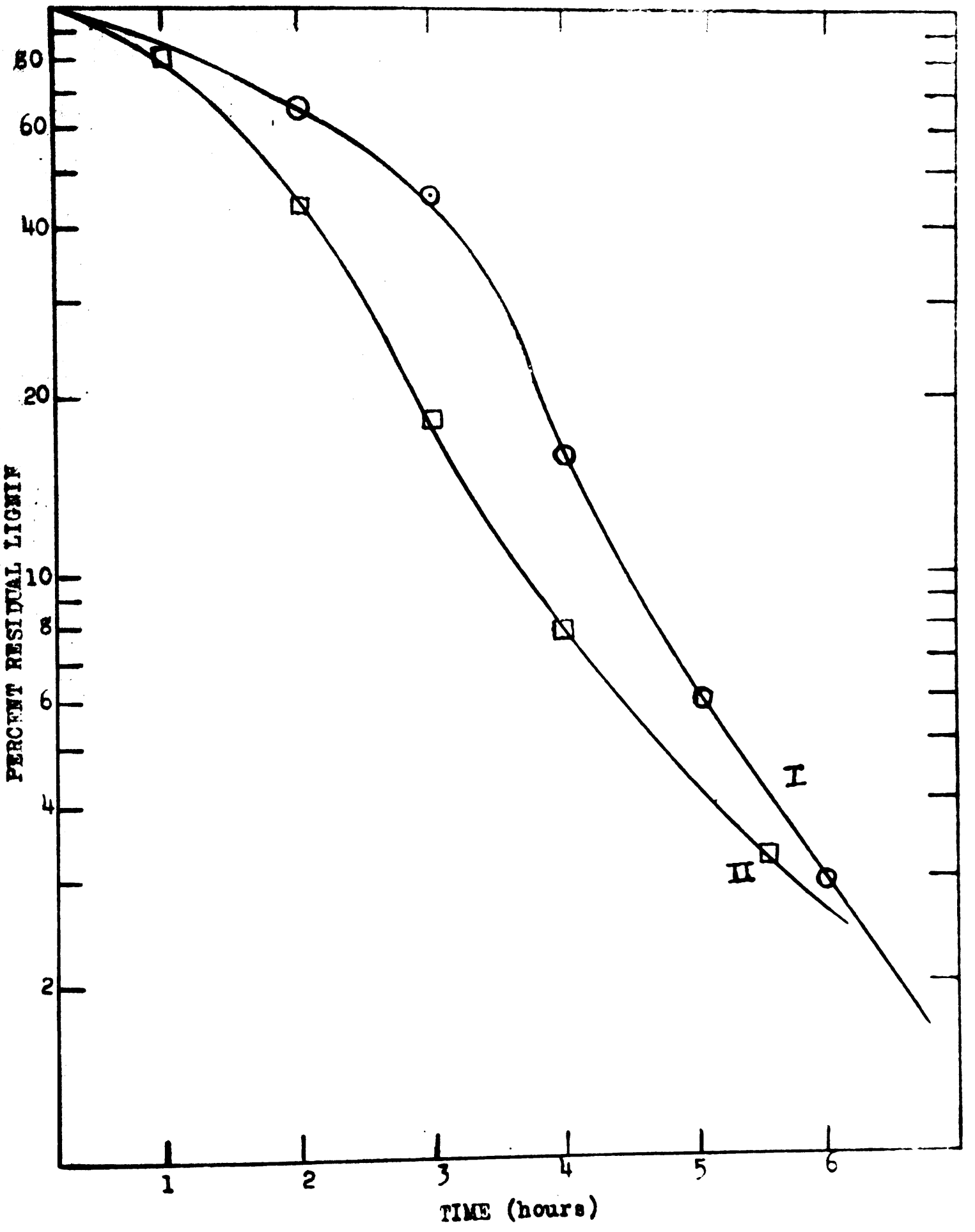


Fig.XIII. - Pulping of spruce lignin. Plot I, Spruce periodate lignin; Plot II, from data obtained by Calhoun by pulping spruce wood.

reactions was obtained from the character of the undissolved lignin residues. In the case of cooks of short duration or at lower temperatures, the dried residues were of much the same appearance as the original lignin. When the cooking reaction was prolonged or carried out at higher temperatures, the residual lignin was recovered as a gummy mass which solidified into a dark resin-like material on drying. The sulfur content of the lignin residues (Fig.XIV) are in agreement with Yorston's (84) observation that uniform sulfonation to the extent of about 5.5% of sulfur seems to be necessary for sulfite pulping.

The results of the sulfite cooks of maple, birch and beech periodate lignins (Table XVII) indicate that there is probably very little difference in the pulping qualities of lignins derived from various species of wood. Differences in the morphological structures probably account for the slight differences that have been noted. Table XVII also summarizes various pretreatments which impaired the capacity of spruce periodate lignin to dissolve in a standard sulfite cook. Pretreatment with acid solutions, or even with hot water, markedly retarded solution of the lignin in sulfite liquor under conditions in which untreated lignin was completely soluble. In order to explain the effect of such pretreatments, it has been assumed (90) that a condensation or polymerization occurred which retards the sulfonation and subsequent solution of the lignin. The exact nature of

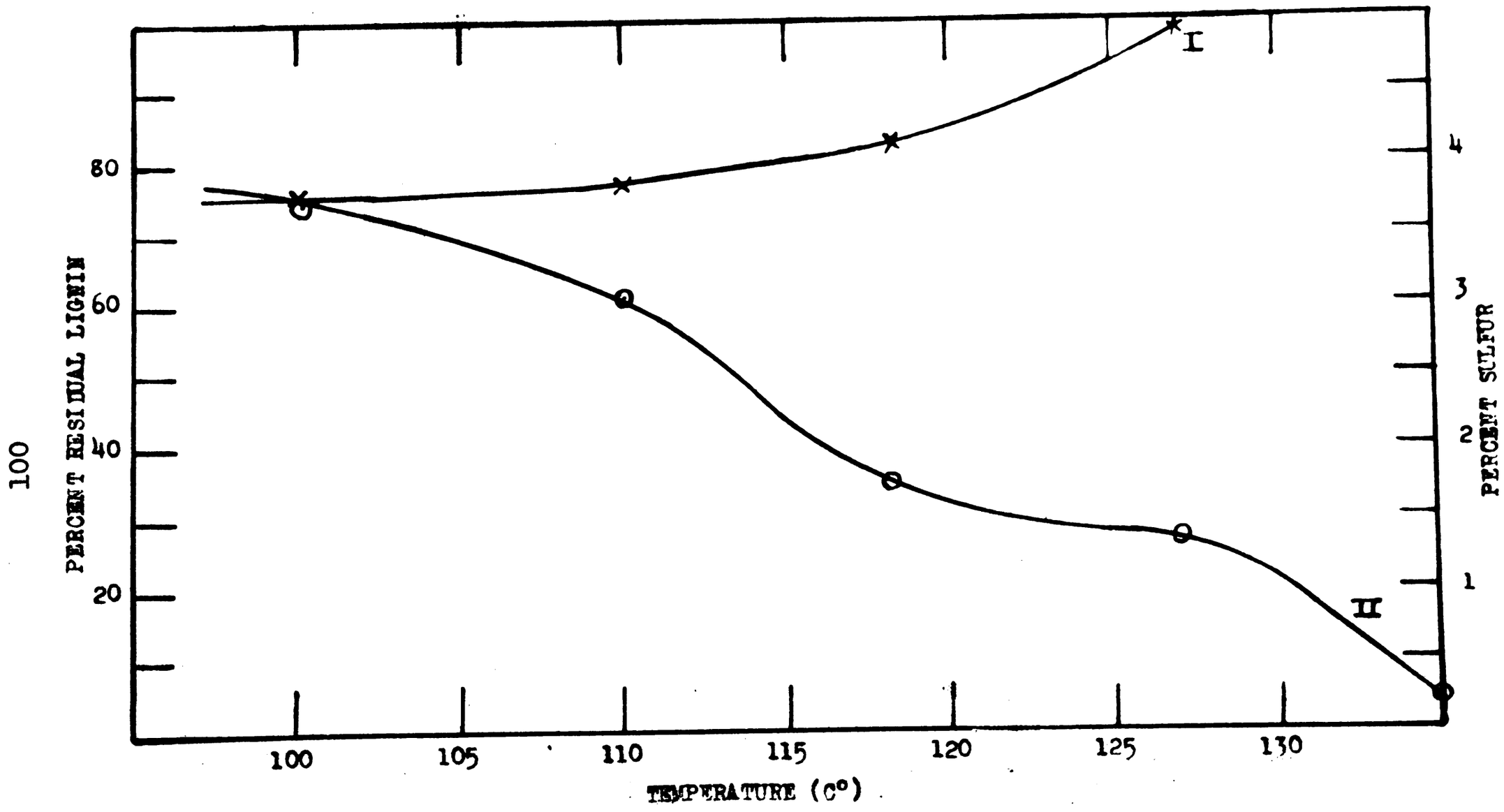


Fig.XIV.-Sulfite pulping of spruce periodate lignin. Plot I, percent sulfur in residual lignin; Plot II, residual lignin as percent of original sample.

TABLE XVII

Pulping[a] of Periodate and Pretreated Periodate Lignins in Sulfite Liquor

Periodate Lignin	Pretreatment	Percent Residue
Spruce	None	2.9
Maple	None	11.4
Birch	None	7.0
Beech	None	8.9
Spruce	Cooked with water for 6 hours at 105°C	79.8
Spruce	Subjected to Klason lignin procedure	98.9
Spruce	Refluxed with N HCl for 4 hours	86.4
Spruce	Subjected to ethanolysis procedure	93.4

(a) All cooks at 135°C for 6 hours in liquor containing 2 percent combined and 6 percent total sulfur dioxide.

the transformation is, however, unknown and a proper explanation must await the elucidation of the structure of lignin itself. The new information contributed by the above experiments is that the pretreatment effect is a function of the lignin alone and is not dependent on the presence of the wood carbohydrates.

The sulfite liquors from periodate lignins, on addition of sulfuric acid, sodium chloride, calcium chloride or β-naphthylamine hydrochloride, precipitated solid lignosulfonic acids in the customary fashion. Another sample of spruce periodate lignin was completely dissolved when cooked with 20% sodium hydroxide solution at 160°C for 8 hours. The very close similarity between periodate lignin and lignin "in situ" in their behavior toward both sulfite and alkali pulping reagents suggests that lignin is not greatly modified during isolation by the periodate method.

D. HYDROGENATION OF SPRUCE PERIODATE LIGNIN

The hydrogenation of lignin has been accomplished under a wide variety of conditions. In most cases high temperatures have been found necessary in order to obtain workable products. Some lignins have required the presence of very active catalysts, and alkali or acid, before hydrogenation occurs. In a series of experiments designed to determine the minimum temperature at which hydrogenation of spruce periodate lignin occurred, approximately 5 gm. of the lignin was suspended in 125 ml. of carefully purified,

anhydrous dioxane and placed in a stainless steel bomb having a volume of 500 ml. In the first experiment, 2.5 gm. of Raney nickel catalyst was added to the suspension and, with an initial hydrogen pressure of 3000 p.s.i., the temperature was raised to 175°C. After six hours, during which no absorption of hydrogen was noted, only a small portion of the lignin (estimated to be less than 10%) had gone into solution. A fresh portion of catalyst was added and the temperature brought to 200°C. After six hours no hydrogen was absorbed and the lignin remained undissolved. Since dioxane is unstable above 210°C in the presence of Raney nickel, the experiment was terminated.

In a similar experiment with copper-chromite catalyst, about 2.5 gm. was added to the lignin suspension and hydrogen admitted to an initial pressure of 1800 p.s.i. The temperature was successively brought to 175, 200, 225 and 260°C for six hour periods but only at the latter temperature did hydrogen absorption become apparent. The product, on distillation from a Claisen flask, gave a water-white distillate and a dark, viscous still residue which in this experiment was very small in amount. When these products were dissolved in methanol, the failure of ferric chloride to color the solution pointed to the entire absence of phenols.

The next experiment was carried out to determine the yield of products boiling above the solvent dioxane. A sample of periodate lignin weighing 5.3 gm. was suspended

in 125 ml. of dioxane and hydrogenated in the presence of 2.5 gm. of copper-chromite catalyst at an initial pressure of 2000 p.s.i. and 260°C for twelve hours. The products were distilled from a Claisen flask under reduced pressure. The distillable portion, b.p. up to 200°C/8 microns weighed 0.96 gm. and thus represented 18.2% of the original lignin. A dark, non-distillable, resinous residue weighed 0.82 gm. or 15.6% of the weight of the lignin hydrogenated. About two-thirds of the lignin was not recovered.

A similar hydrogenation of 39.5 gm. of periodate lignin is described in the Experimental Portion and the results are summarized in Table XVIII. The yield of 3.97 gm. of methanol recovered represents about 80% of the original methoxyl content but the total yield of identified products, based on carbon contents, accounts for only 18.9% of the carbon in the lignin. In addition, unidentified fractions amount to 31.1% of the periodate lignin. It is thus seen that about half of the carbon in the lignin was not recovered and apparently was lost as gaseous products during the hydrogenation. Standard procedures were used in identifying the 4-n-propylcyclohexanol-1 and 4-n-propylcyclohexanediol-1,2 as described in the Experimental Portion. The difficulty experienced in obtaining a good yield of the crystalline urethane derivatives was attributed to the presence of isomers, presumably of a cis-trans nature, in each crude fraction. Table XVIII also contains data obtained by Harris (186) for the hydrogenation of spruce

TABLE XVIII

Hydrogenation of Spruce Periodate and Methanol Lignin

Product	Black Spruce Periodate Lignin		White Spruce Methanol Lignin
	Percent by Weight	Percent of Carbon (a)	Percent by Weight (b)
Water	13.6		10.3
Methanol	10.1	6.1	15.0
4-n-propylcyclohexanol-1	8.0	10.3	21.0
4-n-propylcyclohexanediol-1,2	2.3	2.5	
High-boiling Oil	8.6	10.6	52.0
Non-distillable Resin	10.6	14.5	
Total	53.2	44.0	98.3

(a) Values calculated from carbon contents of original periodate lignin and products.

(b) Data obtained by E. E. Harris from hydrogenation of white spruce methanol lignin in dioxane solution at 250°C with copper chromite catalyst (186).

methanol lignin under conditions comparable to those used in this research. The discrepancy in the total yields of products for the two lignins, although large, may not be of great significance because methanol lignin accounts for only about one-quarter of the total lignin in wood (187). Hibbert and co-workers (133) hydrogenated spruce wood under somewhat similar conditions and recovered high-boiling products, derived from the Klason lignin content of the wood, in the following yields; 22.3% of an alcohol (n_D^{25} = 1.4600 to 1.4700) assumed to be mostly 4-n-propylcyclohexanol-1; 20.7% of a material (n_D^{25} = 1.4700 to 1.4800) probably chiefly 4-n-propylcyclohexanediol-1,2; and 43.2% of an unidentified resin, n_D^{60} = 1.4800. It was specifically pointed out that the yield and nature of the products were dependent upon the pretreatment to which the wood had been subjected and even upon the amount of water in the dioxane medium. The exact correspondence observed in the chemical structures of the products isolated from spruce periodate lignin and spruce wood, particularly the isolation of the same derivatives of propylcyclohexane, is strong evidence that no extensive structural change occurred during the preparation of the periodate lignin.

E. ETHANOLYSIS OF SPRUCE PERIODATE LIGNIN

Preliminary experiments showed that spruce periodate lignin was completely insoluble in neutral solvents, but that in boiling methanol, ethanol, butanol, amyl alchohol

and dioxane containing about 2% of hydrogen chloride the lignin was partially soluble. The dissolved portion imparted a deep wine color to the solvent while the undissolved residue, when dried, was a dark brown, friable material. Repeated treatment of this residue with fresh portions of the acidified solvent resulted in a final residue that was quite insoluble in the reagent. Judging only from visual observations, the solubility increased with the higher alcohols, probably because of their higher boiling-point. Anhydrous dioxane containing 2% of hydrogen chloride appeared to be the best solvent studied. However, a dark residue remained undissolved as in the other cases.

This residue amounted to 48.2% when spruce periodate lignin C was exhaustively extracted, as described in the Experimental Portion, with boiling anhydrous ethanol containing 2% of hydrogen chloride. Table XIX is a summary of the yields of the various ethanolysis fractions observed, together with the yields reported by Hibbert and co-workers (143) for a similar study of spruce wood. The ethanolysis of spruce wood extracted some 35 to 40% of the lignin as a yellow to brown, water-insoluble substance termed ethanol lignin and as water-soluble oils. The higher yield of ethanol lignin (21.7%) from periodate lignin was probably connected with the fact that in this case the ethanolysis was exhaustive, whereas the spruce wood was subjected to a single extraction. The fact that

TABLE XIX

Summary of Data from Ethanolysis of Spruce Lignin

Product	Percent Yield	
	Spruce Periodate Lignin(a)	Spruce Wood(b)
Water-insoluble		
Undissolved Lignin(c)	48.2	64.0
Ethanol Lignin(d)	21.7	10.0
Water-soluble		
Ether-soluble Oils	11.5	18.0
Ether-insoluble Tar	7.3	
Total	88.7	92.0

(a) Yields based on weight of original spruce periodate lignin corrected for moisture and ash content.

(b) Data obtained by H. Hibbert and co-worker from the ethanolysis of extractive-free spruce wood.

(c) Analysis; C, 65.0; H, 5.4; CH_3O, 20.8%

(d) Analysis; C, 63.8; H, 6.4; CH_3O, 20.8%

the insoluble residue from the periodate lignin was resistant to pulping in sulfite liquor under standard conditions (Table XVII) was in agreement with previous observations to the effect that wood residues obtained from ethanolysis experiments are difficultly delignified with sulfite liquor (188). It will be remembered that according to Hibbert (189) the building-units isolated from the ethanolysis products of wood lignins are extremely sensitive materials. These units readily condensed with each other and the re-ethanolysis of ethanol lignin also resulted in the formation of insoluble residues. Such observations, combined with the insolubility of the unextracted lignin in sulfite liquor, led Hibbert to postulate that during ethanolysis both depolymerization and polymerization reactions of lignin building-units occurred concurrently. The present research clearly demonstrates that the formation of this sulfite-insoluble residue is not in any way dependent upon the presence of the holocellulose of wood. Ethanol lignin obtained from periodate lignin was similar in its analysis (Table XIX) to that prepared from spruce wood in the usual manner (190). The yields of water-soluble oils and the total yields of products obtained in each case compare very favorably. The close similarity in behavior toward ethanolic hydrogen chloride is further indication that lignin is not radically modified during isolation with periodate solutions.

F. ALKALINE OXIDATION OF SPRUCE PERIODATE LIGNIN

Preliminary work showed that spruce periodate lignin was unusually sensitive to oxidation by atmospheric oxygen. When small lignin samples were heated with tetralin under reflux the lignin was slowly dissolved, giving highly colored red solutions. However, when the extraction was carried out in an atmosphere of nitrogen, only a very small amount of lignin was dissolved even in several days time, and it thus became clear that the lignin was degraded to a soluble condition in the presence of hot tetralin and air. Complete solution occurred when small samples of periodate lignin were heated in glass-stoppered flasks on the steam bath for several days with 2 to 12% sodium hydroxide solution. The failure of the lignin to dissolve in warm aqueous alkali maintained in an atmosphere of oxygen-free nitrogen again proved that oxidation was the cause of solution. The rate of solution was greatly accelerated by passing a slow stream of air through the warm alkaline suspension.

A sample of spruce periodate lignin weighing approximately 2 gm. was treated with 500 ml. of 2% sodium hydroxide solution in a glass-stoppered flask on the steam bath. The mixture was thoroughly shaken occasionally and after six days the lignin was completely dissolved. Carbonation of the golden-yellow solution gave a voluminous white precipitate which was found to be largely silica dissolved from the glassware. On neutralization with dilute hydrochloric acid a

small amount of a dark brown, amorphous material precipitated and was removed by filtration. The acidified solution, which had a distinct odor of vanillin, yielded an ether extract which was back-extracted with 20% sodium bisulfite solution to remove all carbonyl compounds. After acidification of the bisulfite extract with dilute sulfuric acid, the liberated sulfur dioxide was removed under vacuum. Addition of a filtered solution of 2,4-dinitrophenylhydrazine to the solution gave a red precipitate which was recovered by filtration and dried in the vacuum oven at 55°C. The melting-point of the crude product was 245 to 248°C. Recrystallization from ethanol gave very fine crystals melting at 266°C, not depressed by admixture with an authentic sample of the 2,4-dinitrophenylhydrazone of vanillin.

Table XX summarizes the yields of products obtained from the nitrobenzene oxidation of spruce periodate lignin C outlined in the Experimental Portion. The dry, water-insoluble material obtained by acidification of the alkaline reaction mixture was very readily soluble in ether or alcohol but, strangely enough, was almost completely insoluble in alkali. This insolubility in alkali suggests that the material was formed on acidification and did not exist as such in the original alkaline solution. The yield of vanillin, as estimated from the weight of the crude 2,4-dinitrophenylhydrozone, was very close to that obtained by Hibbert and co-workers from spruce wood

TABLE XX

PRODUCTS FROM NITROBENZENE OXIDATION OF LIGNIN[a]

Fraction	Yield (8m.)	Yield (%)[b]
Water-insoluble precipitate	6.5	32.6
Acidic fraction	1.1	5.5
Phenolic fraction	0.6	3.0
Non-phenolic aldehydes	0.3	1.5
Phenolic aldehydes (as vanillin)[c]		25.0
	Total	67.6

(a) Oxidation of 20.0 gm. of spruce periodate lignin (C)

(b) Based on the weight of original periodate lignin.

(c) Vanillin content of aliquots calculated by multiplying weight of the crude dinitrophenyl-hydrazone precipitate by the factor 0.4578.

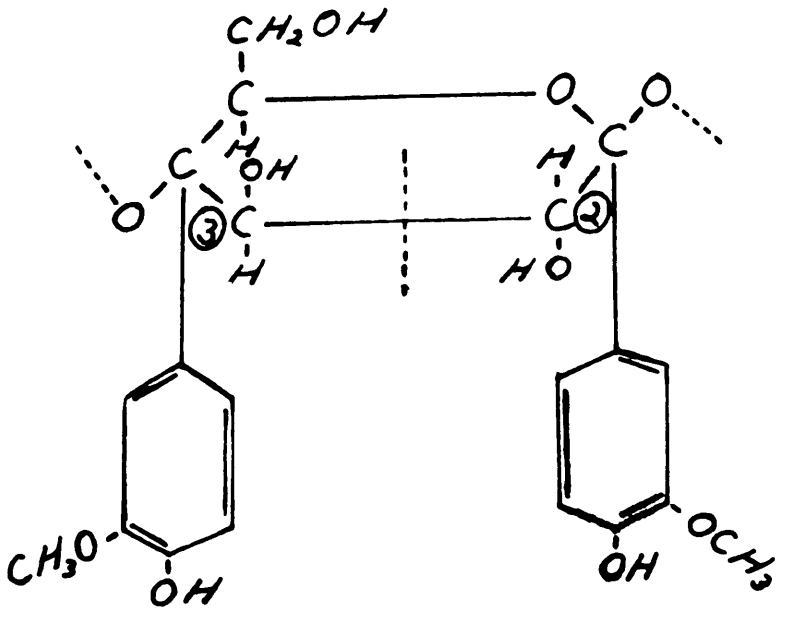
(Table IV). Although the melting-point of the phenylhydrazone was in the range reported by Pearl (125) for crude vanillin 2,4-dinitrophenylhydrazone precipitates, the calculated yield was undoubtedly high. The bisulfite-soluble materials were shown to consist, in part, of a dark, tarry material which probably contributed to the hydrazone formation. It is important to note that the presence of vanillin in the reaction products was definitely established through isolation of the pure dinitrophenylhydrazone and crystalline vanillin. Therefore, the loss of methoxyl that occurred during the preparation of spruce periodate lignin and purification of lignin sulfonic acids (176) was not connected with the complete destruction of the guaiacyl nucleus in the lignin. This observation is of particular interest since it has been reported that vanillin and other methoxyl-containing phenols of the type obtained by degradation of lignins are rapidly attacked by solutions of periodate (191).

G. CONCLUSIONS REGARDING THE NATURE OF LIGNIN "IN SITU"

The changes produced in the lignin complex under the conditions of the periodate isolation appear to be limited to the removal by oxidation of some highly methylated side group and almost assuredly do not involve condensation or polymerization reactions. This belief, combined with the fact that periodate lignins are isolated as highly insoluble, amorphous materials,

is a strong indication that lignin exists "in situ" as a substance of high molecular weight. Hilpert's (152) belief that lignin "in situ" is a carbohydrate or mixture of carbohydrates, is inconsistent with the high carbon content calculated for unchanged lignin and difficult to reconcile with the resistance of lignin to periodate oxidation. Since cyclization during isolation of the lignin with periodate is highly improbable, the detection of considerable amounts of cyclic materials like vanillin in the degradation products of periodate lignin is additional evidence of the existence of aromatic units in lignin "in situ".

One of the most interesting structures proposed for spruce lignin in recent years is that of Jayme and Hanke (67) who suggested a polyhexosan skeleton with two guaiacyl residues substituted in each hexose unit. Although unsaturated structures resulting from dehydration of the hexose unit were also visualized (page 49), they were assumed to revert to the hydroxylated form (XXIX) during extractions with hot water. Such a structure,



which according to Jayme and Hanke, is the principal component of spruce lignin in the conditions of the periodate isolation, would be susceptible to cleavage between the second and third carbon atoms of the hexose unit. The resulting cleavage product should be readily decomposable and there is no obvious reason to believe that it would not be completely disintegrated to soluble products in subsequent stages of the isolation. If solution did not occur, the hydrogenation products from the cleaved structure would probably be methyl (propyl) cyclohexane and ethylcyclohexane derivatives but certainly not the propylcyclohexane derivatives isolated.

The structures suggested by Freudenberg for lignin (page 44) account for its relative stability to the periodate reagent and, perhaps, for the formation of propylcyclohexane derivatives during hydrogenation or vanillin on oxidation. On the basis of the present work, it cannot be assumed, however, that the entire spruce lignin complex conforms to Freudenberg's concept since the large loss of carbon as gaseous substances during hydrogenation (Table XVIII) corresponds to a loss of aromatic units from the Freudenberg structure that is highly improbable. It is difficult to account for the partial loss of lignin substance and methoxyl groups during the periodate isolation on the basis of a uniform polymer such as that visualized by Freudenberg. A more

probable explanation is that lignin is a complex in the true sense of the word and far less homogeneous than is sometimes assumed.

The new periodate lignin, although somewhat oxidized, very clearly duplicates the behavior of lignin "in situ" toward acid ethanol, high pressure hydrogenation, oxidation and in alkali and sulfite cooks. The latter similarity is particularly impressive for, insofar as can be ascertained, other isolated lignins have lost the capacity to dissolve in a normal sulfite cook. Their negative behavior is entirely consistent with the observation that periodate lignins are rendered insoluble by such slight chemical treatments as heating in water at 135°C or in normal hydrochloric acid at 100°C. Wood lignin, therefore, is a highly sensitive complex whose potential solubility is greatly decreased by obscure chemical changes when isolated by methods employing high temperatures or mineral acids. Periodate lignins seem to fulfil, to a great extent, the need for an isolated lignin that is reasonably free from the suspicion of having suffered resinification or chemical change during isolation. They seem to be most promising materials for fundamental studies on the nature of lignin "in situ" as well as for more technical investigations, for example, on lignin sulfonic acids, which at present may be complicated by carbohydrate residues derived from the original wood.

EXPERIMENTAL PROCEDURES

A. ELEMENTARY COMPOSITION OF SPRUCE LIGNIN "IN SITU"

I. Preparation of Materials:

(1) Extractive-free Spruce Wood Meal.

A sound log of black spruce, approximately 140 years in age, was freed of bark, sawn into pieces and all knots were removed. The pieces were put through a chipper and the chips spread out to dry. After 96 hours the chips were ground in a Wiley mill, that portion passing a 40 mesh screen and resting on an 80 mesh screen being retained. The dry wood meal was exhaustively extracted in a metal Soxhlet (no rubber connections) with a boiling mixture of alcohol and benzene in the ratio of 1:2. This extraction required on the average about sixty hours. After thorough air-drying, the wood meal was extracted in a glass Soxhlet with 95% ethanol for four hours, then for a further four hours with hot water and again air-dried. Screening to break up lumps, thorough mixing and storage in large glass-stoppered bottles followed.

(2) Spruce Holocellulose.

Holocellulose was prepared from the extractive-free spruce wood by the method of Kurth and Ritter (15). Wood meal (about 7 gm.) was weighed into a large sintered-glass extraction thimble in which all subsequent operations were carried out. Brief chlorinations were accomplished by placing the extraction thimble in a rubber adaptor fitted

to a suction flask, passing the gas through the flask under slight pressure from a cylinder upward through the thimble, and thoroughly stirring the wood meal with a glass rod. After three to four minutes the flow of chlorine was terminated and the orange-colored wood meal was immediately covered with a solution of 50% pyridine in ethanol. The thimble and contents were then transferred to a glass Soxhlet and extracted with pyridine-ethanol for three hours. The chlorination-extraction cycle was repeated until the orange color developed on chlorination was barely discernible. The residue was then thoroughly washed with alcohol followed by water, dried at 105°C for 6 hours and weighed. Complete removal of lignin was impracticable since over-chlorination results in destruction of the more sensitive hemicelluloses. Considerable difficulty in determining the correct "end-point" was encountered and several preparations were lost through over-chlorination and consequent darkening of the holocellulose residue.

II. Methods of Analysis:

Analyses for moisture, lignin and ash were carried out according to the procedures detailed by the United States Forest Products Laboratory (192) and only deviations from these procedures will be noted.

(1) Ash.

Determinations of ash were carried out by igniting approximately 2 gm. samples of the material to constant

weight over Meker burners.

(2) Moisture.

(a) Unless otherwise stated, moisture contents were determined by heating duplicate 2 gm. samples at 105°C for eight instead of three to five hours, since the shorter periods were found to give less reproducible results.

(b) The moisture content of certain lignin specimens was determined by drying duplicate 2 gm. samples "in vacuo" over phosphorus pentoxide until constant weight was attained. It was found that most of the moisture was removed during the first two or three days but that a slow loss of weight occurred over a period of three weeks.

(c) Mitchell's (193) method of estimating the moisture content of cellulose specimens by the use of the Karl-Fischer reagent was applied to lignin in some cases.

(3) Lignin.

Klason lignin determinations were made by mixing 40 ml. of 72% sulfuric acid (by weight) with 2 gm. wood meal samples. Pre-cooling the acid to 0°C was adopted in order to avoid appreciable heating of the sample before mixing was complete. The period of two hours at room temperature, recommended for the dissolution of carbohydrates, was extended to sixteen hours at 10°C with occasional shaking (65). This change gave minimum, more reproducible results. After dilution of the mixture to 3% acidity and boiling under reflux for three hours, the Klason lignin was recovered by filtration, dried at 105°C for eight hours and weighed.

(4) Holocellulose.

Analyses for holocellulose were carried out by the original method of Kurth and Ritter (15) since it has been reported (17) (65) that more accurate and reproducible results are obtainable from softwoods by this procedure than by the more rapid technique of Van Beckum and Ritter (16).

(5) Carbon and Hydrogen.

Combustion analyses were on the semi-micro scale and, unless otherwise specified, with air dry samples. Moisture and ash determinations were simultaneously carried out on separate samples and the results used to correct the combustion data to a moisture and ash-free basis. All combustion analyses quoted are averages of concordant duplicate or triplicate estimations.

(6) Methoxyl.

The method of Viebock and Schwappach (194) and Viebock and Brecher (195) as modified by Peniston and Hibbert (196) for the analysis of woods and pulps, was adopted for methoxyl determinations.

III. Chemical Instability of Lignin at 105°C:

Duplicate, weighed samples of air-dry spruce Klason lignin contained in weighing bottles were thoroughly degassed in a vacuum desiccator at 1.5 m.m. pressure. A nitrogen chamber was constructed by fitting a glass cylinder of 800 ml. volume.with a large two-hole cork stopper in which glass tubes had been inserted. This

apparatus was placed in an air-oven with the inlet and outlet tubes protruding through a two-hole stopper closing an opening in the top of the oven. The degassed samples were placed in the cylinder and air removed from the apparatus by thorough flushing with a stream of nitrogen purified by passage through an alkaline solution of pyrogallol. The temperature of the oven was then quickly raised to 105°C and the samples heated in a continuous stream of oxygen-free nitrogen. At intervals, the duplicate samples were removed and the loss in weight was determined from one, while small portions were withdrawn from the other for combustion analysis. Heating was continued only after thoroughly flushing the system with nitrogen. Results of the initial combustion analyses were corrected for moisture and ash by heating separate samples for eight hours in the air-oven at 105°C and ignition of the dry residues to constant weight. The results of subsequent analyses were corrected for ash content only. Simultaneously duplicate samples of spruce wood and Klason lignin were heated in air at 105°C, the loss in weight and carbon contents being followed in an entirely similar manner. The data obtained are summarized in Table XI.

B. PREPARATION OF PERIODATE LIGNINS

I. Materials and Apparatus:

(1) Extractive-free wood meals.

The extractive-free spruce wood meal already described was used. Similar birch, beech and maple wood meals were prepared or obtained.

(2) Preparation of trisodium paraperiodate.

Trisodium paraperiodate, $Na_3H_2IO_6$, was prepared by oxidizing sodium iodide, dissolved in strong aqueous sodium hydroxide kept at 80°C, with bromine according to the directions of Lange and Paris (197). The product is highly insoluble in cold alkali and was recovered in 90% of the theoretical yield and in a state of 95% purity by filtration through sintered glass, since filter paper is rapidly destroyed by periodate. The substitution of chlorine at 100°C for the more expensive bromine, and of sodium iodate for sodium iodide, as described by Hill (198), gave a very pure product in about 80% yield.

Aqueous solutions of sodium periodate were prepared by shaking the salt in water slightly acidulated with acetic acid. A small amount of material remained undissolved and was removed by filtration. This insoluble substance proved to be silica removed from the glassware during the heating with hot caustic soda. The acidity was then adjusted to pH 4.1 with glacial acetic acid and the solution was allowed to stand overnight.

Any further precipitate that formed was removed by filtration. After the oxidation of the wood meal, the supernatant solution contained sodium iodate and any remaining paraperiodate. The latter was precipitated by adding a sufficient quantity of caustic soda to the mother liquor and was recovered in a nearly pure condition. The filtrate from this operation, containing sodium iodate, was treated at 80°C with bromine or at 100°C with chlorine in order to remove iodate as the insoluble periodate. This simple recovery system made possible an economic use of the expensive periodate salt.

(3) Continuous-washing apparatus.

The filtration and washing of large quantities of oxidized wood meal proved to be unusually difficult and tedious. Since even dilute periodate solutions rapidly destroy filter paper, ordinary Büchner funnels could not be used and sintered glass filters quickly became clogged with the finest wood particles. In order to cope with this problem, a large continuous-washing device (Fig.XV) was constructed. This apparatus consisted essentially of a 20-litre glass cylinder with a constant-level siphon inlet and a filter outlet attached to the water pump. The filter outlet was constructed from a two-piece Büchner funnel fitted with a screen of 200 mesh copper gauze, which was inverted in the cylinder at the desired level. After removal of the iodate-rich mother liquors from an oxidation by filtration through sintered glass,

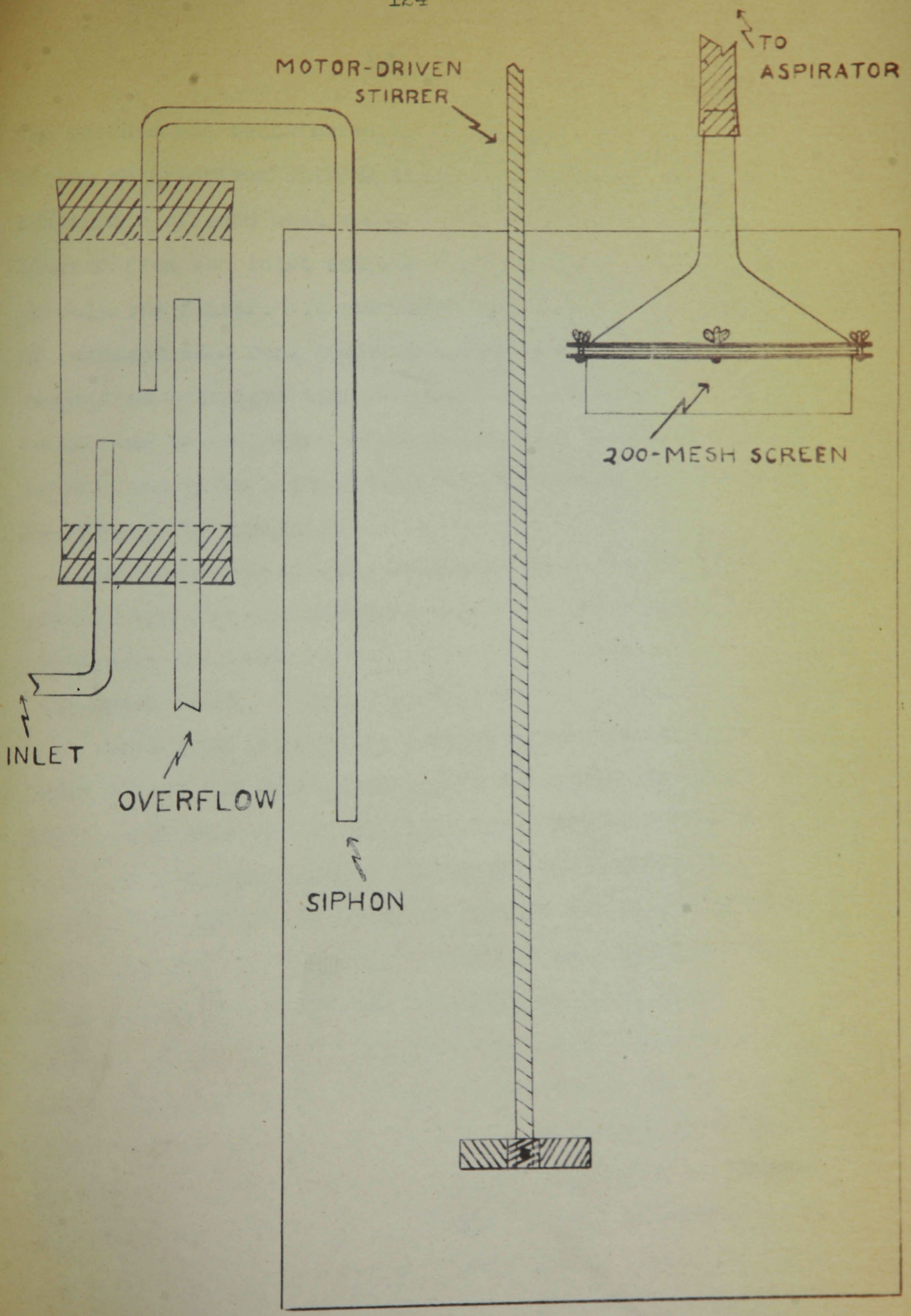

MOTOR-DRIVEN
STIRRER
TO
ASPIRATOR
200-MESH SCREEN
INLET
OVERFLOW
SIPHON

FIG. XV

the residue was transferred to the glass cylinder. A large, motor-driven stirrer thoroughly agitated the suspension of wood meal while fresh tap water was delivered from the inlet and the wash-water was withdrawn through the filter. It was found that large quantities of oxidized wood meal could be washed free of inorganic impurities overnight with no attention. The wood residue could then be collected on large sintered glass filter funnels and given a final wash with distilled water.

II. Methods of Analysis:

Determinations of ash, moisture, lignin holocellulose, carbon, hydrogen and methoxyl were carried out by methods previously outlined.

(1) "Pentosans".

"Pentosan" estimations were by distilling 2 gm. samples with 12% hydrochloric acid and estimating the furfural formed as the phloroglucide. All technical details were as described by the Forest Products Laboratory (192). The method developed by Hunter, Wright and Hibbert (180) was used in separating from the furfural distillate of periodate lignin any formaldehyde that was formed during the heating with hydrochloric acid. Continuous extraction with ether was employed to remove the furfural, along with a small portion of the formaldehyde, from the distillate. After evaporating the ether extract to dryness the residue was taken up in a small amount of water and the hydrazones precipitated by addition of 2.4-dinitrophenylhydrazine solution. The dry mixture,

consisting chiefly of the dinitrophenylhydrazone of furfural and a small amount of that of formaldehyde, was extracted with cold chloroform whereupon the formaldehyde derivative was dissolved while the furfural dinitrophenylhydrazone remained as a residue. The bulk of the formaldehyde was estimated by adjusting the acidity of the extracted distillate to pH 4.6 (181) and adding dimedon reagent. After allowing the precipitate to settle by standing 24 hours at 10°C it was collected on a tared filter, dried and weighed.

(2) Analysis of Periodate Solution.

Trisodium paraperiodate was estimated in aqueous solution by a slight modification of the method of Fleury and Lange (199). Aliquots of 5 ml. were transferred by pipette into a 125 ml. Erlenmeyer flask, diluted with distilled water up to 30 ml. and made just alkaline to phenolphthalein with normal caustic soda. The addition of freshly prepared sodium bicarbonate solution, or of a small piece of dry ice, adjusted the pH of the mixture to the proper value of 8 to 9 as shown by the faint pink color of the phenolphthalein indicator. An excess of solid potassium iodide was then added together with 10 ml. of standard 0.1 N arsenious acid, the latter being run in accurately from a pipette. After ten minutes, the excess arsenious acid was back-titrated with decinormal iodine. The chemistry involved is expressed in the following

equations:-

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

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

Since iodates do not liberate iodine from iodides in neutral or basic solution, only the periodate present enters into the reaction. The amount of periodate present is calculated by the following formulae:-

$$\text{Blank} = \frac{\text{Ml. of } H_3AsO_3 \times \text{N. of } H_3AsO_3}{\text{N. of } I_2} \times 10$$

$$\text{Grams of Periodate} = \frac{(\text{Blank-Titre}) \times 2.94 \times \text{N. of } I_2}{1000 \times 2 \times 10 \times 0.1000} \times \frac{T}{V}$$

where T is the total volume of the solution and V the volume of the aliquot.

III. Large-scale Oxidation of Spruce Wood:

A sample of spruce wood meal weighing 101.9 gm. (corrected for 7.46% moisture) was oxidized with 2 litres of a solution containing approximately 65 gm. of trisodium paraperiodate buffered to pH 4.1 with glacial acetic acid. The temperature was maintained at 20°C by immersing the container in the water bath and the mixture was slowly stirred for 60 hours. The oxidized residue was recovered by filtration and was washed with water with tedious repetitions until the washings, on acidification, failed to liberate iodine from potassium iodide. When iodate and any excess periodate had been thoroughly removed in this manner, the residue was boiled under reflux for three hours with 8 litres of water at a pH close to 7. After recovery by

filtration and washing with water, the residue was re-oxidized with the same amount of sodium paraperiodate solution. At no point in the cycle was the residue permitted to dry in air. After six alternate oxidations and hydrolyses the product analyzed 86.0% Klason lignin. The consumption of periodate was followed by titrating 5 ml. aliquots of the original periodate solution and the undiluted filtrate after each oxidation period had elapsed. The data for these analyses are given in Table XXVII and the consumption of periodate is plotted against the number of oxidations in Fig. VIII. The time allowed for oxidation was too long in this experiment, as all the reagent present was consumed in each of the first three oxidations.

The product from the oxidation (Spruce periodate lignin A) was stored in a flask under water until used. A small amount of toluene was placed on the surface of the water to prevent the growth of molds. Samples were removed as required and were freed of water by solvent exchange with anhydrous methanol and benzene, the final drying being under vacuum. A complete analysis of this material is summarized in Table XV.

C. SULFITE PULPING OF PERIODATE LIGNINS

I. Materials and Apparatus:

(1) Sulfite liquor.

The quantity of calcium oxide calculated to give the required concentration of combined sulfur dioxide was suspended in distilled water. Sulfur dioxide was bubbled through the suspension until the desired total sulfur dioxide concentration, as determined by titration of aliquots of the solution, was attained.

(2) Digestor vessels.

Experimental cooks were carried out in glass-lined, Monel metal bombs of 150 ml. capacity, sealed with lead gaskets. The desired temperatures were attained by immersing the bombs in an oil-bath controlled by a bimetallic thermoregulator.

II. Methods of Analysis:

(1) Sulfite liquor.

Analysis for free and combined sulfur dioxide content of liquors was carried out by the method of Palmrose (200). Aliquots of 2 ml. were diluted with water to a total volume of 50 to 100 ml. and starch-potassium iodide solution was added. The solution was then titrated to the first permanent blue color with N/8 potassium iodate solution. After destroying the blue color with a drop of N/10 thio-sulfate solution and adding methyl red or bromthymol blue indicator, the solution was titrated with N/8 sodium hydroxide solution. The sulfur dioxides concentrations were calculated from the

following equations:

0.2 (Volume of Iodate) = Total SO_2

0.2 (Volume of Sodium Hydroxide) = Combined SO_2

The concentration of free sulfur dioxide was the difference of the results obtained as above.

(2) Sulfur.

The sulfur content of partially cooked lignins was determined by the Carius method (201) using samples weighing about 30 mg.

III. Experimental Cooks:

Throughout these experiments liquor containing 5% combined and 1% free sulfur dioxide was used. A sample of lignin weighing approximately 2 gm., together with 100 ml. of liquor, was sealed in a bomb which was then placed in the oil-bath at the temperature at which the cook was to be conducted. At the end of the reaction, the bomb was immediately removed and cooled by plunging in cold water. Semi-cooked lignins were recovered as very highly swollen materials which quickly blocked sintered glass filters and made filtration impossible. Separation of residual lignin from the liquors and washing was therefore accomplished by centrifuging. After thorough washing, the swollen residue was collapsed by washing once with ethanol, collected in a tared sintered glass filter crucible, washed with ethanol, dried "in vacuo" at 55°C and weighed.

Data on the rate of pulping of spruce periodate

lignin were obtained by carrying out a series of cooks at a bath temperature of 135°C for periods of two to seven hours. In Fig.XIII the residual lignin, expressed as a percentage of the weight of the original lignin (uncorrected for ash), is plotted against time. A series of cooks was conducted at temperatures ranging from 100 to 135°C for a constant time of six hours. The percentage of undissolved lignin (uncorrected for ash) is plotted against temperature in Fig.XIV, which also contains the sulfur analyses of the residues plotted against temperature. In both series of cooks spruce periodate lignin (C) was used. The pulping qualities of various pre-treated lignins, as well as birch, beech and maple periodate lignins were compared by subjecting each to a standard sulfite cook at 135°C for six hours. The results of these cooks are summarized in Table XVII.

D. HYDROGENATION OF SPRUCE PERIODATE LIGNIN

I. Materials and Apparatus:

(1) Copper-chromite catalyst.

The catalyst was prepared according to the directions of Folkers and Connor (202) and stored in a stoppered bottle until used.

(2) Anhydrous dioxane.

The method recommended by Weisberger and Proskauer (203), in which commercial dioxane was heated under reflux with hydrochloric acid in an inert atmosphere, was used. The final distillation was from metallic sodium.

(3) Karl-Fischer reagent.

The Karl-Fischer reagent, prepared by addition of liquid sulfur dioxide to a cold solution containing methanol, pyridine and iodine as directed by Smith, Bryant and Mitchell (204), was used in estimating water. Water determinations were carried out with the Wilkens-Anderson titrimeter in which the Karl-Fischer reagent, the standard water solution and the solution being titrated were adequately protected against contamination by atmospheric moisture at all stages of the procedure. The solution was mechanically stirred during titration and the end-point detected electrometrically.

(4) Cooke-Bower column.

A slightly modified Cooke-Bower (205) column having an efficiency of 28 theoretical plates was used for separating the hydrogenation products of lignin. Introduction of a ground-glass bearing in the receiver assembly (Fig.XVI) permitted more satisfactory work at reduced pressures and the addition of a ground-glass joint at the cold finger obviated the possibility of contamination from the rubber stopper formerly used. Adequate control of the temperature of the electrically-heated still-pot and column was accomplished through a system of rheostats inserted in the electrical heating circuits.

II. Methods of Analysis:

(1) Estimation of water.

The water-equivalent of the Karl-Fischer reagent was

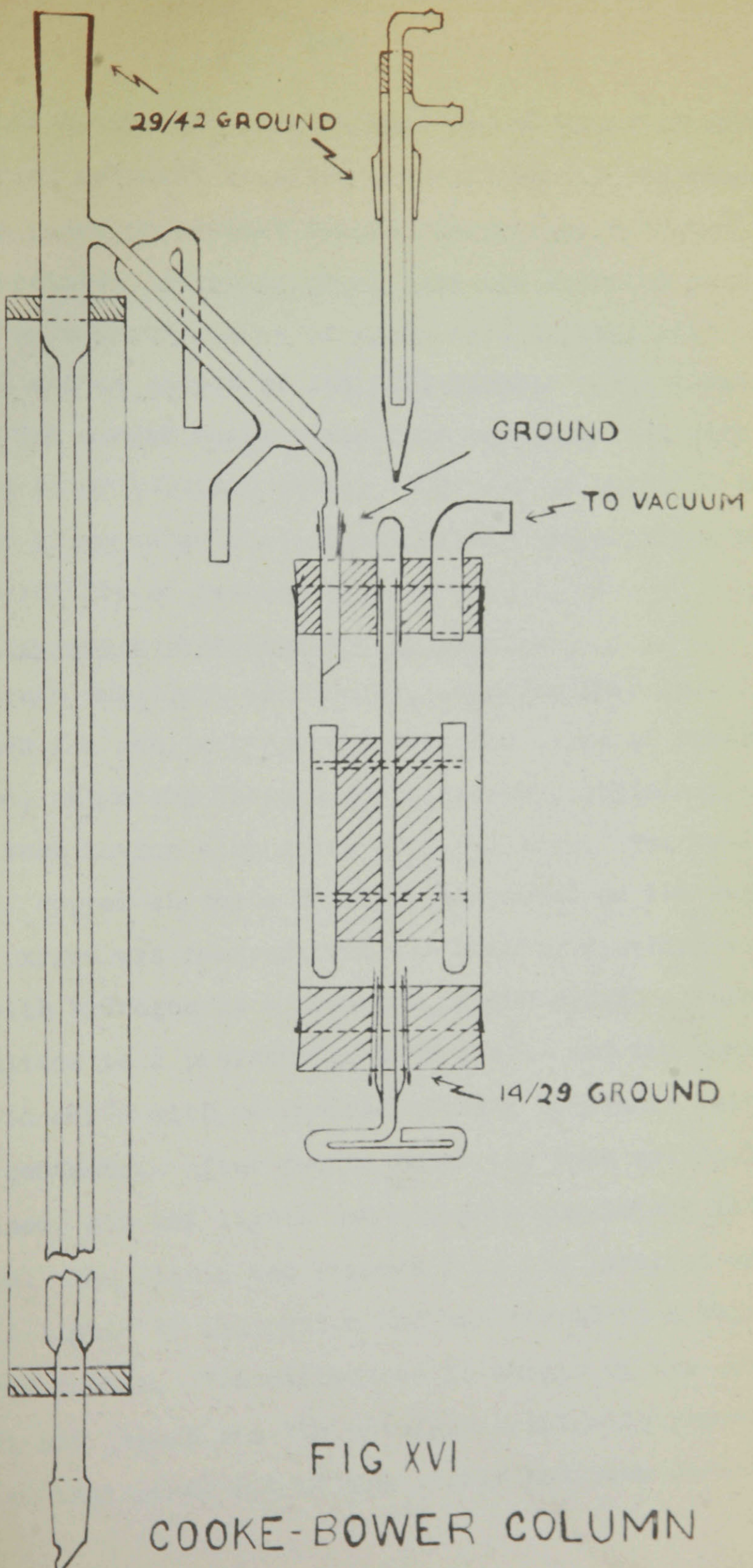


FIG XVI

COOKE-BOWER COLUMN

determined by standardization against solutions of carefully dried methanol to which a known quantity of water had been added or against weighed amounts of hydrated sodium acetate. Aliquots (5 ml.) of the solution containing an unknown amount of water were diluted with anhydrous methanol and an excess of the Karl-Fischer reagent was added. The excess reagent was then back-titrated with the standard water solution (204). Analysis of methanol solutions of known water content indicated high accuracy and reproducibility of results by this method.

III. Large-scale Hydrogenation of Lignin:

Spruce periodate lignin (B), weighing 39.5 gm., corrected for ash, was suspended in one litre of freshly purified, anhydrous dioxane contained in a stainless steel Aminco bomb having a capacity of 2.5 litres. Twenty-five grams of copper-chromite catalyst was added to the suspension. Oxygen was removed from the bomb by flushing three times with hydrogen at a pressure of 600 p.s.i. Hydrogen was admitted to a pressure of 1975 p.s.i. and the bomb was heated to 260°C with mechanical rocking to assure agitation of the contents. After twenty hours the bomb was cooled and opened, but the lignin had not gone completely into solution. The liquid was removed from the catalyst and residual lignin by filtration and was stored in a tightly stoppered bottle. The difference in weight of the combined catalyst and lignin and the catalyst originally present indicated that about 60% of the lignin had been dissolved.

This residue was suspended in one litre of fresh dioxane and an additional 25 gm. of catalyst was added. A second hydrogenation (initial pressure of 2425 p.s.i.) proved sufficient to convert all the remaining lignin into soluble products. The combined, filtered liquors were colored slightly yellow. The course of the hydrogenation was followed by frequent temperature and pressure readings which are recorded in Table XXXII.

Separation and Identification of Hydrogenation Products

Fractional distillation of the hydrogenate at atmospheric pressure through a modified Vigreaux column yielded; Fraction1, b.p.56 to 80°C; Fraction 2, b.p.80 to 101°C; Fraction 3, b.p.101 to 104°C; and Fraction 4, the residue, boiling above 104°C. The latter on distillation under reduced pressure from a Claisen flask, yielded Fraction 4A, 12.2 gm., b.p. below 200°C at 8 m.m. pressure, and Fraction 4B, 3.4 gm., b.p.115 to 200°C at 10 microns pressure. The still residue, Fraction 5, 4.2 gm., was a dark resinous material that solidified on cooling to an amorphous mass. The further fractionation of these fractions gave the following results;

Fraction 1 was slowly distilled through the Cooke-Bower column at atmospheric pressure. The fraction boiling from 65.5 to 67°C was retained and the remainder, which distilled continuously with rising temperature, was combined with Fraction 2. The colorless distillate, 3.97 gm., b.p. 65.5 to 67°C, n_D^{25} = 1.3368, gave a 3.5-dinitrobenzoate

and α- naphthylurethane of methanol showed no depression from the recorded values of 107°C and 123.5°C, respectively (206).

Fraction 2. The fraction boiling from 80 to 101°C contained the water formed during the hydrogenation, since during previous operations exposure of the hydrogenate to moisture had been carefully avoided. The estimation of water was made by diluting Fraction 2 to a definite volume with anhydrous methanol and titrating 5 ml. aliquots with the Karl-Fischer reagent as previously described. The water content of the fraction was estimated to be 5.36 gm. or 13.59% of the weight of the lignin hydrogenated.

Fraction 3. This fraction had distilled at 101°C with the exception of the last few drops which were recovered as the temperature rapidly rose to 104°C. The boiling-point and refractive index (n_D^{25} = 1.4236) indicated that the fraction was almost pure dioxane.

Fraction 4A. The portion of the hydrogenate boiling below 200°C at 8 m.m. pressure was carefully fractionated through the Cooke-Bower column. The pressure was reduced to 7 m.m. and with the cold-finger temperature at 20°C, the still-pot and column were heated until the column was flooded. The temperature was then lowered and again slowly raised until the column was operating under total reflux at a rate of approximately 30 drops per minute. After the elapse of one hour to assure equilibrium conditions within the column, the temperature of the cold-finger was slowly

raised by introducing warm water and distillation was carried out at a reflux ratio of approximately 100:1. As distillation proceeded, the temperature of the still-pot and column was slowly raised in order to maintain the rate of reflux. Samples of the distillate weighing approximately 0.2 gm. were collected. When it was necessary to interrupt the distillation, equilibrium within the column was restored as described before continuing. Sub-fractions 1 to 46 were collected below a still-pot temperature of 140°C. At this point the pressure was reduced to 1 m.m. and sub-fractions 47 to 61 were collected below the same still-pot temperature. The refractive index of sub-fractions 1 to 22 (n_D^{25} = 1.412 - 1.417) indicated that all consisted of some slightly impure dioxane not removed in the initial, rough separation. All were shown to boil very near 101°C at atmospheric pressure. The fractionation curve obtained by plotting refractive index against grams (cumulative) of distillate for the remainder of the sub-fractions (Fig.XVII, Table XXXIII) possessed two distinct "flats" corresponding to two main components in the distillate.

Isolation of 4-n-propylcyclohexanol Derivatives

Sub-fraction 36 was selected as typical of the "flat" comprised by sub-fractions 28 to 46 inclusive. The α-naphthylurethane was prepared according to the directions of Schriner and Fuson (206) and after repeated

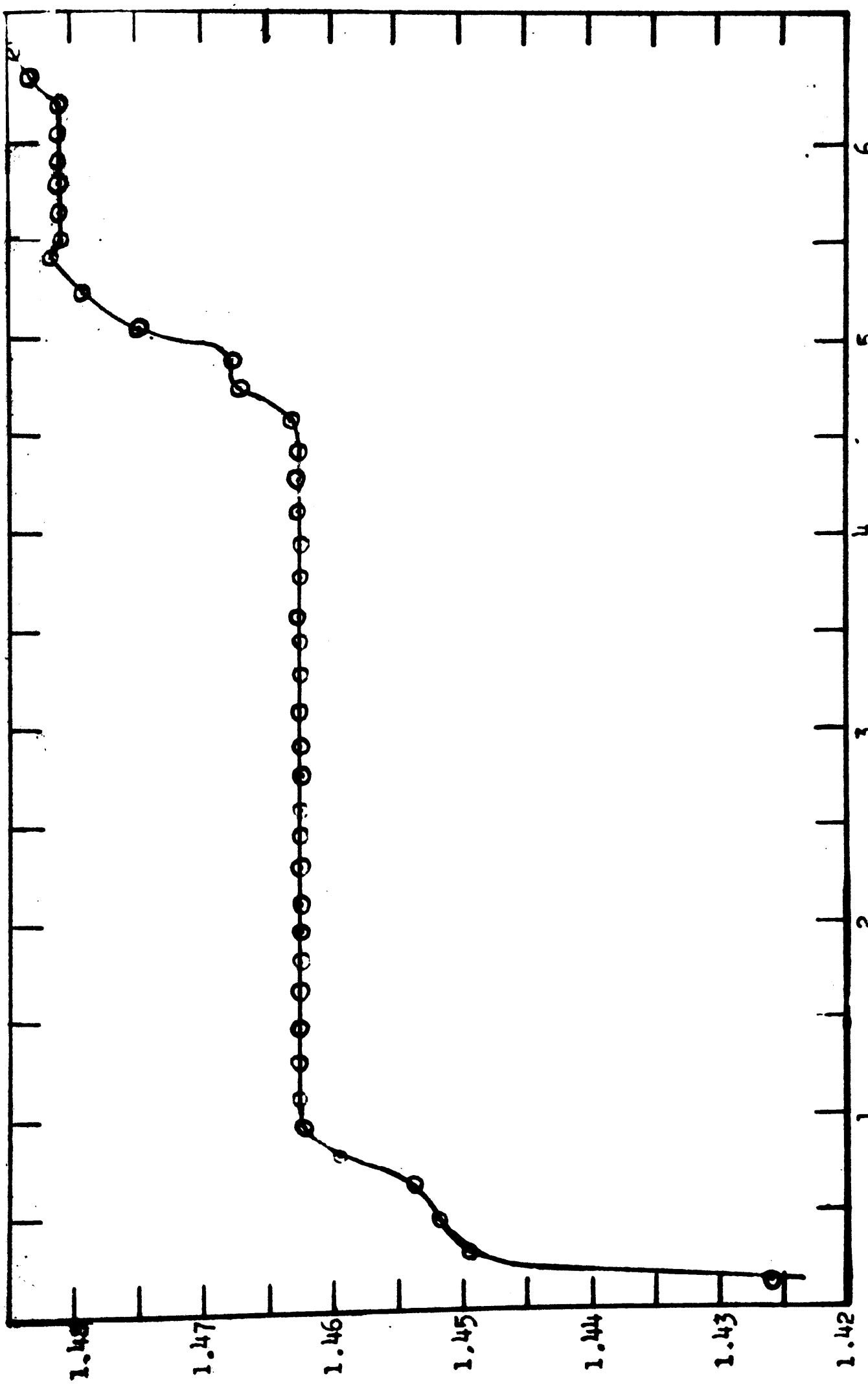


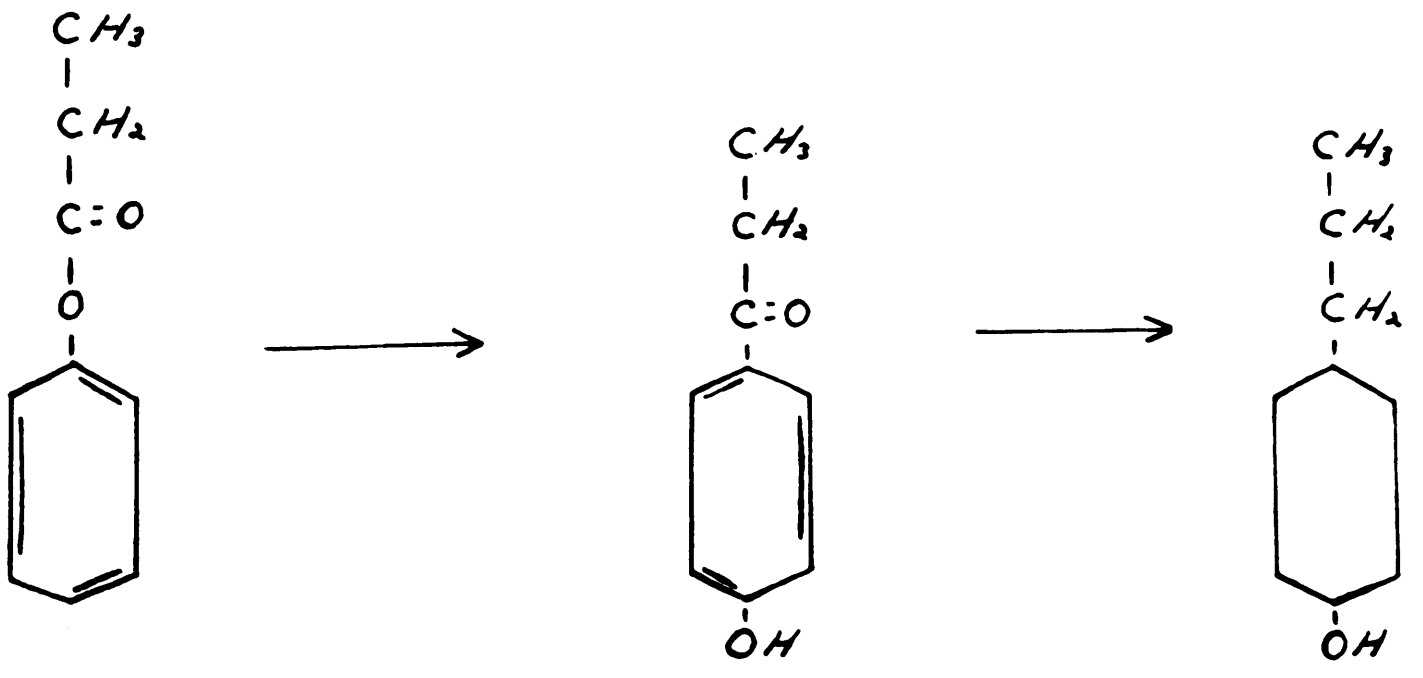
Fig.XVII. - Distillation curve of periodate lignin hydrogenation products

recrystallization from chloroform melted at 133 to 134°C. The phenylurethane was similarly prepared and after recrystallization melted at 130 to 131°C. These melting-points were not depressed by admixture with authentic samples of the ∝- naphthylurethane and phenylurethane of 4-n-propylcyclohexanol-1.

Analysis: Sub-fraction 38; C, 75.9; H, 12.4%. Calculated for 4-n-propylcyclohexanol-1; C, 76.1; H, 12.7%.

Synthesis of 4-n-propylcyclohexanol-1

Phenyl propionate was prepared and rearranged to p-hydroxypropiophenone through the Fries reaction as described by Rosenmund and Lohfert (207). The product, recrystallized from methanol and having the correct melting-point of 146 to 147°C, was obtained in a yield of 27.8%, in good agreement with a yield of 31% reported by Rosenmund. The p-hydroxypropiophenone, 24.1 gm., was hydrogenated in 125 ml. of dioxane over 15 gm. of copper-chromite catalyst



at 250°C and initial hydrogen pressure of 1800 p.s.i. for ten hours. The theoretical amount of hydrogen had then been absorbed. The catalyst was removed by filtration and the solvent evaporated under reduced pressure. Distillation of the residue from a Claisen flask at 7 m.m. pressure gave 19.2 gm. of a colorless liquid, b.p. 92 to 95°C, n_D^{25} = 1.4605, in an overall yield of approximately 23%. The phenylurethane and α- naphthylurethane were prepared and, after recrystallization from chloroform, melted at 130 to 131°C and 134.5 to 135°C, respectively. These melting-points agree with previous published values (130).

Isolation of 4-n-propylcyclohexanediol-1,2

Sub-fractions 55 and 56 were selected as typical of the "flat" comprised by sub-fractions 55 to 58 of Fig. XVII. Analysis: Calculated for a propylcyclohexanediol, $C_9H_8O_2$; C, 68.4; H, 11.4%. Found for sub-fraction 56; C, 68.1; H, 10.6%. The α- naphthylurethane was prepared from sub-fraction 55 and after repeated recrystallization from chloroform melted at ≥17.5 to ≥19°C. This figure corresponded to the melting-point of the bis-α-naphthylurethane of 4-n-propylcyclohexanediol-1,2 (130). In order to confirm the adjacent position of the two alcohol units, sub-fraction 54 was subjected to oxidation with 0.005 N sodium paraperiodate solution (208). The disappearance of periodate was followed with the usual arsenite-iodine titration and it was found that 0.1021 gm. of the diol con-

sumed 0.1901 gm. of periodate, i.e., one mole of the diol consumed almost exactly one mole of periodate. This result proved that the compound was a 1,2 - glycol and removed the possibility that it was 3-(4-hydroxycyclohexyl)-propanol-1.

Fraction 4B: The high-boiling material, obtained as a light yellow, viscous oil, was soluble in ether, dioxane and methanol. No coloration was observed on treatment of a methanolic solution with ferric chloride and phenolic substances were presumed absent. Analysis: Found; C, 76.4; H, 11.4; CH_3O, 1.91%.

Fraction 5: The dark, non-distillable resin was soluble in ether, dioxane and methanol, but quite insoluble in water. Tests for phenolic groups on methanol solutions were negative. Ether solutions were highly fluorescent under ultra-violet light. Analysis: Found; C, 84.3; H, 11.8; CH_3O, 2.17%. Very probably both fractions 4B and 5 were mixtures. The yields of the various hydrogenation products are summarized in Table XVIII.

E. ETHANOLYSIS OF SPRUCE PERIODATE LIGNIN

Spruce periodate lignin (C), weighing 9.2 gm. corrected for moisture, was placed in a litre round-bottom flask fitted with a ground-glass condenser in reflux position. A two-hole stopper in the top of the condenser carried a calcium chloride drying-tube and a and a glass inlet tube extending to a point about two

inches below the neck of the flask. About 500 ml. of anhydrous ethanol containing 2% of hydrogen chloride gas was added to the flask and air was removed from the apparatus by thorough flushing with a current of oxygen-free nitrogen continued for a period of two hours. After being heated under reflux on a steam-bath in a continuous stream of nitrogen for 48 hours, the liquid was cooled and the insoluble residue was collected on a fritted glass filter. The extract was neutralized with excess sodium carbonate and was stored in a stoppered container. The residual lignin was re-extracted with boiling ethanolic hydrogen chloride three times in the manner described. At the end of the fourth extraction the extract, which in the first operation was colored deep red, was only light yellow in color and the undissolved lignin appeared to be inert to further treatment. The residue was thoroughly washed with water and dried through solvent-exchange with dry methanol and benzene. The combined, neutralized extracts were filtered and concentrated to about one-third volume under reduced pressure.

The concentrated extract was poured in a thin stream into two litres of water with rapid stirring, whereupon a tan-colored precipitate separated from the solution. After allowing the ethanol lignin to settle overnight, it was recovered by filtration, thoroughly washed with water, dissolved in acetone and reprecipitated by pouring into seven volumes of rapidly stirred water. The ethanol

lignin was allowed to settle, collected on a filter, washed with water and dried under vacuum at 55°C. The friable, light brown powder was reserved for analysis. The combined solutions and washings were continuously extracted with benzene until no further colored materials were removed by the extractant. After drying the extract over anhydrous sodium sulfate, the solvent was removed under reduced pressure, leaving a dark red, viscous oil. This residual oil was taken up in acetone and poured into five volumes of petroleum ether, whereupon a red tar separated. The petroleum ether solution was removed from the tar by decantation and the ether-soluble oils recovered by evaporation of the solvent. The yields of the products obtained are summarized in Table XIX.

F. ALKALINE OXIDATION OF SPRUCE PERIODATE LIGNIN

Spruce periodate lignin (C), weighing 20 gm., was suspended in 1.5 litres of 8% sodium hydroxide solution contained in a stainless steel autoclave. After adding 50 ml. of nitrobenzene, the autoclave was sealed and heated at 160°C for three hours with mechanical rocking. On cooling and opening the autoclave, it was found that the lignin had been completely converted into soluble products. The alkaline reaction mixture was filtered, extracted with ether to remove excess nitrobenzene and other nitrogenous products, and acidified to pH 3 with dilute sulfuric acid. A dark, amorphous precipitate

separated from the solution and was recovered by filtration, washed and dried "in vacuo" at 55°C. The acidulated solution, which smelled strongly of vanillin, was then continuously and exhaustively extracted with ether. The ether extract was resolved into carbonyl, acidic, phenolic and neutral components by shaking successively with saturated sodium bisulfite, 20% sodium bicarbonate and 5% sodium hydroxide solutions.

Acidic Fraction: The aqueous bicarbonate solution was acidified to pH 5 with dilute sulfuric acid and, after warming under reduced pressure to remove as much carbon dioxide as possible, was back-extracted with ether. The ether extract was dried over anhydrous sodium sulfate, filtered and the solvent removed by evaporation. A small quantity of colored, solid material remained.

Phenolic Fraction: The ether solution obtained by extraction of the acidified aqueous sodium hydroxide extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. As before the solid residue was colored and consisted, in part, of crystalline materials. The methanolic solutions of the substance became highly colored on addition of ferric chloride solution, an indication that phenols were present.

Carbonyl Fraction: The aqueous sodium bisulfite solution was acidified and sulfur dioxide was removed under reduced pressure at room temperature. Addition of sodium hydroxide solution to pH 10 was followed by ether extraction to remove non-phenolic carbonyl components. After adjusting

the acidity of the aqueous solution to pH 3 with dilute sulfuric acid, the volume was increased to two litres with distilled water. Two aliquots of 250 ml. each were withdrawn and the remainder was continuously extracted with ether for 48 hours. On addition of approximately 100 ml. of 2,4-dinitrophenylhydrazine solution (209) to each of the aliquots, a brick-red, voluminous precipitates formed. After warming the solutions on the steam bath for one hour the precipitates were recovered by filtration through tared sintered glass crucibles, dried for four hours at 105°C and weighed. The crude dinitrophenylhydrazine melted at 240 to 247°C and, after twice recrystallizing from 95% ethanol, melted at 262 to 264°C. The melting-point (210) of an authentic sample of the 2,4-dinitrophenylhydrazone of vanillin (266°C) was not depressed by admixture with the crystals obtained from the periodate lignin. The ether extract from the remainder of the solution was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. This dark-colored residue was extracted with hot water, leaving a residual, dark, tarry material. On cooling the water extract, light-colored crystals were obtained which, after drying, melted at 79.5 to 81°C. Admixture with authentic samples of vanillin resulted in no depression of the melting-point from the recorded value of 81 to 82°C. The yields of the various products obtained by alkaline oxidation of spruce periodate lignin are summarized in Table XX.

TABLE XXI

Oxidation of Spruce Holocellulose. Fig.II. plot I

Time	Iodine Titre (a)	$Na_3H_2IO_6$ Consumed by Sample (b)		$Na_3H_2IO_6$ Consumed per Gram of Sample	
0	7.54 ml.	0.000 gm.	0.00000 moles	0.000 gm.	0.00000 moles
20	7.60	0.059	0.00020	0.122	0.00041
40	7.64	0.103	0.00035	0.214	0.00073
95	7.73	0.199	0.00067	0.413	0.00140
155	7.78	0.273	0.00092	0.565	0.00192
245	7.85	0.354	0.00120	0.734	0.00250
350	7.90	0.425	0.00144	0.879	0.00299
460	7.92	0.444	0.00152	0.921	0.00313

A sample weighing 0.4830 gm. (or 0.0030 moles as anhydroglucose) was oxidized with 190 ml. of a solution containing 1.9519 gm. (or 0.0066 moles) of $Na_3H_2IO_6$.

Oxidation of Spruce Wood. Fig.II. plot II

0	7.51 ml.	0.000 gm.	0.00000 moles	0.000 gm.	0.00000 moles
20	7.61	0.050	0.00017	0.087	0.00029
50	7.71	0.150	0.00051	0.259	0.00088
95	7.77	0.228	0.00077	0.394	0.00133
155	7.81	0.295	0.00100	0.509	0.00173
245	7.86	0.365	0.00124	0.630	0.00214
350	8.00	0.473	0.00161	0.816	0.00277
475	8.04	0.504	0.00171	0.869	0.00296

A sample weighing 0.6305 gm. (or 0.0038 moles as anhydroglucose) was oxidized with 180 ml. of a solution containing 1.8644 gm. (or 0.0063 moles) of $Na_3H_2IO_6$.

TABLE XXI (Contd.)

Oxidation of Spruce Klason Lignin. Fig.II. plot III

Time	Iodine Titre	$Na_3H_2IO_6$ Consumed by Sample		$Na_3H_2IO_6$ Consumed per Gram of Sample	
0 min.	6.54 ml.	0.000 gm.	0.00000 moles	0.000 gm.	0.00000 moles
25	6.55	0.019	0.00006	0.000 gm.	0.00000
60	6.57	0.069	0.00023	0.166	0.00056
115	6.58	0.098	0.00033	0.236	0.00080
185	6.59	0.129	0.00044	0.311	0.00106
340	6.60	0.154	0.00052	0.370	0.00126
435	6.61	0.157	0.00053	0.379	0.00129

A sample weighing 0.4162 gm. (or 0.0025 moles as anhydroglucose) was oxidized with 185 ml. of a solution containing 1.8816 gm. (or 0.0064 moles) of $Na_3H_2IO_6$.

(a) Analyses were carried out on 5 ml. aliquots using 10 ml. of 0.7061 N-arsenious acid and 0.9732 N-iodine from a semi-micro buret.

(b) All oxidations at pH 4.1 and 20°C.

TABLE XXII

Oxidation of Spruce Klason Lignin. Fig.III. plot I

Time	Iodine Titre(a)	$Na_3H_2IO_6$ Consumed by Sample (b)		$Na_3H_2IO_6$ Consumed per Gram of Sample	
0 min.	7.53 ml.	0.000 gm.	0.00000 moles	0.000 gm.	0.00000 moles
50	7.58	0.074	0.00035	0.434	0.00149
95	7.62	0.170	0.00058	1.004	0.00341
155	7.64	0.202	0.00068	1.191	0.00404
245	7.68	0.269	0.00091	1.584	0.00538
350	7.71	0.331	0.00112	1.947	0.00662

A sample weighing 0.3404 gm. (or 0.0021 moles as anhydroglucose) was oxidized with 170 ml. of a solution containing 1.7512 gm. (or 0.0060 moles) of $Na_3H_2IO_6$.

Oxidation of Spruce Holocellulose. Fig.III. plot II

0 min.	7.53 ml.	0.000 gm.	0.00000 moles	0.000 gm.	0.00000 moles
20	7.61	0.033	0.00011	0.052	0.00018
50	7.70	0.120	0.00040	0.190	0.00064
95	7.82	0.216	0.00073	0.341	0.00116
155	7.93	0.305	0.00103	0.482	0.00164
230	7.94	0.354	0.00120	0.560	0.00190
320	8.07	0.445	0.00151	0.703	0.00239
450	8.10	0.458	0.00156	0.724	0.00246

A sample weighing 0.6335 gm. (or 0.0039 moles as anhydroglucose) was oxidized with 150 ml. of a solution containing 1.5246 gm. (or 0.0056 moles) of $Na_3H_2IO_6$.

TABLE XXII (Contd.)

Oxidation of Spruce Wood. Fig.III. plot III

Time	Iodine Titre	$Na_3H_2IO_6$ Consumed by Sample		$Na_3H_2IO_6$ Consumed per Gram of Sample	
0 min.	8.05 ml.	0.000 gm.	0.00000 moles	0.000 gm.	0.00000 moles
20	8.29	0.115	0.00039	0.112	0.00038
50	8.38	0.198	0.00067	0.193	0.00065
95	8.55	0.314	0.00107	0.306	0.00104
157	8.65	0.395	0.00134	0.385	0.00131
235	8.74	0.469	0.00159	0.457	0.00155
325	8.80	0.528	0.00179	0.515	0.00175
440	8.84	0.564	0.00190	0.550	0.00186

A sample weighing 1.0260 gm. (or 0.0063 moles as anhydroglucose) was oxidized with 170 ml. of a solution containing 1.5011 gm. (or 0.0055 moles) of $Na_3H_2IO_6$.

Oxidation of Spruce Klason Lignin. Fig.III. plot IV

0 min.	6.91 ml.	0.000 gm.	0.00000 moles	0.000 gm.	0.00000 moles
40	6.93	0.030	0.00010	0.094	0.00032
90	6.94	0.053	0.00018	0.164	0.00056
150	6.95	0.066	0.00022	0.206	0.00070
250	6.95	0.066	0.00022	0.206	0.00070
370	6.95	0.066	0.00022	0.207	0.00070
470	6.96	0.067	0.00022	0.208	0.00071

A sample weighing 0.3221 gm. (or 0.0020 moles as anhydroglucose) was oxidized with 160 ml. of a solution containing 0.7644 gm. (or 0.0026 moles) of $Na_3H_2IO_6$.

TABLE XXII (Contd.)

(a) Analyses were carried out on 5 ml. aliquots using 10 ml. of 0.7061 N arsenious acid and 0.9732 N iodine from a semi-micro burette.

(b) All oxidations at pH 4.1 and 20°C

TABLE XXIII

Oxidation of Spruce Wood at pH 4.1 and 20°C. (Fig.IV)

Oxidation	Iodine Titre(b) Initial	Final	$Na_3H_2IO_6$ Added(a)	$Na_3H_2IO_6$ Consumed
1	6.15 ml.	8.23 ml.	17.53 gm.	7.996 gm.
2	6.17	7.78	17.45	6.189
3	6.11	7.70	17.68	6.112
4	5.74	7.25	19.10	5.804
5	6.14	6.60	17.56	5.612
6	6.14	7.54	17.56	5.382
7	5.81	7.07	18.83	4.843
8	6.30	7.44	16.95	4.382
9	6.22	7.32	17.26	4.228
10	6.53	7.61	16.06	4.151
11	6.38	7.42	16.64	3.998
12	6.16	7.44	17.49	3.920
13	6.44	7.41	16.41	3.729
14	6.58	7.50	15.87	3.536
15	6.24	7.13	17.18	3.421
16	6.44	7.29	16.41	3.267
17	6.12	6.94	17.64	3.152

(a) As determined by analysis.

(b) Analysis with 5 ml. aliquots from a total volume of 1400 ml. using 0.1000 N arsenious acid and 0.0934 N iodine.

TABLE XXIV

Klason Lignin Content of Oxidized Wood in Various Stages of Oxidation (Fig.V)

Oxidation	Amount Withdrawn	Sample for Analysis	Residue	Percentage Klason Lignin
0				28.77%
5	0.7836 gm.	0.7593 gm.	0.2889 gm.	38.04
7	1.5295	1.4735	0.5972	40.53
10	2.0282	1.9585	0.8955	45.72
13	1.0680	1.0631	0.5579	52.48
17	1.0012	0.9983	0.6060	60.70

Analyses carried out on samples dried through solvent exchange and under vacuum in the standard manner.

TABLE XXV

Oxidation of Spruce Wood at pH 4.1 and 20°C in 200 ml. of Periodate Solution (Fig.VI)

Run No.1 (Fig.VI, plot 5)

Time (hours)	Iodine Titre[a] (ml.)	$Na_3H_2IO_6$ present gm.	$Na_3H_2IO_6$ consumed[b] by Sample gm.	moles	$Na_3H_2IO_6$ consumed per gram of Sample gm.	moles
0	7.59	0.865	0.000	0.0000	0.000	0.00000
6	8.08	0.541	0.323	0.0011	0.167	0.00057
12	8.25	0.429	0.435	0.0014	0.225	0.00076
24	8.46	0.290	0.564	0.0019	0.297	0.00101
48	8.71	0.125	0.739	0.0025	0.383	0.00130
72	8.80	0.086	0.778	0.0026	0.403	0.00137
96	8.82	0.052	0.812	0.0027	0.420	0.00143
120	8.90	0.000	0.865	0.0029	0.448	0.00152
Run No.2 (Fig. VI, plot 4)						
0	5.19	2.449	0.000	0.0000	0.000	0.00000
6	6.15	1.815	0.633	0.0061	0.317	0.00108
12	6.49	1.591	0.858	0.0029	0.430	0.00146
24	6.80	1.386	1.063	0.0036	0.533	0.00181
48	7.28	1.069	1.380	0.0046	0.692	0.00235
72	7.56	0.884	1.565	0.0053	0.784	0.00266
96	7.79	0.733	1.716	0.0058	0.861	0.00292
120	8.07	0.548	1.901	0.0064	0.953	0.00324
Run No.3 (Fig.VI, plot 3)						
0	2.24	4.397	0.000	0.0000	0.000	0.00000
6	3.58	3.512	0.884	0.0030	0.431	0.00146
12	3.99	3.242	1.155	0.0039	0.563	0.00191
24	4.48	2.918	1.479	0.0050	0.721	0.00245
48	5.23	2.423	1.974	0.0067	0.962	0.00327
73	5.85	2.014	2.383	0.0081	1.162	0.00345
96	6.28	1.730	2.667	0.0090	1.300	0.00442
120	6.64	1.492	2.905	0.0098	1.416	0.00481

TABLE XXV (Contd.)

Run No.4 (Fig.VI, plot 2)

Time (hours)	Iodine Titre[a] (ml.)	$Na_3H_2IO_6$ present gm.	$Na_3H_2IO_6$ consumed[b] by Sample gm.	moles	$Na_3H_2IO_6$ consumed per gram of Sample gm.	moles
0	7.75	6.636	0.0000	0.0000	0.000	0.00000
6	9.38	5.559	1.076	0.0036	0.544	0.00185
12	0.85	5.315	1.320	0.0044	0.668	0.00227
24	1.57	4.840	1.796	0.0061	0.909	0.00309
48	2.65	4.127	2.509	0.0085	1.270	0.00432
72	3.32	3.684	2.951	0.0100	1.494	0.00508
96	3.63	3.479	3.156	0.0107	1.598	0.00543
120	3.98	3.248	3.387	0.0115	1.715	0.00593

Run No.5 (Fig.VI, plot 1)

0	2.98	9.886	0.000	0.0000	0.000	0.00000
22	4.65	8.683	1.202	0.0040	0.627	0.00213
5	5.90	7.857	2.028	0.0068	1.057	0.00359
8	6.35	7.660	2.225	0.0075	1.160	0.00394
18.5	7.54	6.774	3.111	0.0105	1.622	0.00551
30	8.30	6.273	3.612	0.0122	1.884	0.00640
43.5	8.91	5.870	4.015	0.0136	2.094	0.00712
72	9.82	5.269	4.616	0.0155	2.407	0.00819

(a) Analyses on 5 ml. aliquots using 0.1123 N iodine and 0.1000 N arsenious acid. In the first three runs 10 ml. of arsenious acid was used but in the first two titrations of No.4 and all of No.5 20 ml. of arsenious acid was used.

(b) Corrected for amounts withdrawn in sampling.

TABLE XXVI

Progressive Oxidation of Spruce Wood (Fig.VII)

Oxidation	Iodine Titre Initial	Iodine Titre Final	$Na_3H_2IO_6$ Added	$Na_3H_2IO_6$ Consumed
1	6.13	9.32	4.3922	3.0659
2	6.25	8.93	4.2769	3.0558
3	6.18	8.80	3.7926	3.0089
4	5.86	8.42	3.5600	3.0299
5	5.86	8.16	3.5600	2.6413
6	5.80	6.81	3.6289	1.1598
7	5.90	6.80	3.5141	1.0336

Analyses for periodate carried out using 5 ml. aliquots and 10 ml. of 0.1000 N arsenious acid. The normality of the iodine for the first two oxidations was 0.0934 and in the remainder 0.1116.

TABLE XXVII

Progressive Oxidation of Spruce Wood[a] (Fig. VIII)

Oxidation	Iodine Titre[b] Initial	Final	Periodate Added	Periodate Consumed
1	3.70 ml.	8.85 ml.	34.18 gm.	34.18 gm.
2	3.48	8.85	35.65	35.65
3	3.58	8.85	34.98	34.98
4	3.40	8.80	36.18	35.91
5	3.74	7.22	33.92	23.14
6	3.98	5.39	32.32	9.38

(a) Oxidation of 101.9 gm. of wood meal with 2 litres of 1.7% periodate solution.

(b) Analyses employed 0.1131 N iodine and 10 ml. of 0.1000 N arsenious acid.

TABLE XXVIII

Progressive Oxidation of Spruce Wood(a) (Fig. IX)

Oxidation	Iodine Titre(b) Initial	Final	Periodate Added	Periodate Consumed
1	2.73 ml.	0.00 ml.	95.60 gm.	95.60 gm.
2	2.16	0.00	100.3	100.3
3	2.16	0.00	100.3	100.3
4	2.14	7.32	101.2	100.9
5	3.47	6.12	89.88	79.99
6	4.56	3.30	81.19	48.81

(a) Oxidation of 226 gm. of wood meal with 2 litres of 5% periodate solution.

(b) Analyses employed 20 ml. of 0.08427 N arsenious acid in initial titrations and 10 ml. in final titrations. Back-titrations with 0.1143 N iodine.

TABLE XXIX

Progressive Oxidation of Spruce Wood[a] (Fig. X)

Oxidation	Iodine Titre[b] Initial	Final	Periodate Added	Periodate Consumed
1	7.63 ml.	0.00 ml.	203.4 gm.	203.4 gm.
2	7.63	0.00	203.4	203.4
3	7.63	0.00	203.4	203.4
4	7.63	8.57	203.4	194.2
5	7.63	4.10	203.4	107.0

(a) Oxidation of 497.6 gm. of wood meal with 6 litres of 3.4% periodate solution.

(b) Analyses employed 0.1106 N iodine and 10 ml. of 0.1000 N arsenious acid.

TABLE XXX

Progressive Oxidation of Spruce and Hardwoods[a] (Fig. XI)

Oxidation	Iodine Titre[b] Initial	Final	Periodate Added	Periodate Consumed
Spruce				
1	9.80 ml.	9.00 ml.	20.3 gm.	19.4 gm.
2	9.80	7.57	20.3	16.7
3	9.80	6.64	20.3	14.5
4	9.80	3.72	20.3	7.3
5	9.80	2.60	20.3	4.6
Beech				
1	9.80	8.83	20.3	19.8
2	9.80	7.80	20.3	17.3
3	9.80	7.60	20.3	16.8
4	9.80	5.55	20.3	11.8
5	9.80	3.18	20.3	6.0
Birch				
1	7.42	7.42	26.0	26.0
2	7.42	7.08	26.0	21.2
3	7.42	6.10	26.0	18.8
4	7.42	0.90	26.0	6.1
5	7.42	8.84*	26.0	3.4
Maple				
1	7.42	0.00	26.0	26.0
2	7.42	7.22	26.0	23.8
3	7.42	7.14	26.0	21.4
4	7.42	3.10	26.0	11.5
5	7.42	9.65*	26.0	5.4

(a) Approximately 35 gm. of wood meal used in each case.

(b) Analyses employed 0.1106 N iodine. All initial and those final titrations marked with an asterisk used 20 ml. of 0.1000 N arsenious acid; elsewhere 10 ml. of arsenite was used.

TABLE XXXI

Oxidation of Periodate and Klason Lignin Specimens (Fig. XII)

Oxidation	Iodine Titre Initial	Iodine Titre Final	$Na_3H_2IO_6$ Added	$Na_3H_2IO_6$ Consumed
No. 1	Oxidation of periodate lignin (86.0% Klason lignin)			
1	3.57 ml.	4.94 ml.	7.0388 gm.	1.8092 gm.
2	3.55	4.64	7.0652	1.4395
3	3.55	4.30	7.0652	0.9904
4	3.55	4.18	7.0652	0.8320
5	3.55	4.10	7.0652	0.7263
No. 2	Oxidation of Klason lignin			
1	2.91 ml.	6.67 ml.	3.9552 gm.	2.4827 gm.
2	3.55	5.75	3.5326	1.4526
3	3.57	5.86	3.3194	1.3121
4	3.55	5.35	3.5326	1.1886
5	3.55	5.20	3.5326	1.0895
No. 3	Oxidation of periodate lignin (86.0% Klason lignin)			
1	3.77	4.88	3.3716 gm.	0.7382 gm.
2	3.90	4.75	3.2851	0.5653
3	3.77	4.49	3.3716	0.4799

(a) Analyses carried out on 5 ml. aliquots using 10 ml. of 0.1000 N arsenious acid and 0.1131 N iodine.

TABLE XXXII

High-pressure Hydrogenation of Spruce Periodate Lignin

Time	Pressure	Temperature	Moles of hydrogen in bomb
Hydrogenation No.1			
0 hours	1975 p.s.i.	295°A	3.06
1	2750	421	3.00
1.5	3100	463	3.06
2	3400	501	3.11
2.5	3550	523	3.10
3	3600	535	3.12
4	3425	532	2.94
6	3450	534	2.96
15	3215	533	2.76
17	3200	536	2.73
20	3200	535	2.74
Final	1550	294	2.41
			———
		Hydrogen absorbed	0.61 moles
Hydrogenation No.2			
0	2430	293	3.80
0.5	2900	344	3.86
1.5	3900	463	3.86
2	4200	495	3.88
3.5	4500	535	3.89
5.5	4500	534	3.88
10.5	4450	535	3.81
20.5	4150	533	3.56
Final	2130	294	3.32
			———
		Hydrogen absorbed	0.48 moles
		Total hydrogen absorption	1.09 moles

TABLE XXXIII

Fractionation(a) of Lignin Hydrogenation Products (Fig. XVII)

Fraction	Weight	n_D^{25}
1	0.2432 gm.	1.4123
2	0.2494	1.4131
3	0.2873	1.4133
4	0.2161	1.4139
5	0.2327	1.4139
6	0.2403	1.4142
7	0.2085	1.4150
8	0.2731	1.4159
9	0.2674	1.4160
10	0.2341	1.4166
11	0.2586	1.4165
12	0.2131	1.4167
13	0.2194	1.4166
14	0.1996	1.4166
15	0.2017	1.4167
16	0.2100	1.4165
17	0.1873	1.4167
18	0.1932	1.4167
19	0.1911	1.4168
20	0.1765	1.4169
21	0.1846	1.4167
22	0.1423	1.4263
23	0.1731	1.4493
24	0.1658	1.4520
25	0.1732	1.4538
26	0.1508	1.4597
27	0.1487	1.4623
28	0.1631	1.4627
29	0.1793	1.4627
30	0.1691	1.4626
31	0.1821	1.4627
32	0.1578	1.4626
33	0.1483	1.4626
34	0.1479	1.4626
35	0.1862	1.4626
36	0.1710	1.4625
37	0.1391	1.4626
38	0.1685	1.4627
39	0.1509	1.4627
40	0.1826	1.4627
41	0.1784	1.4626
42	0.1743	

TABLE XXXIII (Contd.)

Fraction	Weight	n_D^{25}
43	0.1699 gm.	1.4626
44	0.1664	1.4627
45	0.1607	1.4629
46	0.1799	1.4628
47	0.1586	1.4630
48	0.1327	1.4672
49	0.1673	1.4678
50	0.1596	1.4750
51	0.1473	1.4791
52	0.1704	1.4816
53	0.1637	1.4809
54	0.1290	1.4809
55	0.1418	1.4810
56	0.1376	1.4808
57	0.1511	1.4809
58	0.1408	1.4809
59	0.1211	1.4833
60	0.1468	1.4856
61	0.1429	1.4839
62	0.1388	1.4844
Total recovered	11.1661	

(a) The Cooke-Bower column was used.

CLAIMS TO ORIGINAL RESEARCH

(1) The composition of spruce lignin "in situ" has been calculated by a refined method from the composition of the extractive-free wood and its holocellulose fraction and is within ± 1% of; C, 67.5; H, 6; CH_3O, 14%.

(2) Both spruce wood and spruce Klason lignin have been shown to decrease in carbon content by 2 to 3%, based on the weight of lignin, on heating at 105°C. The loss of carbon is not dependent upon the presence of atmospheric oxygen and probably occurs by a slight thermal decomposition.

(3) Lignin has been isolated by a method involving oxidation of the extractive-free wood with aqueous solutions of periodate at pH 4.1 and 20°C, solution of the oxidized carbohydrates in boiling water near pH 7 and recovery of the insoluble "periodate lignin" residue by filtration. The yield of carbohydrate-free periodate lignin from sprucewood was nearly quantitative but those from three hardwoods ranged from 75 to 85% of theory. Much of the morphological structure of the original wood was preserved in the isolated lignin.

(4) Isolated spruce periodate lignin has been shown to duplicate spruce lignin "in situ" very closely in its behavior toward ethanol containing hydrogen chloride gas, toward high-pressure hydrogenation, toward alkaline oxidation with nitrobenzene and in insolubility toward

neutral solvents.

(5) It has been demonstrated that periodate lignins, to a greater extent than any other isolated lignin, also resemble the lignin in wood in their capacity to dissolve under the conditions of standard sulfite and alkali cooks. The ability to dissolve in sulfite liquor was lost when the periodate lignin was pre-treated with acid solution or with water at a high temperature.

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