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The alkaline oxidation of lignin

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THE ALKALINE OXIDATION OF LIGNIN

BY

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INTRODUCTION

The proof of structure of lignin as it occurs in plant tissue is rendered exceedingly difficult by the fact that it cannot be separated from the original plant tissue without modification. That there is modification is shown by the variation in the properties of the lignins isolated from the same plant material, as well as by the variation in properties of lignin from different plant materials, by different methods. A further difficulty encountered is that none of the isolated lignins can be purified by recrystallization since all are amorphous solids of high molecular weight. As a result of these difficulties, each investigator selects a lignin from a certain plant, isolated by a certain specified method. The results obtained and their interpretation differ widely from one another.

Since oat hulls and cornstalks contain a very high percentage of siliceous ash, the acid methods for the preparation of lignin, in which the cellulose and the pentosans are removed by acid hydrolysis, are not desirable for the preparation of these lignins. The alkali lignins, which are of technical importance in the preparation of cellulose pulp, are rather costly because the excess alkali cannot be recovered as such. Since ammonia is a weaker alkali, and since it is easily recovered by volatilization, its use for the preparation of alkali lignin was investigated. The structure of the ammonia lignin thus obtained was studied. As these structural studies

advanced, they gradually centered around the problem of step-wise oxidation of the lignin.

The oxidation methods generally applied to proof of structure are of little value in interpreting the structure of the original lignin, since these reagents either oxidize the substance to simple organic acids or to indefinite amorphous products of high molecular weight. A method of oxidation which could be applied quantitatively was desired. The data presented in this thesis show that, of the oxidation methods, the alkaline oxidation is best adapted for the study of the chemical constitution of ammonia lignin.

HISTORICAL REVIEW

General Definition of Lignin

Lignin may be defined from two points of view. To the botanist, it represents the woody or lignified cellular tissue appearing abundantly in the plant kingdom. By lignified cellular tissue, the botanist means those tissues which are insoluble in ammoniacal cupric hydroxide solution, which do not give the customary cellulose reactions, which are colored red with phloroglucinol-hydrochloric acid solutions, and which are colored yellow with aniline sulfate solution. Lignified cell walls are those which contain lignin.

Webster's New International Dictionary defines lignin as "a substance, or a mixture of substances which, with cellulose, constitutes the essential part of woody tissue. It is related physiologically to cellulose, but its chemical nature is not yet clear".

To the chemist, lignin represents the high-molecular weight, non-carbohydrate portion of the cellular tissue after it has been freed of tannins, resins, and similar secondary constituents. It is very resistant to the usual carbohydrate hydrolysis but is soluble in hot alkali sulfites with the formation of sulfonic acids. It can be further defined as that portion which contains methoxyl groups and approximately 20% more carbon than the carbohydrates. It would appear, therefore, from the high carbon content, that lignin represents a

highly unsaturated cyclic substance. From the fact that potassium hydroxide fusion yields protocatechuic acid and guaiacol, the lignin complex also appears to be a phenolic methyl ether.

Due to the fact that lignin cannot be isolated unchanged nor can it be crystallized, very little is known about the greater part of the molecule. Although much emphasis has been placed on certain functional groups, very little is known of others. It is impossible to state whether or not it is homogeneous. Just what changes occur during isolation are also unknown. From the standpoint of constitution, the following logical scheme of possibilities must be considered:

1. That lignin is a mixture

    a. of unrelated substances,

    b. of closely related substances.

2. That lignin is a chemical compound

    a. of aliphatic nature,

    b. of homocyclic nature,

    c. of heterocyclic nature,

    d. of a combination of any two or all three of the preceding.

In connection with the study of the structure and composition of lignin there arises the question of purity in isolation. This question of purity is an important factor, as the methods of removal of the lignin from the tissue include the

use of drastic reagents.

Methods of Preparation

Lignin may be removed from the plant tissue either by hydrolysis of the carbohydrates or by solution of the lignin. The strong mineral acid methods may be classed with the hydrolysis method, while the solution procedure can be subdivided into the alkali and the organic solvent methods. Other methods which have been developed are more or less a combination of the above procedures. The acid methods most commonly mentioned and described in detail in books treating on the chemistry of cellulose and wood are the 72% sulfuric acid and the 42% hydrochloric acid methods.

The principle of the sulfuric acid method is the solution of the carbohydrates under carefully regulated conditions of time, temperature and concentration of acid. The method is of technical importance in the determination of the relative amounts of lignin in woody tissues. If the temperature goes above 15°C. or the time is extended beyond eighteen hours or the acid concentration is more than 72%, carbonization of the hydrolyzed carbohydrates results. If, however, any one of the conditions is reversed, incomplete hydrolysis of the carbohydrates occurs. A set of conditions which will give a minimum yield of lignin is to be preferred. (39)(52).

The hydrochloric acid method for the preparation of lig-

nin involves the same principles as the sulfuric acid method. The two processes give yields of lignin which do not differ by more than one-half of one per cent. The hydrochloric acid method involves the use of a concentrated fuming acid (42% or sp. gr. 1.209). Since less carbonization occurs, this method is to be preferred for the preparation of lignin to be used for the study of constitution. However, the use of such a strong acid is inconvenient as the hydrolysis must often be repeated two or more times in order to completely remove the carbohydrates.

The use of these two methods for the preparation of lignin to be used in the study of structure has been criticized from the standpoint of purity, since both methods involve the use of strong dehydrating agents. Criteria of purity which have frequently been used give preference to a product which has the highest methoxy content, the lowest copper number, and which yields no furfural when distilled with 12% hydrochloric acid. Since lignin contains about 18% more carbon than do carbohydrates, the high carbon content has also been used as a criterion of purity. These criteria may seem to be sufficient to characterize the lignin as it appears in plant tissue, but the results are not in agreement with those of the alkali lignins.

The alkali methods depend primarily on solution of the lignin from woody materials by heating with caustic alkalies, or, in the case of less resistant tissues such as straws and

cornstalks, with alkali carbonates. The lignin and pentosans are dissolved, while the cellulose remains undissolved. The alkaline solution is filtered to remove the cellulose, and the pentosans are removed from the dissolved lignin by precipitation with methyl or ethyl alcohol. The remaining alkaline solution is neutralized with dilute acid in order to precipitate the lignin. Fractional precipitation by several methods was found to yield fractions which were identical in carbon content, methoxy content, pentosans, and copper number. However, these values usually do not agree with those obtained with the acid lignins.

In order to obtain an especially "pure" lignin, Freudenberg (14) has combined the acid and the alkali methods under less drastic conditions. His method consists of treating the tissue alternately with ammoniacal cupric hydroxide solution and 1% sulfuric acid at 100°C. He repeats the process several times to be certain that all the non-lignic substances are removed. The lignin obtained has the highest carbon and methoxy contents of all the methods mentioned. The lignin which is recovered, however, constitutes but one-fourth of the lignin in the original tissue.

The sulfonic acid lignins, which are formed in the technical production of paper from sulfite pulp have been studied by the Swedish investigator, Klason. Since most of the original work is published in the Swedish journals, it was necessary to have recourse to the summaries of his work as found in

the publications of Fuchs (15), Kürschner (36), Hess (24), Schorger (48), and Abderhalden (1). The yields are not over 30% of the total lignin removed, so use of these lignins for the proof of structure has been severely criticized. The methoxy and carbon contents, after correcting for the presence of the sulfonic acid group, were found to be quite constant for different preparations, but they are somewhat different from the values obtained for other lignins.

From the fact that chlorine dioxide reacts with lignin by addition, Erik Schmidt has perfected a quick method for the quantitative determination of lignin by merely weighing the sample before and after chlorination. The method is, however, of little use, either technically or theoretically.

The use of neutral organic solvents for the preparation of lignin has been studied quite thoroughly. Some hydroxy compounds are capable of dissolving lignin quite easily; a catalyst is necessary in every case.

Phenols, in the presence of 0.1% of strong mineral acid, will dissolve lignin at 100°C. The solvent power of phenol is undoubtedly due to chemical combination with the lignin; a compound may be assumed to be formed, because the carbon content of the product is increased and the methoxy content lowered. This fact has been verified by Hillmer (30), who used various substituted chloro- and bromophenols. The amount of halogen present in the isolated lignin varied according to the conditions used, but approximately one atom of halogen from

the halogenated phenols entered the lignin complex for every
1000 units of molecular weight.

Glycerol resembles the phenols in its behavior toward
lignified tissue. Its action on spruce wood has been studied
by Hibbert and his co-workers (28)(29) using glycerol at 110°C.
with hydrochloric acid as a catalyst. Only about one-third of
the lignin in spruce wood was removed by this procedure. There
was apparently some reaction between the glycol and the lig-
nin, since some ethylene glycol can be removed by acid hydroly-
sis. A considerable portion of the product was composed of
pentosans. After purification by acid hydrolysis, the glycol
lignin was soluble in cold, one per cent sodium hydroxide, and
insoluble in sodium carbonate solution; it reduced Fehling's
solution, and was shown to be free of pentosans by Tollen's
phloroglucinol hydrochloride method.

These methods of removing lignin from plant tissue repre-
sent only the best methods now in use. Many modifications of
these methods exist, but they have not been generally accepted
by lignin chemists. The chief objection to their use is that
the purity of the lignin obtained is very doubtful.

Concepts of Structure

A number of formulae have been presented for the struc-
ture of lignin. Each investigator has used methods and reac-
tions which he has considered necessary and valid for the proof

of structure. These workers used lignins isolated from different plant material by several different methods, and each having slightly different, but characteristic, properties.

A reasonable explanation of some of the differences in the formulae proposed is that the lignins isolated might not be pure. In each case, only a few reactions of limited significance were performed to prove the purity of the lignin being studied. The following illustrate the tests of purity of lignin: Fractional precipitation of alkali lignin gives fractions having approximately the same carbon, hydrogen and methoxy contents. For other lignins, some workers consider the product with the highest methoxy content to be pure. Others consider several reprecipitations to be sufficient to remove the impurities present. Some use the non-reduction of Fehling's solution as an indication of purity, meaning thereby the absence of pentosans and hydrolyzed sugars. The absence of furfural by Tollen's phloroglucinol method for pentosans has also been used to indicate that the lignin is free from extraneous material. Some consider the absence of sugars in the filtrate of a lignin treated with acid to indicate purity. Even the fact that the color of the isolated lignin was the same as the original tissue has been used as an additional criterion of purity. In almost every case, no mention was made of the fact, one which cannot be neglected, that, since an organic reaction has taken place, decomposition products may be present in the prepared lignin. For this reason, too much emphasis should not

be placed on the postulated structures which will be given below. Some of the differences in structure may be due to the removal or modification of some constituent in the lignin complex during isolation. The lignin in any plant may be composed of several types of molecules, some of which might be removed by one kind of treatment, and others by different treatments.

The preceding explanations are given merely to show the difficulties and errors entering into the study of the structure of lignin. Only a few of the many variations reported are presented. There is a great body of literature pertaining to lignin chemistry, yet investigators cannot agree on what constitutes the greater part of the lignin molecule. For this reason, the methods by which the following formulae were derived will not be given.

Willstätter and his co-workers isolated lignin from spruce wood by 42% hydrochloric acid hydrolysis of the carbohydrate material present (55). They treated the lignin and also cellulose with a mixture of iodine and red phosphorus (53), and from the elementary composition of the reduced products, concluded that carbohydrates and lignin are very similar in chemical composition.

In the light of the results obtained by Wilstätter and Zechmeister (55), Schrauth (50) assumes that, since carbohydrates give hydroxymethylfurfural with strong acids, lignin may be formed by the condensation of three molecules of 5-hydroxy-

methylfurfural to give a benzophenanthrene derivative,

$C_{18}H_{18}O_g$:
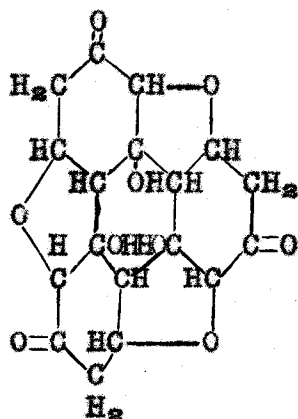
In a study of the structure of sulfonic acid lignins of spruce wood, Dorée and Hall (9) found the following groups to be present in the empirical formula:

$$C_{21}H_{18}O_2 \begin{cases} SO_3H \\ (OH)_2 \\ CHOH \\ CHO \\ CH_2OH \\ (OCH_3)_2 \end{cases}$$
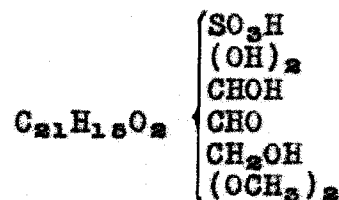
Cross, Bevan and Beadle (6) have submitted the following general or schematic constitutional formula as typical for the lignin of spruce wood. They drew their conclusions from the formation of acetic acid in the hydrolysis, and from the results of their study of the chlorinated lignin.
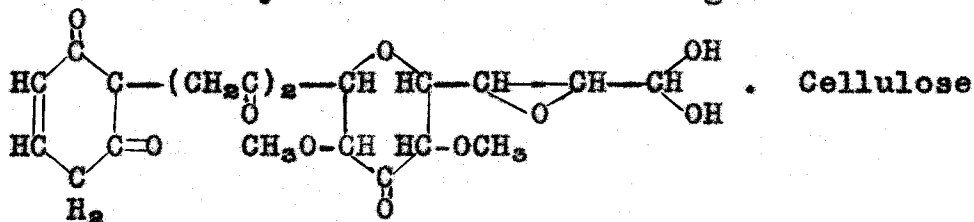
Fuchs and Daur (16), in a recent study, have expressed the constitution of lignin in the plant tissue in the following way:

$$\text{C}-\text{OCH}_3 \quad \text{H} \quad \text{H}$$
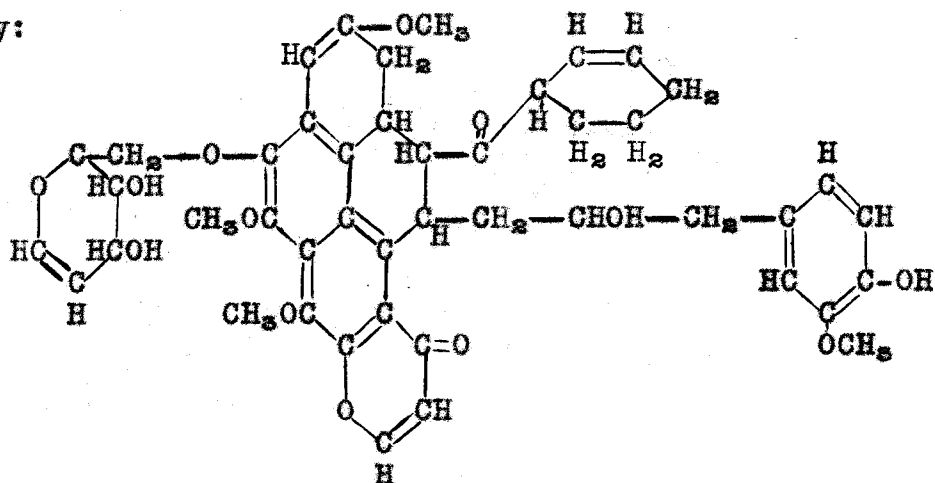
Herzog and Hillmer (22)(23) have tried to correlate the absorption spectra of known compounds with that of lignin. Their data support a structure closely related to those presented by Klason (33) and Freudenberg (14).

Kürschner (36) believes that the lignins consist principally of vanillin-like groups. He reports, and has patented, several processes for recovering vanillin from lignin.

Hawley and Harris (21) treated Cross and Bevan cellulose at 125°C. for several days and obtained an insoluble product closely resembling natural lignin in physical and chemical properties. Methoxyl groups, however, were absent in their synthetic product.

On extracting sulfite liquor from spruce wood with ether, Holmberg (31) obtained a crystalline compound which he found to be diguaiacyl tetramethylenecarbinol carboxylic acid:

$$HOC \begin{matrix} H & H \\ C=C \\ | & | \\ C & CH \\ | \\ OCH_3 \end{matrix} C - \overset{H}{\underset{}{C}} - \overset{H}{\underset{}{C}} - CH_2OH$$
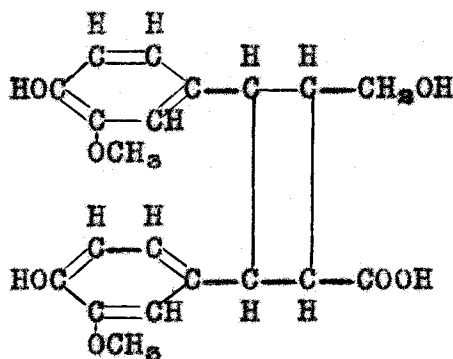
The yields, however, were less than one per cent of the total lignin present. This compound may have been derived from some extraneous material present in the wood.

Klason's general formula has been slightly modified from time to time to agree with experimental data. Klason has studied the sulfonic acid lignins of spruce wood, and he considers lignin to be a condensation product of coniferyl aldehyde. The following formula is one of a dozen or more slightly modified structures which he has proposed (33):
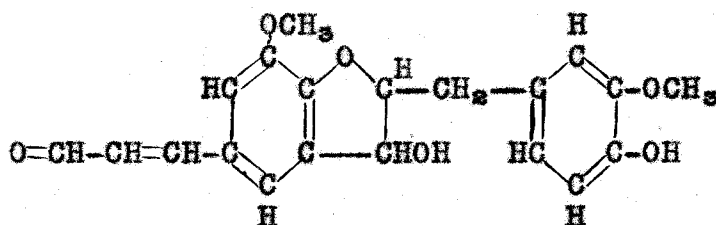
Freudenberg and co-workers (14) have made a very careful study of spruce lignins and have represented the structure of this lignin by the following general scheme:

9-10-11
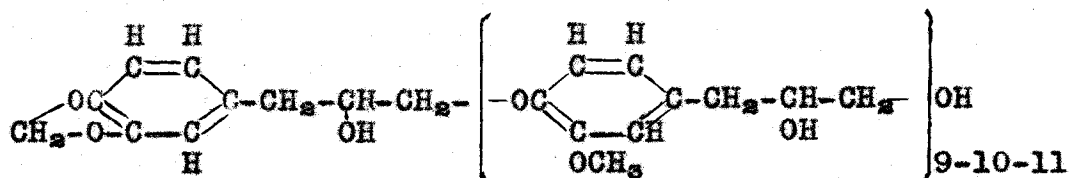
The most recent and best evidence that lignin is of an aromatic nature is given by Phillips and Goss (41) who have oxidized their ethylated alkali lignin with 5 N nitric acid and have obtained p-ethoxybenzoic acid. This fact shows that phenolic rings were present in the lignins studied.

Review of Literature

Powell and Whittaker (42) made a careful study of the uniformity of alkali lignin from flax by fractional precipitation with hydrochloric acid. They could obtain no difference in methoxyl content, or carbon and hydrogen contents of the different fractions. Freudenberg (14), on the other hand, believes that there can be no assurance of the uniformity of alkali lignin. He believes that carbonyl groups and phenolic groups do not exist in lignin, and that decomposition products of the carbohydrates are incapable of separation from the lignin.

Alkali lignins have been found to contain almost as high a methoxyl content as acid lignins: That of rye straw ranged from 14.5% to 15.8% (4); that of flax lignin, 14.90% (42); and that of spruce lignin, 15.5% (44). These results are in close agreement with the methoxyl contents calculated from the determination of the lignin and methoxyl contents of the acid-hydrolyzed tissue.

Hägglund (20) has investigated the quantitative oxidation

of lignin with Fehling's solution, and reported a copper number of 7.9. This value could not be decreased, even by hydrolysis of the lignin with concentrated hydrochloric acid. By using this method, Powell and Whittaker (42) calculated that there was one aldehyde group present for every 830 units of molecular weight in lignin.

The alkaline oxidation of lignins with potassium permanganate yields only acetic and oxalic acids, according to the work of Heuser and Samuelson (25). Hibbert and Marion verified these results in their study of glycol-lignin.

Pringsheim and Magnus (45) treated wood with eight per cent sodium hydroxide solution in the cold, and obtained 3.5% of acetic acid. They found that xylan yielded no acetic acid under the same conditions, so concluded that the acetic acid had its origin in the lignin of the wood.

König and Rump (34) treated lignins with an ammoniacal solution of hydrogen peroxide in the presence of lime water, and found that the lignin complex was gradually destroyed. Small amounts of acetic, formic and carbonic acids were obtained. Other oxidation products could not be isolated. By oxidation of alkali lignin with an alkaline solution of hydrogen peroxide, Anderzen and Holmberg (2) obtained 1.0-1.6% of acetic acid, some malonic and a trace of succinic acid.

The simplest form of the alkaline oxidation of lignin is its fusion with potassium hydroxide in the presence of air. According to Heuser and Winswold (27), 16% to 19% of proto-

catechuic acid, 2.0% of catechol, some oxalic acid and lignin acids were obtained by the potassium hydroxide fusion of lignin at 240°C. Below 240°C., the principle product of fusion is an indefinite substance called lignin acid. In the presence of a catalyst such as iron, the proportions of protocatechuic acid and catechol were almost completely reversed. The catechol was assumed to result from the decomposition of protocatechuic acid by the loss of carbon dioxide.

Various other substances have been reported in traces from the oxidation of lignin. Fischer (10) reported the presence of benzene pentacarboxylic acids in lignin oxidized under pressure in the presence of caustic soda. Fischer, Schrader, and Friedrich (11) reported the presence of additional acids: benzoic, phthallic, isophthallic, mellitic, trimellitic, succinic, oxalic and fumaric acids. Such products are of little value, however, for the determination of the constitutional nature of lignin, since they may result from combination of molecules of entirely different substances at the temperature of the alkaline fusion.

In chlorinating alkali lignins from wheat straw, Powell and Whittaker (43) observed that hydrogen chloride was liberated when the chlorination was carried out in carbon tetrachloride. The product contained 5.2% of methoxyl and 35.1% of chlorine. However, when this product was dissolved in alkali and reprecipitated with acid, the chlorine content decreased to 20.8%. Although no explanation was offered for the removal

of chlorine by aqueous alkali solution, it is in agreement with
the experimental data presented in this thesis for the presence
of a methyl carbinol in lignin.

Cross and Bevan (5) observed that treatment of brominated
lignin with alkali partially removed the bromine. They report
that iodine was without chemical reaction on lignin (5), al-
though they had previously observed that iodine was loosely
bound to the extent of 12.9% to 13.3% in jute lignin obtained
by the treatment of jute fibers with $\underline{N}/10$ iodine solution.
Treatment with $\underline{N}/50$ solution of iodine left 6% of loosely bound
iodine.

Nitration of lignin has been accomplished in two ways. In
the method of Fischer and Tropsch (12), the lignin was treated
with 5 $\underline{N}$ nitric acid at about 80°C. until the product became
uniformly orange-colored. The yields were low, because a con-
siderable amount of oxidation takes place along with the nitra-
tion. Because of the ease of nitration, lignin was concluded
to have a phenolic structure. In the second method, that of
Powell and Whittaker (42), concentrated nitric acid alone, or a
mixture of equal parts of concentrated nitric and sulfuric
acids, at 0°C. was used as the nitration medium. In each case,
approximately one-half of the methoxyl content was removed. The
nitrogen content of the product was 4.5%. The nitrolignins were
reduced with zinc and hydrochloric acid, and the reduced pro-
duct was found to contain no nitrogen.

Practically all of the different kinds of isolated lignins

have been methylated. Heuser, Schmidt and Gunkel (26), in methylating Willstätter lignin, found that a product containing 24.5% of methoxyl could not be dissolved in hot alkali solution. Several other workers have methylated lignins and have found that the methylated products all had methoxyl contents of 23% to 28%. Holmberg and Wintzell (32) methylated two separate lignin fractions with different methoxyl contents and found that the methylated products contained within 0.2% of the same amount of methoxyl. Powell and Whittaker (42) obtained a methylated lignin containing 25.6% of methoxyl. Diazomethane does not methylate alkali-insoluble lignins, according to Freudenberg (14).

Alkali lignin has been demethylated by Holmberg and Wintzell (32) by use of hydroiodic acid (sp.gr. 1.7) at 130°C. A dark powder was obtained, which was soluble in alkali. A more thorough study of this reaction was made by Beckmann, Liesche and Lehmann (3). Their preparation was found to be free from methoxyl groups, but contained chemically bound iodine.

According to the work of Dorée and Hall (9), lignin sulfonic acid has the following formula:

$$C_{21}H_{18}O_2 \begin{cases} SO_3H \\ (OH)_2 \\ CHOH \\ CHO \\ CH_2OH \\ (OCH_3)_2 \end{cases}$$
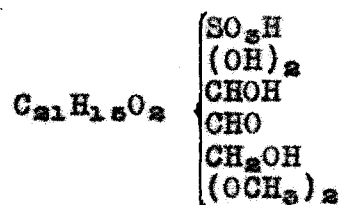
After oxidizing the lignin sulfonic acid with 5% nitric acid, they believed that one of the methoxyl groups had been removed,

that the primary alcoholic and the aldehyde groups had been oxidized to carboxylic groups, that the secondary alcoholic group had been converted into a ketone, and that two nitro groups had been introduced.

EXPERIMENTAL

Preparation of Ammonia Lignin from Cornstalks

There are two methods for the preparation of lignin by the use of aqueous ammonia. The method to be used depends on the kind of material to be treated and upon the type of lignin to be studied.

In the first method, 100 g. of mechanically separated cornstalk fibers (38) were treated in a bomb (38) with 1000 cc. of aqueous ammonia (sp.gr. 0.90) for eight hours at a pressure of 100 pounds per square inch and a temperature of 110°C. After cooling, the contents of the bomb were removed to a four-liter beaker, filtered twice through an eight-inch Büchner funnel; a tightly woven cloth filter was used for the first filtration and a cloth filter covered with regular filter paper for the second filtration. The composition of the remaining residue is given by Peterson (38).

The filtrate, containing lignin and pentosans in solution, was evaporated to approximately 500 cc. Sufficient concentrated sulfuric acid was then added to the filtrate to give a four per cent solution of the acid. Hydrolysis of the precipitated pentosans was carried out according to the method of Morrow (37). An open beaker, instead of a reflux condenser, was used in the hydrolysis, as frequent stirring was necessary to prevent decomposition due to superheating at the bottom of the vessel. After three hours of hydrolysis, the lignin was filtered off,

washed several times with hot water until the wash water was free of the sulfate ion, and dried for two hours at 105°C. Pentosans in lignin: 0.84% - 0.85%. Table I shows the results obtained in five separate cooks.

The pentosans were determined by the phloroglucinol method of Schorger (48).

TABLE I.

ANALYSIS OF CRUDE PULP AND AMMONIA LIQUORS
FROM CORNSTALK FIBERS

Num- ber	Sample Weight in g.	Crude Pulp Weight in g.	Pentosans in Crude Pulp, %	Dried Liquor in g.	Lignin Obtained in g.
1	100	70.0	17.5	29.6	12.0
2	100	73.2	18.5	26.2	10.5
3	100	75.0	20.5	24.0	10.0
4	100	73.0	----	25.9	10.5
5	100	72.2	----	27.4	11.0

Preparation of Ammonia Lignin from
Oat Hulls

Approximately 3000 g. of dry oat hulls were covered, in a steam heated stone crock, with a 0.6 $\underline{N}$ solution of hydrochloric acid and hydrolyzed for four hours at 90°C. The hydrolyzed hulls were then transferred to a large stone filter and washed

with tap water until free of chlorides. Nearly one-half of the
original weight of the material disappeared on hydrolysis. In
order to treat a large amount of material with ammonia, a
special iron bomb was constructed. It is heated by an inner
coil connected with the high-pressure steam line. The air
dried, hydrolyzed oat hulls were transferred to the bomb and
digested with 1:1 ammonia (one part by weight of concentrated
aqueous ammonia of sp. gr. 0.90 to one part by weight of water),
and heated at 100 pounds pressure for eight hours. While the
digester is still hot, most of the ammonia is recovered by con-
densing the vapors in cold water. The digester was cooled al-
most to room temperature and the remaining ammoniacal liquor
was drained off from the bottom, at once, into a stone filter.
The residue was washed twice with water and the liquors all
combined. The liquor was filtered through cloth on a Büchner
funnel. The lignin in the liquor was recovered by one of two
methods: by evaporation of the solution to dryness under re-
duced pressure, or by warming to about 50°C. and precipitating
the lignin with hydrochloric acid. Sulfuric acid was not used
in place of hydrochloric acid because any adsorbed sulfate in-
terferes with the determination of the methoxy groups present
by the Zeisel method.

The quality of the lignin prepared depends, to a large ex-
tent, on the conditions of precipitation from the ammoniacal
liquor and on the technique of filtration. After the lignin
has been properly precipitated, it is allowed to settle for

several hours, preferably until settling is complete. The super-
natant liquid is very carefully decanted or siphoned off. The
lignin precipitate remaining is filtered by suction and washed
with water until free from chlorides. The lignin is then trans-
ferred to a large watch glass and dried at about 40°C. until it
loses its plastic character. The drying may be completed by
heating at 105°C. for two hours. Yields, 135 - 150 grams.
Methoxy content: 12.5% - 13.1%.

Properties of Ammonia Lignin

Ammonia lignin is a light brown, fluffy, amorphous powder.
It is insoluble in alcohol, water and five per cent sodium ace-
tate solution, and very slightly, if at all, soluble in five
per cent sodium carbonate solution from which it may be com-
pletely precipitated by saturation with carbon dioxide. A
characteristic property of freshly-prepared lignin (a character-
istic of all alkali lignins) is that at 60°C. or above it soft-
ens in water. If the dry lignin is allowed to stand for a
month or longer, it gradually loses this property until it no
longer melts in boiling water. The dry lignin melts with de-
composition at 180°C. The oat hull lignin contained 3.7% ash
whereas the cornstalk lignin contained only 0.1% ash.

Although the dry ammonia lignin is apparently insoluble in
cold water, it cannot be quantitatively recovered. The follow-
ing procedure illustrates the difficulty encountered in purify-

ing lignin by reprecipitation: 0.5 g. of ammonia lignin were
dissolved in 25 cc. of 1.0 $\underline{N}$ sodium hydroxide. The solution
was allowed to stand for two hours at 60°C., then cooled to
room temperature and the lignin precipitated with a slight ex-
cess of 1.0 $\underline{N}$ sulfuric acid. The lignin was allowed to settle,
then was filtered on a weighed Gooch crucible, washed free of
sulfates and dried at 103°C. The amount recovered from two
samples was 0.4081 g. and 0.4372 g. or 81.6% and 87.4% respec-
tively.

The Alkaline Oxidation of Lignin

The oxidation of an alkaline solution of lignin with chlorine.

The lignin is dissolved in an excess of 1 $\underline{N}$ alkali, solu-
tion taking place much more readily when the lignin is previous-
ly dispersed thoroughly in a few cubic centimeters of water.
Chlorine gas is slowly introduced into the alkaline solution of
lignin, taking care to prevent the solution becoming acid,
since chlorine substitution immediately occurs in acid medium.
As the oxidation proceeds, the oxidized lignin gradually becomes
soluble and it remains in solution when the solution is acidi-
fied. Considerable evolution of carbon dioxide was observed
when the solution was acidified. In addition, an unreactive
gas was evolved, and the alkaline solution had a characteristic
odor.

The oxidation of an alkaline solution of lignin with bromine.

The oxidation of an alkaline solution of lignin with liquid bromine is carried out in essentially the same manner as with gaseous chlorine. The oxidation apparently does not proceed as far with bromine as it does with chlorine. The alkaline solution of oxidized lignin had a very penetrating odor. When the reaction mixture is acidified, there is an orange red precipitate. The amorphous product was filtered on a fluted filter. A white crystalline solid appeared to be intermingled with the lignin precipitate. To isolate and identify this white crystalline product, the following procedure was carried out: Approximately five grams of ammonia lignin prepared from oat hulls were treated in a 500 cc. Erlenmeyer flask with an alkaline solution of bromine. The solution was allowed to stand for one hour; if the dark solution had not changed to a light straw color, more alkaline bromine solution was added, and the solution allowed to stand for another hour. The reaction mixture was steam distilled and the distillate condensed in a flask packed in ice. Snow white crystals appearing in the distillate were isolated and identified as carbon tetrabromide, of melting point 92°C. The carbon tetrabromide was found to be insoluble in water, acids, and cold, dilute sodium hydroxide; alcoholic silver nitrate precipitated silver bromide on warming, and alcoholic potassium hydroxide yielded potassium bromide and potassium carbonate. According to Dehn (7)(8), the course of the reaction is given by the following equations:

$$R(CHOH)CH_3 \text{ and } RCOCH_3 \xrightarrow{\text{NaOBr}} RCOOH + CHBr_3 + NaOH$$

$$CHBr_3 + NaOBr \longrightarrow NaOH + CBr_4$$

Konschegg (35) isolated carbon tetrabromide from an alkaline oxidation of humin and humic acids with sodium hypobromite.

The oxidation of an alkaline solution of lignin with iodine.

When an alkaline solution of lignin was oxidized with iodine under the conditions for the quantitative determination of acetone (19), it was observed that a large amount of oxidation occurred. A systematic investigation of the effect of time and temperature on the oxidation showed that the reaction was quantitative under slightly different conditions from those used in the determination of acetone. The following table shows the affect of time on the amount of oxidation. The sample in each case weighed 0.1500 g. Approximately three minutes' time was required for the addition of the potassium iodide-iodine solution from a burette; the time interval for the oxidation was begun one and one-half minutes before the total volume of iodine solution had been added. The titration of the blank was equivalent to 38.50 cc. of 0.1100 $\underline{N}$ $Na_2S_2O_3$.

TABLE II.

THE EFFECT OF TIME ON THE OXIDATION OF AMMONIA LIGNIN FROM OAT HULLS

Methoxy 13.1 %
Ash 3.65%

Sample	Time of Oxidation	Excess $Na_2S_2O_3$ cc.	cc. 0.110 $\underline{N}$ $Na_2S_2O_3$ cc.	cc. $\underline{N}/10$ Iodine per g. of lignin cc.
1	14.5 min.	13.90	24.60	180.5
2	23.5 "	13.50	25.00	183.5
3	31.5 "	13.35	25.15	184.5
4	36.5 "	12.85	25.65	188.0
5	54.5 "	12.85	25.65	188.0
6	65.0 "	12.85	25.65	188.0
7	18.5 hrs.	12.85	25.65	188.0
8	68.0 "	12.85	25.65	188.0
9	92.0 "	12.90	25.60	187.8

The oxidation of ammonia lignin from oat hulls at 75°C. for two hours gave inconsistent results. Three samples were oxidized and the following values, calculated in cc. of $\underline{N}/10$ iodine per gram of lignin, were obtained: 175.3, 176.4, 178.5.

Several samples of lignin were treated with an acid solution of iodine, and practically no oxidation occurred. The oxidation values were equivalent to 0.2 cc. of $\underline{N}/10$ iodine per gram of lignin.

In addition to the oxidation, it was observed that a small amount of glistening, yellow plates appeared shortly after oxidation. The following procedure for isolating this solid was carried out: Five grams of ammonia lignin were treat-

ed with a concentrated solution (containing 25 g.) of sodium
hydroxide. The solution was cooled to room temperature and
slightly more than the theoretical amount of iodine which can
be reduced was added (8.5 g.), with constant shaking. The solu-
tion was allowed to stand for one hour with occasional shaking,
and was then transferred to a separatory funnel and extracted
twice with alcohol-free ether. The ether was evaporated and
the residue dissolved in alcohol or a mixture of alcohol and
petroleum ether and a solid was fractionally crystallized. The
properties of the solid so obtained were compared with those of
a known sample of iodoform, and the two were found to be iden-
tical. Mixed melting points likewise showed no lowering. The
iodoform was also characterized by a phenyl isonitrile deriva-
tive.

Microscopical examination of the crystals of iodoform ap-
pearing in the alkaline solution after the iodine oxidation
showed them to be identical in form to those from a known sam-
ple of iodoform.

The above procedure was considered necessary because these
crystals were prepared by a method independent of any organic
solvent, such as ethyl alcohol, or any other organic substance
which will produce iodoform. Melting points, mixed melting
points and the isonitrile derivative showed that the crystal-
line product observed after the oxidation was iodoform.

To determine approximately how much iodoform was present,
5.0 g. of lignin were oxidized under conditions similar to those

used in the quantitative oxidation of lignin. The iodoform
crystals were allowed to settle over night. The solution was
decanted and that remaining was filtered through an alundum
crucible. The precipitate was washed free from iodides, then
dissolved in alcohol. The amount of iodoform present in the al-
coholic solution was determined quantitatively by precipitating
the iodine from the iodoform by use of silver nitrate, and weigh-
ing as silver iodide. Five grams of lignin gave 0.1526 g. of
silver iodide, which is equivalent to 0.0851 g. of iodoform.
Since the solubility of iodoform in water is approximately 0.01 g.
per 100 cc. at 25°C., only about half of the iodoform from the
reaction is converted to silver iodide.

The preparation of oxidized lignin.

Oxidized lignin may be prepared by two different methods,
which give products having the same properties. The first
method was used to show that the ammonia treatment in the prep-
aration of ammonia lignin was not the cause of the formation of
lignin in the oxidation procedure.

The first method was as follows: Approximately 450 g. of
ground acid-hydrolyzed oat hulls containing 27.46% lignin were
treated with one liter of 25% sodium hydroxide. One hundred
ninety-two grams of iodine and 315 g. of potassium iodide were
dissolved in some water and diluted to approximately 2.5 liters.
The potassium iodide-iodine solution was added slowly over an

hour's time to the alkaline oat hull mixture. The total volume
was approximately four liters. This mixture was allowed to
stand over night. A known amount of the mixture was tested for
the presence of free iodine by acidifying the solution. As
soon as the oxidation was complete, the solution was filtered
by suction, using a Büchner funnel containing a double cloth
filter, and was washed twice with water. The wash water was
added to the filtrate. The filtrate was acidified with hydro-
chloric acid, and the precipitated oxidized lignin filtered by
suction. The precipitate was washed free from iodides; this
washing required nearly a day's time, since the product was
amorphous. When the solution is acidified with hydrochloric
acid, very little free iodine should be present, as it tends to
be adsorbed on the precipitated oxidized lignin. On duplicate
determinations, the methoxy content was found to be 7.25% and
7.23% by the Zeisel method (56); the ash content was 0.57% and
0.50%.

The following procedure was used for the second method:
Ten grams of ammonia lignin prepared from oat hulls (methoxy
13.1%) were dissolved in about 400 cc. of 1.0 N sodium hydrox-
ide. The theoretical quantity of 0.2 N iodine solution was ad-
ded and the solution was allowed to stand for two hours, after
which it was acidified with 1.0 N sulfuric acid. The precipi-
tated lignonic acid was filtered by suction and washed free
from sulfates, the latter process requiring approximately two
days. The product was dried as described in the preparation of

ammonia lignin. Yield, 8.5 g. Analysis, 7.4% and 7.6% methoxy
and 0.1% ash. Chlorine water liberated no iodine from the oxi-
dized product.

TABLE III.

COMPARISON OF PROPERTIES OF OXIDIZED LIGNIN
WITH THOSE OF ORIGINAL LIGNIN

Property	Lignin	Oxidized Lignin
Solubility		
in water ....:	Insoluble	Insoluble
in 5% NaOAc ....:	Insoluble	Insoluble
in dilute acids ......:	Insoluble	Insoluble
in dilute alkalies ...:	Soluble	Soluble
in Na_2CO_3 ....:	Insoluble	Soluble
in $NaHCO_3$ ....:	Insoluble	Soluble
Melting point		
of dry material ......:	Decomposes at 180°C.	Decomposes at approximately 190°C.
in water ....:	Softens at 60°C.	No softening, even in boiling water.
Fehling's solution .....:	Copper reduced.	Copper not reduced
KI-I_2 solution		
in acid ....:	No oxidation	No oxidation
in alkali ....:	Oxidation	No oxidation
$FeCl_3$-$K_4Fe(CN)_6$ ....:	Precipitate of Turnbull's Blue	No effect
Sodium fusion ....:	No iodine	Iodine test
Alkali-soluble product :		
Effect NaCl ....:	No precipitate	Precipitate of Na-salt
Methylated product		
Per cent methoxy .....:	26.0%	22.2%
Soluble in NaOH ......:	Insoluble	Soluble
Per cent methoxy in al-:		
kali-solution products:	——	17.4%

Oat hull lignin prepared by the caustic alkali method (40) was found to have practically the same oxidation value as that prepared by the ammonia method. Iodoform was also characterized in the same manner as in the oxidation of ammonia lignin.

Preparation of Lignin Derivatives and Their
Alkaline Oxidation

Methylation of Lignin and Its Quantitative Oxidation.

The methylation was carried out by the method described by Powell and Whittaker (42) and successfully used by Phillips and Goss (41). Five grams of lignin were added to 100 g. of 10% sodium hydroxide solution and the reaction mixture heated to 60°C. under a reflux condenser. The solution was stirred mechanically while 32 g. of dimethyl sulfate were added slowly from a dropping funnel. After all the dimethyl sulfate had been added, the stirring and heating were continued for a half hour. The reaction product was removed by filtration and the methylation repeated. After the second methylation, the reaction product was filtered and washed free from sulfates with distilled water. The product was dried in the oven at 100°C. Yield, approximately five grams. Analysis: 25.94% and 26.02% methoxy. The alkaline-iodine oxidation values were equivalent to 6.92 cc. and 7.09 cc. of 0.1 $\underline{N}$ iodine solution per gram of sample.

Nitration of oat hull lignin and methyl lignin.

Five grams of lignin were added, in small portions, and
with constant stirring, to a mixture of equal parts of nitric
and sulfuric acids cooled to 0°C. The mixture was kept at 0°C.
for one hour, then poured into ice water. The precipitated
nitro compound was washed free from acid and dried at 50°C.
The resulting nitrolignin is a red powder, partially soluble
in acetone or alcohol, soluble in sodium hydroxide, and may be
acetylated readily. Methoxy content 4.69% and 4.73%. The ox-
idation equivalent with an alkaline iodine solution could not
be determined accurately since no definite end point could be
obtained. The values obtained were equivalent to 19 cc. to
48 cc. of 0.1 $\underline{N}$ iodine solution per gram of nitro-lignin. Io-
doform was present in the alkaline-iodine oxidized solution.

Methyl lignin containing 25.98% methoxy was nitrated by the
method described above. Nitromethyl lignin is a red powder,
slightly soluble in alcohol and only partially soluble in sodium
hydroxide solution. Found: 15.37% and 15.42% methoxy and
8.00% and 8.15% nitrogen.

Reduction of ammonia lignin from oat hulls with HI.

Ten grams of lignin were treated with 40 cc. of constant
boiling hydroiodic acid (57% b.p. 127°C.) in a 200 cc. round bot-
tom flask and refluxed for two hours. An oil bath was used to
keep the temperature at 130°C. At the end of the reaction, the

product was cooled, poured into approximately 200 cc. of water and filtered immediately by suction, washing several times with distilled water. The wash water was tested for free iodine with a starch solution, in order to make sure that the reduced lignin contained no excess iodine, which would interfere with the alkaline iodine oxidation. The precipitate was washed into a one-liter beaker with dilute alkali and reprecipitated with hydrochloric acid. The precipitate was allowed to settle over night, then was filtered by suction and washed free from chlorides with distilled water. Approximately two days were required for this filtration and washing. The precipitate was transferred to a large watch glass and dried at room temperature. As soon as it was dry enough to crumble, it was ground fine in a mortar and finally dried for two hours at 103°C.

The reduced product was black in color and contained considerable amounts of chemically combined iodine which was detected by heating in a test tube with concentrated sulfuric acid. Upon oxidation of the reduced product with alkaline iodine solution, iodoform crystals appeared in amounts similar to those appearing in the original lignin alkaline iodine oxidation.

A quantitative alkaline iodine oxidation was made, and the oxidation values obtained were found to be at least as great or greater than the values for the original lignin. The end point could not be determined on account of the dark color of the reduced lignin interfering with the sharp color change of the

starch-iodine solution. The oxidation equivalents ranged from 180 to 225 cc. of 0.1 N iodine solution per gram of sample.

Elimination of the iodoform reaction by means of aniline and the alkaline oxidation of the resulting product.

Three grams of ammonia lignin from oat hulls were treated with 11 cc. of freshly distilled aniline and heated in a small balloon flask at 160°C. for two hours. The mixture was cooled, treated with chloroform to precipitate the lignin, and the lignin filtered off by suction. The precipitate was washed thoroughly with dilute hydrochloric acid to free it of excess aniline, and dried by the usual procedure, first in air and then in an oven at 105°C. for eight hours. Oxidation with an alkaline iodine solution did not yield iodoform, since iodoform crystals could not be identified by microscopical examination of the oxidized solution, and since the iodoform odor was absent. The oxidation values were 170 cc., 173 cc. and 180 cc. of 0.1 N iodine solution per gram of material tested.

The effect of dilute acid on the oxidation value of ammonia lignin from oat hulls.

Eight and five-tenths grams of oat hull lignin were hydrolyzed at 60°C. with 100 cc. of 0.5 N sulfuric acid for 20 hours. The hot solution was filtered and the filtrate neutralized with

calcium carbonate. The calcium sulfate and the excess carbonate
were removed by filtration, and the filtrate was evaporated to
10 cc. The solution was partially decolorized with charcoal
(Norite) and the filtrate tested in a polariscope for sugars.
No rotation was observed in the solution, and no osazone was
formed when a sample was treated with phenylhydrazine acetate
in acetic acid.

The precipitate remaining after the acid hydrolysis was
thoroughly washed and oxidized with an alkaline iodine solution
in the usual manner. The oxidation values were equivalent to
181.0 cc. and 183.5 cc. of 0.1 $\underline{N}$ iodine solution.

Oxidation of ammonia lignin from oat hulls with Fehling's solution and the alkaline oxidation of the oxidized product.

Two 0.250 g. samples of ammonia lignin from oat hulls were
oxidized by Fehling's solution by the Schaffer-Hartmann method
(51). The cuprous oxide was filtered and weighed by the ordinary
gravimetric procedure. The amount of cuprous oxide obtained was
0.0325 and 0.0353 g. corresponding to a dextrose equivalent of
5.42% and 5.48%.

Approximately five grams of the lignin were oxidized in
the manner described above. After the oxidation, the solution
was cooled and the cuprous oxide filtered off; the solution was
acidified with sulfuric acid. The precipitated lignin was sep-
arated by filtration with suction, and washed on the filter with

distilled water until the wash water was free from sulfates. The oxidized lignin was transferred to a large watch glass and dried in the usual manner: first in the air and then in an oven at 100°C. for two hours. A 0.2 g. sample of the dried product was analyzed qualitatively for copper and only a trace was found. Oxidation values on the oxidized product obtained by alkaline iodine oxidation were equivalent to 153.1 cc., 153.6 cc. and 154.1 cc. of 0.1 $\underline{N}$ iodine solution per gram of sample. Crystals of iodoform were found in the oxidized solution.

Alkaline iodine oxidation of sulfuric acid lignin of oat hulls, spruce and aspen.

Oat hull lignin, spruce lignin and aspen lignin were prepared by the 72% sulfuric acid method of Peterson, Walde and Hixon (39). Acid hydrolyzed hulls were used in the preparation of the oat hull lignin. Both the spruce wood and the aspen wood samples were ether-alcohol-extracted according to Schorger (49) in order to remove resins and gums. The oxidation values for oat hull lignin were calculated on the ash-free basis and those for the spruce and aspen on the ether-alcohol extracted basis.

TABLE IV.

OXIDATION VALUES OF OAT HULL, SPRUCE AND ASPEN LIGNIN PREPARED BY SULFURIC ACID METHOD

Lignin		Oxidation Value, in cc. of 0.1 N Iodine per gram
Oathull	I	156.5
	II	160.8
	III	161.5
Spruce	I	66.6
	II	70.2
Aspen	I	112.1
	II	111.8

Iodoform crystals were identified microscopically in the alkaline oxidized solution of each of these three lignins. The odor of iodoform was also detected.

Cold alkali extracts from acid hydrolyzed oat hulls and ether-alcohol extracted spruce and aspen woods each gave small precipitates of iodoform when oxidized with iodine in the alkaline solution.

Methylation of oxidized oat hull lignin.

The oxidized lignin was methylated twice with dimethyl sulfate as has been previously described for the methylation of lignin. Five grams of oxidized lignin yielded approximately five grams of the methylated product. The oxidized lignin con-

tained 7.23% of methoxyl before methylation, and 22.19% methoxyl
after methylation. The methylated product was insoluble in
cold, 10% sodium hydroxide, but when warmed to approximately
80°C. it began to go into solution. At 90°C. it was completely
soluble in the alkali in less than one minute. The alkali-sol-
uble product was reprecipitated with dilute sulfuric acid and
washed free from sulfates. Analysis, 17.42% and 17.36% methoxy;
10.18%, 10.46% and 10.75% iodine.

The Effect of Dilute Acid Hydrolysis on the Alkaline Iodine Oxidation Value of Several Plant Tissues

In order to oxidize quantitatively the alkali-insoluble
plant tissues, the regular alkaline iodine procedure was slight-
ly modified: The samples were prepared as described by Peter-
son, Walde and Hixon (39). The acid hydrolysis was made with
1.0 $\underline{N}$ hydrochloric acid at ten pounds pressure for six hours.
The time of oxidation was increased to four hours and the end
point in the titration was considered as the point at which the
starch solution remained colored for five minutes. The lignin
was determined by the modified 72% sulfuric acid method (39);
the methoxy content was determined by the Zeisel method (56),
and the oxidation values were based on the dry material analyzed.
The following table shows the results for five different plant
tissues.

TABLE V.

THE EFFECT OF ACID HYDROLYSIS ON THE ALKALINE-IODINE OXIDATION EQUIVALENT, LIGNIN CONTENT AND METHOXY CONTENT OF SEVERAL PLANT TISSUES.

Tissue	Oat Hull %	Rye Straw %	Flax Straw %	Arti- choke Stalks %	Corn- Stalks %
ORIGINAL TISSUE					
Lignin ....:	19.07	21.04	22.87	23.99	24.74
Methoxy ....:	2.62	3.51	3.47	4.51	3.11
Calc. Methoxy in Lignin:	13.74	16.68	15.17	18.80	12.57
$N/10$ Iodine Equivalent,:					
cc./gram sample ....:	16.56	27.58	17.90	18.42	39.90
$N/10$ Iodine Equivalent,:					
cc./gram Lignin ....:	86.80	131.10	78.30	76.80	161.30
HYDROLYZED TISSUE					
a.Unhydrolyzed Tissue.:	52.54	57.38	60.85	56.22	50.90
Lignin ....:	27.46	33.86	34.53	37.23	43.79
Methoxy ....:	3.77	4.92	4.92	6.01	4.34
Calc. Methoxy in					
Lignin ....:	13.73	14.53	14.25	16.14	9.91
$N/10$ Iodine,					
cc./gram sample ..:	47.71	57.11	35.52	51.85	71.50
$N/10$ Iodine,					
cc./gram Lignin ..:	171.20	168.60	103.90	139.20	163.30
b.Calc. on Basis of					
Original Tissue					
Lignin ....:	14.43	19.43	21.01	20.93	22.29
Methoxy ....:	1.98	2.82	2.99	3.38	2.21
LOSS DUE TO HYDROLYSIS					
Lignin ....:	4.64	1.61	1.86	3.06	2.45
Methoxy ....:	0.64	0.69	0.48	1.13	0.90
Methoxy in Lignin ...:	0.01	2.15	0.92	2.66	2.66
FRACTION OF TOTAL					
HYDROLYZED					
Lignin ....:	24.33	7.65	8.13	12.75	9.90
Methoxy ....:	24.40	19.60	13.80	25.10	28.90

DISCUSSION OF RESULTS

For preparation of lignin by the ammonia process, the second method, involving preliminary acid hydrolysis, was found to be much more satisfactory for obtaining a uniform, light-colored lignin. The first method, in which acid hydrolysis followed the alkaline treatment, was originally used for the preparation of cornstalk pulp, but was found to be inconvenient for the preparation of lignin because the pentosans present peptized the lignin and prevented its precipitation. Since the freshly-precipitated lignin fuses at about 70°C., the pentosans cannot be hydrolyzed without precipitating the lignin in a resinous mass from which it is difficult to wash the acid, the ammonium salts and the pentoses. In the second method, the pentosans are removed in the hydrolysis preceding the ammonia cook. The removal of these pentosans increased the relative amount of lignin recovered from the hydrolyzed material.

Apparently part of the less-resistant lignin also disappeared upon hydrolysis. It was found that hydrolysis of the oat hulls for four hours with a 0.6 $\underline{N}$ hydrochloric acid solution was sufficient to hydrolyze approximately 45% of the total weight of the oat hulls. The reducing sugars formed by hydrolysis gave a maximum reducing value.

The use of an alkaline solution of chlorine was found to be of little value in the study of the oxidation of lignin since the oxidation did not have a definite end point, and the products of the oxidation were usually simple acids which have lit-

tle bearing on the structure of the original lignin. Then, too, when the solution was acidified to precipitate the oxidized products, the excess chlorine liberated produced indefinite halogen-substituted products containing as much as 40% of chlorine. Approximately one-half of the chlorine was removed from such chlorinated compounds by dissolving in alkali.

An alkaline solution of bromine was found to react with lignin much more slowly than chlorine in alkaline solution, but much more rapidly than iodine. The products resulting from the oxidation were similar to those from the chlorine oxidation, and contained as high as 50% of bromine. Approximately two-fifths of the bromine was removed from the compound by dissolving it in alkali. The carbon tetrabromide isolated was undoubtedly formed as a secondary product from the oxidation of bromoform.

An alkaline solution of iodine was found to react quantitatively with lignin to form a quite definite, oxidized product. Although the product may not have been uniform in composition, the methoxy contents were approximately the same for lignins prepared from different ammonia cooks. The methoxy contents were the same as that of oxidized lignin prepared directly from acid-hydrolyzed hulls (without the ammonia treatment) by the action of an alkaline solution of iodine.

One of the general criticisms applied to alkali lignins is that during the preparation, the alkalies cause a marked change in the properties and structure. The use of ammonia for the

preparation of lignin from oat hulls has apparently not affect-
ed the lignin in two of its properties: The iodine equivalent
of the lignins in the acid hydrolyzed oat hull is of very near-
ly the same value as that from the prepared ammonia lignin. The
methoxy contents of the oxidized lignin were likewise the same
by either method.

The use of cold, dilute alkali and iodine on acid hydro-
lyzed oat hulls makes possible the preparation of a definite
oxidized product which has not been subjected to any drastic re-
agents. Such a preparation is to be desired for a study of the
structure of lignin.

Oxidation at higher temperatures proved to be unsatisfac-
tory. The oxidation values were inconsistent, and lower than
the values found at room temperature. The iodoform resulting
from the oxidation was destroyed at the higher temperatures.

The formation of the iodoform was probably due to a second-
ary alcoholic group which can be oxidized to a methyl ketone.
Although sugars give the iodoform reaction when heated with an
alkaline iodine solution, the presence of sugar-like substances
could not be detected in the prepared lignin. If the amount of
oxidation occurring in the lignin were entirely due to the pres-
ence of sugars, the calculated amount of sugar present in the
lignin would exceed 180% of glucose. It is at once evident that
such a condition cannot exist.

The reduction of Fehling's solution has frequently been
used to indicate the presence of sugars accompanying the lignin.

This property has recently been found to be characteristic of the alkali lignins (47). The formation of iodoform from lignin oxidized by Fehling's solution would indicate, although it is not conclusive proof, that an aldehyde group is present in addition to the group producing the iodoform. Powell and Whittaker (42) claim that there is one aldehyde group present in flax stem lignin to every 800 units of molecular weight. However, the amount of copper reduced does not parallel the loss in the oxidation value as determined by iodine.

The reaction of an alkaline iodine solution on aldehydes is as follows:

$$R—\underset{O}{\overset{}{C}}—H + 2NaOH + I_2 \longrightarrow R—\underset{O}{\overset{}{C}}—OH + 2NaI + H_2O$$

This reaction has been used for the quantitative determination of sugars by Willstätter and Schudel (54).

The methylation of lignin indicated clearly that the points of oxidation were definitely centered around the hydroxyl groups. The oxidation equivalent fell from 188.0 cc. of $N/10$ iodine for lignin to 7.0 cc. of $N/10$ iodine for methylated lignin. Since the iodination of lignin was prevented, the group taking part in the formation of iodoform must have been changed during methylation in such a way that it could no longer form iodoform.

The general reaction of an alkaline iodine solution with a phenol is as follows:

$$\text{C}_6\text{H}_5\text{OH} + \text{NaOH} + \text{I}_2 \longrightarrow \text{C}_6\text{H}_4(\text{I})\text{OH} + \text{NaI} + \text{H}_2\text{O}$$
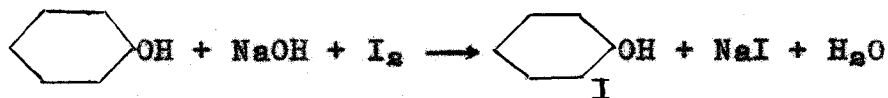
The introduction of iodine into the lignin molecule probably takes place in a similar manner, since Phillips and Goss (41) have shown that alkali lignins have phenolic groups. Another possible explanation, one which is frequently given for the alkaline bromine or chlorine oxidation of aromatic derivatives is as follows:

$$\text{R}-\underset{\overset{|}{\text{OH}}}{\text{CH}}-\text{CH}_3 + \text{NaOH} + \text{I}_2 \longrightarrow \text{R}-\underset{\overset{\|}{\text{O}}}{\text{C}}-\text{CH}_3$$

$$+ \text{NaI} + \text{H}_2\text{O}$$

$$\xrightarrow{\text{NaOI}} \text{R}-\underset{\overset{\|}{\text{O}}}{\text{C}}-\text{CI}_3 + \text{NaI} + \text{H}_2\text{O}$$
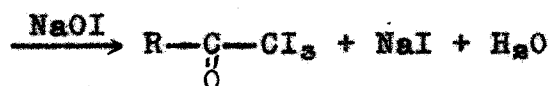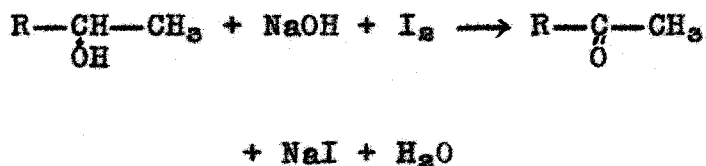
It is known that when the ortho positions are substituted by groups which cause steric hindrance, the trihalogenated ketone tends to be stable (13)(17)(18), though acetophenone, which is unsubstituted, yielded iodoform when subjected to alkaline iodine oxidation under the usual conditions.

The methoxy groups are generally considered to be aromatic ethers. As iodophenols are formed when free phenolic hydroxyl groups are present, the iodine equivalent of a lignin which had been demethylated with hydroiodic acid should be increased considerably. Although the quantitative results were not very accurate, the iodine equivalents for the demethylated product were somewhat higher than those of the original lignin. The

demethylation apparently caused a certain amount of decomposition
to take place, as well as side reactions, as the demethylated
product was quite dark in color and contained chemically bound
iodine. Beckmann, Liesche, and Lehmann (3) reported iodination
of lignin during demethylation with hydroiodic acid.

Since it was suspected that decomposed sugars of some sort
might be present, an attempt was made to find out the effect of
acid hydrolysis of ammonia lignin on the iodine equivalent. If
such reducing substances were present in the lignin to any great
extent, the iodine equivalent should be decreased considerably,
since sugars have much smaller reducing power per gram than does
lignin. If hydrolyzable material were causing the formation of
iodoform, the latter would not occur upon oxidation of a hydro-
lyzed product which had been washed free of reducing material.
The iodine equivalent decreased only about 6 cc. per gram of
lignin, and iodoform was found in the oxidized solution. This
is in agreement with the fact that traces of sugars could not
be detected, either with a polariscope, or by phenylhydrazine
(as an osazone).

A few quantitative tests were made on other lignins and it
was found that the oxidation value varied according to the
method used for its preparation and on the kind of plant from
which it originated. Oat hull lignin prepared by the 72% sul-
furic acid method was found to have a lower iodine equivalent
than that prepared by the alkali process. Spruce and aspen lig-
nins prepared by the 72% sulfuric acid method gave oxidation

values much lower than lignin from oat hulls; however, iodoform was identified in the alkaline oxidized solution of each.

Since methylation prevented alkaline iodine oxidation, it must be concluded that the action is associated with the free hydroxy groups; the question immediately arises as to whether all hydroxy groups might take part in the alkaline oxidation. When lignin was first oxidized and then methylated, the methoxy content was found to increase from 7.4% in the oxidized lignin to 22.19% in the methylated oxidized lignin, or correcting for the presence of iodine in the lignin complex, 24.8% of methoxy group. This is almost equal to the value, 26.0%, for the unoxidized methylated lignin. Such an increase in the methoxy content shows that most of the hydroxyl groups had not been changed. It would appear, therefore, that the alkaline oxidation had not greatly changed the hydroxyl content of lignin.

Since the methylated oxidized lignin was soluble in sodium hydroxide solution at approximately 90°C., in contrast to methylated lignin (not oxidized), which is insoluble in boiling sodium hydroxide solution, it must contain a methyl ester. The saponified product is soluble in cold alkali. The methoxy content was found to be decreased, from 22.19% to 17.39%. This decrease represents the portion of the lignin molecule which can be saponified.

By assuming that there is one carboxyl group to the molecule of oxidized lignin, the average minimum molecular weight, as calculated from the decrease in methoxy content during sa-

ponification, is 600. But from the assumption that there is one
iodine atom to each molecule of the oxidized lignin complex, the
molecular weights are calculated to be 1180, 1210, and 1245.
Since the ratio of molecular weight from iodine content to that
from methoxy decrease is two to one, it is evident that there
are two carboxyl groups present to every atom of iodine.

The solubility of the methylated oxidized lignin in alkali
and the presence of the carboxyl group show that the secondary
alcohol group belonged to the lignin molecule, otherwise the
product would not have been totally soluble in alkali.

One of the peculiarities of the oxidation is that the meth-
oxy content decreased from 12.5% to approximately 7.4%. Of
course, the increase in iodine alone would have decreased the
methoxy content about 1.1%. This fact supports the previous
conclusion that the methoxy groups are not all similarly joined
in the lignin molecule.

The oxidation value of nitrolignin cannot be ascertained
quantitatively, since no definite endpoint could be determined.
However, the presence of iodoform in the alkaline solution in-
dicated that the group yielding iodoform was not destroyed dur-
ing the nitration.

If the 72% sulfuric acid method is a criterion of the
amount of lignin in plant tissues, then the results of dilute
acid hydrolysis of several different plant tissues show that
apparently some less-resistant lignin is hydrolyzed.

The amount of lignin disappearing on hydrolysis varies with

the kind of tissue hydrolyzed. The decrease in methoxy content upon hydrolysis does not parallel the decrease in lignin content as determined by the 72% sulfuric acid method. This discrepancy could be explained by the presence of pectic substances which contain methoxyl groups and which are easily hydrolyzed by dilute acids. The oxidation values of the acid-hydrolyzed tissues were generally higher than those of the unhydrolyzed tissues. If the alkaline iodine oxidation equivalent is calculated for the lignin in the plant tissue, the values are of the same order of magnitude as those for the isolated lignin. This fact can be used as evidence that a large oxidation equivalent for isolated lignin is not due to impurities.

SUMMARY

1. Lignin can be prepared from oat hulls and corn stalks by the use of a volatile alkali. Acid hydrolysis, preceding rather than following the alkaline treatment, is recommended.

2. The alkaline halogen oxidation of ammonia lignin has been studied. The alkaline iodine oxidation proceeds quantitatively and forms a definite product, containing iodine and carboxyl groups. What appears to be the same compound can be formed by first treating oat hulls by mild acid hydrolysis, washing out the hydrolyzed carbohydrates and then oxidizing the tissue with a dilute alkaline iodine solution. Since the oxidized lignin can be formed by the latter method, which is independent of any drastic treatment, it seems probable that the oxidized lignin has not undergone any extensive rearrangements.

3. Iodoform has been isolated as one of the oxidation products of the alkaline oxidation of lignins prepared by the acid, the caustic alkali and the ammonia methods.

4. Carbon tetrabromide was isolated as one of the oxidation products resulting from the oxidation of lignin with an alkaline bromine solution.

5. Since methylation of lignin prevented alkaline oxidation and the formation of iodoform, and since alkaline oxidation produced a carboxyl group, a secondary alcoholic group of the type $CH_3CH(OH)-$ is probably present.

6. Methylated lignin, nitrolignin, hydroiodic acid-demethyl-

ated lignin, and lignin oxidized with Fehling's solution have been oxidized with an alkaline iodine solution.

7. No sugar-like bodies could be found in the ammonia lignin.

8. Acid hydrolysis of plant tissues removes part of the less resistant lignin and part of the methoxy groups and increases the iodine equivalent. The reactions of lignin and the methoxy contents do not parallel each other. The iodine equivalent in the acid hydrolyzed tissue is of the same order of magnitude as that of the isolated lignin.

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