[CONTRIBUTION FROM FOREST PRODUCTS LABORATORY,[1] FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Some Chemical Reactions of Maple and Spruce Lignin

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The methods used for the study of the chemistry of wood lignin have differed principally in the procedures used for the isolation of lignin. These may be divided into two general classes: the removal of the carbohydrates and extractives, leaving the lignin as a residue, and the alteration of lignin chemically so that it may be separated from the carbohydrates by treatment with certain solvents. All methods are open to the criticism that changes occur during the isolation, producing properties and chemical reactions not found in the original lignin. If, however, these changes are of a superficial and not a fundamental nature, they may aid rather than hinder the development of the problem, provided of course that the nature and extent of the changes can be determined. Lignin which has been subjected to the action of active oxidizing agents or heated in the presence of acids or alkalies may be fundamentally changed. The action of cold 70 to 72% sulfuric acid[2] on extractive-free wood, however, has been shown to produce quantitatively a lignin free from carbohydrates.

It has been observed that the hardwoods differed from softwoods in lignin content. Therefore both types were studied in order to determine the differences in the lignins isolated from them. Sugar maple and white spruce were chosen as representatives of the two types.

Comparison of Sulfuric Acid Lignin with Lignin Prepared by Other Methods.—In considering the different methods used for the preparation of lignin, various investigators[3,4,5,6] have stressed the importance of retaining all the methoxyl, hydroxyl, acetyl or methylene oxide groups in the original wood lignin, however small the effect produced by their loss would be on the lignin molecule, but have neglected to consider the fact that some of the carbohydrates had not been removed and that their method was not quantitative. The preparation at the Forest Products Laboratory of hydrochloric acid lignin by the revised methods of Hägglund[7] failed to give a product free from cellulose as determined by the Cross and Bevan method. The yield of lignin was 1 to 1.5% higher than that obtained by the modified sulfuric acid method, showing that because of the low solubility of cellulose in hydrochloric acid, this reagent was incapable of effecting a complete removal of the carbohydrate fraction in the time specified. Also the methoxyl content of the hydrochloric acid lignin was low, showing some splitting of the ether linkage.

Lignin prepared by the cuprammonium method from several different kinds of wood was found to contain in each case a residue of Cross and Bevan cellulose. The yields were low, due to the solubility of lignin in the reagents used, and therefore it was impossible to study any of the quantitative relationships between isolated lignin and native lignin by the use of this method.

Lignin prepared by the older sulfuric acid methods has been subjected to many criticisms. Paloheimo[8] stated that sulfuric acid produced charring. This was overcome by controlling the conditions for the isolation.[2] Euler[9] objected to the sulfuric acid method because it would split off acetic acid. Ritter and Kurth[10] have prepared a carbohydrate fraction in which they were able to account for all the acetyl groups in the wood and therefore it was not necessary to consider this objection. Freudenberg[11] found that under certain conditions acid treatment split formaldehyde from compounds containing the methylene oxide grouping and suggests that the sulfuric acid may remove methylene oxide groups during the isolation of lignin. A determination of methylene oxide groups in sulfuric acid lignin according to the method of Freudenberg and Harder[5] gave for spruce lignin 1.6% and for maple 0.8%. These values are slightly higher than those reported by Freudenberg[11] on pine and beech wood.

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[1] Maintained at Madison, Wis., in coöperation with the University of Wisconsin.

[2] E. C. Sherrard and E. E. Harris, *Ind. Eng. Chem.*, **24**, 103 (1932).

[3] E. Hägglund, *Cellulosechemie*, **4**, 73 (1923); *Biochem. Z.*, **207**, 1–7 (1929).

[4] P. Klason, *Cellulosechemie*, **4**, 81 (1923).

[5] K. Freudenberg and M. Harder, *Ber.*, **60B**, 581 (1927).

[6] H. Hibbert, *Pulp and Paper Mag. Can.*, International No., 125–127 (1928); *Can. J. Res.*, **2**, 357 (1930).

[7] E. Hägglund and H. Urban, *Biochem. Z.*, **207**, 1–7 (1929).

[8] L. Paloheimo, *Biochem. Z.*, **165**, 463 (1925).

[9] A. C. von Euler, *Cellulosechemie*, **4**, 1 (1923).

[10] Geo. J. Ritter and E. F. Kurth, "Holocellulose, The Total Carbohydrate Fraction of Extractive-free Maple Wood," *Ind. Eng. Chem.*, **25**, 1250 (1933).

[11] K. Freudenberg and F. Sohns, *Ber.*, **66B**, 262–269 (1933).

To determine the action of fuming hydrochloric acid and cold sulfuric acid on the methoxyl groups of lignin, samples of methylated lignin were treated at the Forest Products Laboratory with cold 70% sulfuric acid and 42% hydrochloric acid. In the sulfuric acid none of the methoxyls were lost, while in hydrochloric acid, methylated maple lignin lost 2.6% of its methoxyl. These observations indicate that lignin produced by 70% sulfuric acid under carefully controlled conditions is satisfactory because it is obtained in quantitative yields, it is free from carbohydrates, and so far as constituent groups are concerned it is little changed.

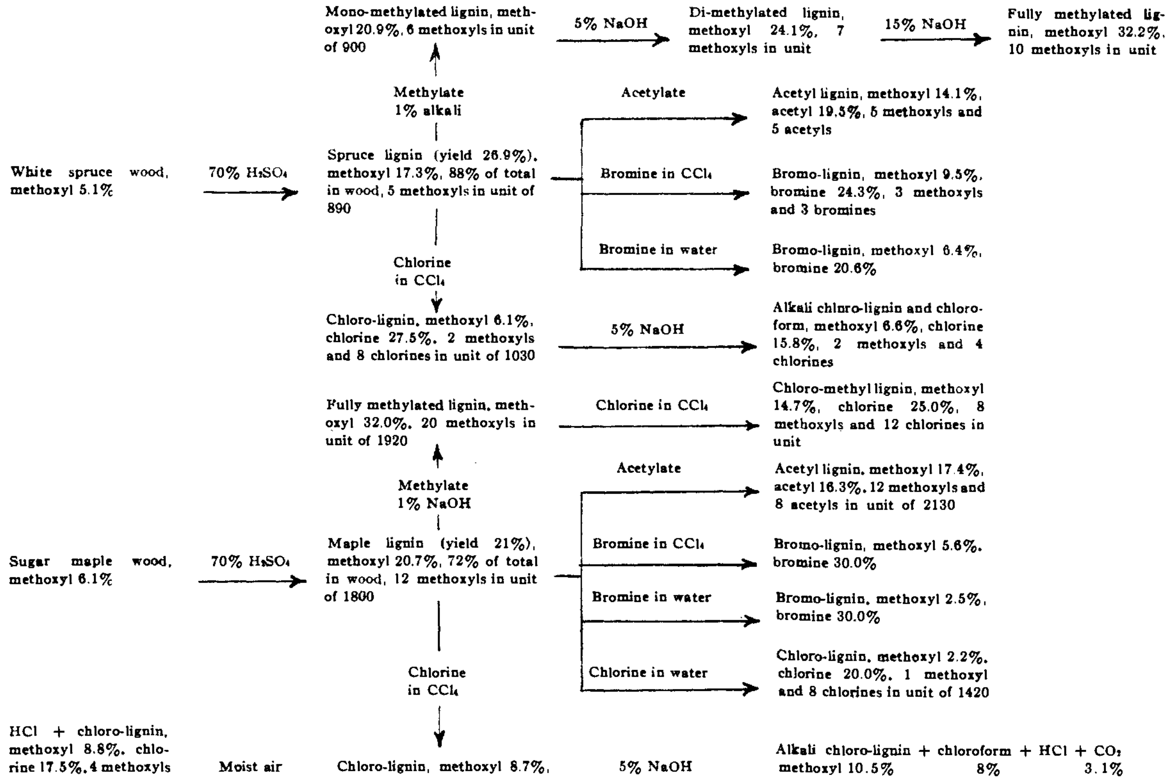

Fig. 1.—Reactions of spruce and maple lignins.

Reactions of Spruce and Maple Lignin.—The results of methylation, acetylation, bromination and chlorination are given in Fig. 1. The unit molecular weight of 890 was chosen for spruce after a study of the composition of spruce lignin derivatives had been made. When the isolated spruce lignin, containing 17.3% methoxyl, was methylated in 1% alkali, a methylated derivative with 20.9 methoxyl was obtained. If one methoxyl was introduced by this reaction the molecular weight of this methylated derivative would be about 860. The second stage of methylation in 5% alkali gave a derivative with 24.1% methoxyl. If this increase over the methoxyl content of 17.3 was due to the introduction of two methoxyls the molecular weight of the dimethylated derivative would be about 910.

The values found are in best agreement if the isolated lignin, monomethylated, dimethylated, and fully methylated lignin contain 5, 6, 7 and 10 methoxyl groups, respectively. This would require a weight of about 890 for the isolated spruce lignin. A slight error in the methoxyl determinations may easily account for the differences found. These values for the number of methoxyl groups suggest that spruce lignin obtained by the sulfuric acid method must be similar to that obtained by the action of alcohols in the presence of an acid.[12]

The unit weight for maple was determined in a similar manner. A study of other lignin derivatives made it necessary to use a value about double the unit weight obtained for spruce. Maple lignin chlorinated in water gave a product which,

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[12] F. Brauns and H. Hibbert, *Pulp and Paper Mag. Can.*, **34** 187–188 (1933).

when the methoxyl content is calculated as one per molecule, gave a molecular weight of approximately 1400. Chloro-maple lignin obtained from the chlorination of maple lignin in carbon tetrachloride lost hydrogen chloride, on exposure to moist air, corresponding to one molecule of hydrogen chloride from a molecular weight of 1440. Work in the future may make it necessary to use multiples of these weights. The unit molecular weights shown are calculated from the percentage composition of the lignin derivative. A comparison of the values found with the values calculated from a molecular weight of 890 are given in Table I.

TABLE I

A COMPARISON OF THE METHOXYL AND ACETYL CONTENT OF SPRUCE LIGNIN PREPARED BY DIFFERENT METHODS

	Type of spruce lignin	Found, %		Calcd. from a mol. wt. of 890 for isolated spruce lignin			
		OCH_3	Acetyl	OCH_3 groups	OCH_3, %	Acetyl groups	Acetyl, %
I	Isolated lignin	..	..	5	17.4		
	Willstätter lignin	14.2					
	Cuprammonium lignin[5]	17.0					
	Glycol lignin[a,b]	17.7					
	Sulfuric acid lignin[2,c]	17.3					
II	Monomethylated lignin	..	..	6	20.4		
	Glycol lignin[a]	22.2					
	Cuprammonium lignin[5]	21.0					
	Sulfuric acid lignin[c,2]	20.9					
III	Fully methylated lignin	..	..	10	32.24		
	Glycol lignin[a]	33.1					
	Cuprammonium lignin[5]	32.0					
	Sulfuric acid lignin[c,2]	32.15					
IV	Acetylated lignin	..	..	5	14.05	5	19.5
	Glycol lignin[a]	14.7					
	Sulfuric acid lignin[c,2]	14.0	19.5				

[a] The values published in Ref. 12 have been corrected for glycol content so that the lignins may be compared on the same basis. [b] B. Rassow and H. Gabriel, *Cellulosechemie*, **12**, 290 (1931). [c] This paper.

As shown in Fig. 1, the methylation of spruce lignin proceeded in a stepwise manner, depending on the concentration of alkali. This indicated the presence of several hydroxyl groups showing distinct differences in properties. One hydroxyl group was capable of being methylated in 1% alkali. This reactivity would indicate a phenolic hydroxyl group, as has been suggested by many investigators, or some enolic grouping. However, lignin which had been chlorinated had lost its ability to be methylated and therefore must have lost its hydroxyl groups. Phenolic hydroxyls are usually resistant to chlorination, while the enolic or ethylenic linkage would be capable of being methylated easily and also be destroyed by chlorination. Such a grouping would also account for a number of other reactions characteristic of lignin. It is capable of existing in a keto form as suggested by Friedrich.[13,14] The keto form would show the reactions of the carbonyl group as found by Hägglund.[15] One such group in a molecule of 900 is in agreement with his findings. The increased stability on methylation, the color changes produced by methylation or acetylation, and the ability of methylated lignin to combine with a larger amount of chlorine than unmethylated lignin are also properties characteristic of compounds containing keto-enolic groups. The 15% of phenolic nucleus in lignin indicated by Hibbert and Taylor[16] as calculated from the reactions of HOCl on glycol lignin could be applied in the same manner to one enolic group stabilized by the presence of a glycol residue in a unit weight of 900.

The percentage of methoxyl contained in fully methylated spruce and maple lignins is about the same, showing that the total number of hydroxyl groups available for methylation is about the same, but in original spruce lignin half of these hydroxyls are methylated whereas in maple three-fifths are methylated. There is also a great difference in the ease of methylation. Maple lignin is completely methylated in 1% alkali while concentrations of over 15% alkali are required to fully methylate spruce lignin.

The high concentration of alkali required for the methylation of the other four of the hydroxyl groups in spruce shows that these groups were much less reactive. Their reactivity resembles that of primary and secondary alcohols.

The acetylation of the two lignins confirms the results of the methylation and further shows that the hydroxyl groups were present in the lignin and not formed by the action of alkali. The acetylation was a very slow reaction as would be the case when a tautomeric compound is acetylated.

Bromine and chlorine react very much the same way on lignin. Both cause the loss of hydroxyl groups as was determined by the fact that bromo or chloro-lignin could not be methylated or acetylated. Both reagents showed

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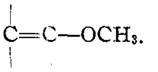
(13) A. Friedrich, *Z. physik. Chem.*, **168**, 50–67 (1927).

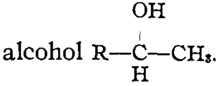
(14) Also B. Rassow and K. W. Wagner, *Papierfabr.*, **63**, 103 (1932).

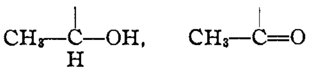
(15) E. Hägglund, T. Johnson and L. H. Trigg, *Cellulosechemie*, **11**, 30 (1930).

(16) H. Hibbert and K. A. Taylor, *Can. J. Res.*, **4**, 240 (1931).

the property of removing some of the methoxyl groups. Bromine showed less capacity to combine with lignin and the products were less definite. Therefore the reactions of chlorine with lignin were studied more fully. The products obtained from the chlorination of spruce and maple lignins in carbon tetrachloride showed that there was a great difference between these two lignins. Spruce lignin combined with about twice as much chlorine as maple lignin. The yield of chloro-spruce lignin, corrected for the loss of methoxyl and hydroxyl groups, indicated that the reaction was principally addition. This was further demonstrated by the fact that very little hydrogen chloride was liberated. The yield of chloro-maple lignin was slightly low and some unidentified products were found in the carbon tetrachloride. This latter is in agreement with the apparent degradation of the lignin molecule shown in Fig. 1. If the principal reaction of chlorine on spruce lignin in a dry organic solvent is addition, the loss of methoxyls can be accounted for if they are attached to unsaturated groups as  $\underset{|}{\overset{|}{C}}=C-OCH_3$. When chlorine is added to such a group the addition compound, due to the accumulation of negative groups on one carbon, loses the methyl group, forming a compound containing the carbonyl group. When the chlorination takes place in water the final amount of chlorine introduced is about the same as when carbon tetrachloride was used, but the reaction is quite different. Oxidation, substitution and addition take place. This reaction splits off not only the methoxyls attached to ethylenic groups but also methoxyls attached to groupings that required oxidation as well as chlorination to split the ether linkage. Substitution reactions in this case were favored, the reaction being similar to that which occurs when ethyl ether is chlorinated in the presence of moisture. If this were a primary or secondary alcohol grouping, chlorination in water would produce carbonyl and carboxyl groups. The presence of carbonyl groups has been reported[17] in chlorinated lignin. Evidence of the presence of carboxyl groups was also found and will be reported in a later paper.

A third type of grouping to which methoxyl was attached did not lose methoxyl when acted upon by chlorine. This may have been an aromatic or some other tertiary carbon not susceptible to oxidation.

There is evidence that one of the hydroxyls in lignin is part of the secondary alcohol  $R-\underset{H}{\overset{OH}{C}}-CH_3$. Since lignin contains only carbon, hydrogen and oxygen, the formation of iodoform or chloroform when lignin is treated with alkaline solutions of iodine or chlorine, respectively, and of chloroform when chloro-lignin is treated with alkali, suggests the presence of one of the groups



$$CH_3-\underset{H}{\overset{|}{C}}-OH, \quad CH_3-\overset{|}{C}=O$$

If lignin is methylated before the treatment, chloroform is not formed. Therefore the group containing the hydroxyl must have been responsible for this reaction. The presence of one methyl-to-carbon linkage in a molecule of 900 was shown by Freudenberg and Sohns[11] by the oxidation of spruce lignin with moist chromic oxide when he obtained a 6% yield of acetic acid.

Table I shows a comparison of the values obtained on spruce with those of other investigators. The values in the table show that spruce lignins prepared by different methods and by various investigators are similar in respect to methylation and acetylation.

Relationship between Lignin and Carbohydrates in Wood.—To determine the possible changes taking place during isolation by the modified sulfuric acid, extractive-free wood was subjected to several of the chemical reactions used in the study of lignin.

Table II shows the degree of methylation of the lignin after successive treatments of the wood by hydrolytic agents. It was found that maple lignin could be methylated in a very dilute solution of alkali whereas cellulose required a high concentration. Maple wood was methylated under these conditions and no methoxyl was added to the lignin isolated from the methylated wood. The methylated wood was subjected to a mild hydrolysis and methylated again. The lignin isolated from this methylated wood showed an increase in methoxyl content. The alternate hydrolysis and methylation was continued and after each hydrolysis more methoxyl could be introduced into the lignin. Other hydrolytic agents had a similar effect, acetolysis permitting a complete methylation. From these experimental data it was concluded that the hydroxyls of lignin

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[17] Geo. J. Ritter, R. L. Mitchell and R. M. Seborg, *Ind. Eng. Chem.*, **24**, 1285 (1932).

TABLE II

EFFECT OF HYDROLYTIC AND SIMILAR AGENTS ON THE METHYLATION OF LIGNIN IN ORIGINAL EXTRACTIVE-FREE WOOD

	Material	Treatment	Methoxyl content of lignin isolated from wood after treatment, %
1	Original wood	Methylated in 1% alkali	21.5
2	Methylated wood from No. 1	Hydrolyzed with 1% acid and methylated in 1% alkali	25.4
3	Methylated wood from No. 2	Hydrolyzed with 1% acid and methylated	27.1
4	Methylated wood from No. 3	Hydrolyzed with 1% acid and methylated	28.0
5	Original wood	Benzoylation followed by methylation	23.2
6	Original wood	Acetylation followed by methylation	32.0
7	Original wood	Heated with glacial acetic acid	21.5
	Methoxyl content of lignin isolated from original wood		21.5

were not free for methylation until after hydrolysis and that lignin must be attached to some of the carbohydrates, possibly the hemicellulose, in the wood cell. Such a chemical combination between lignin and carbohydrates is not difficult to conceive in the cell-wall lignin which is intimately mixed with a large proportion of carbohydrates, but structurally it is not commonly considered that there are any carbohydrates in the middle lamella with which the lignin can combine. Recently, however, evidence[10] has been offered for the presence of carbohydrates in the middle lamella.

Further evidence for a carbohydrate-lignin compound was found in the chlorination of wood. A combination of carbohydrate with lignin would stabilize the enolic form of certain groups in lignin which are more unsaturated than isolated lignin as was shown by the greater amount of chlorine taken up by fully methylated lignin.

Table III shows a comparison of the amounts of chlorine taken up by wood lignin, isolated lignin and methyl lignin.

TABLE III

CHLORINATION OF MAPLE LIGNIN IN WATER

Percentages calculated in terms of dry isolated lignin

Condition of lignin	Combined chlorine,[a] %	Chlorine as HCl,[b] %
Original wood	37	86.0
Isolated lignin	23[c]	86.5
Isolated lignin (methylated)	35[c]	..

[a] Chlorine was determined by dissolving lignin from wet residue after extracting hydrochloric acid in hot sodium sulfite solution, heating with silver nitrate and nitric acid to destroy the chloro-lignin, and titrating the excess silver.

[b] Hydrochloric acid was determined by titration of washings from residue.

[c] The differences in the percentage of chlorine shown in Table III and Fig. 1 are due to the fact that none of the combined chlorine was lost by the splitting out of hydrogen chloride in the products shown in Table III.

The amount of hydrochloric acid formed by substitution or oxidation was the same for original lignin and isolated lignin, while the combined chlorine is much higher, indicating more unsaturated groups in the original lignin. Lignin as it occurs in wood and methylated lignin have approximately the same degree of unsaturation.

The results from the study of the methylation and chlorination of wood indicate that the principal difference between original lignin and isolated lignin is that certain groups exist in opposite tautomeric forms. Original lignin in the enolic form is combined with carbohydrates while isolated lignin exists principally in the keto form.

Summary

Lignin isolated by the cold sulfuric acid method was found to be produced quantitatively, to be free from carbohydrates, and to contain all of the methoxyl not accounted for in the carbohydrates of the wood. Hydrochloric acid lignin contained carbohydrates and had lost methoxyl groups. Cuprammonium lignin contained carbohydrates and was not quantitative.

A study of the properties of sugar maple and white spruce lignin showed that lignins from hardwoods and softwoods were not the same. The number of acetyl, methoxyl, chlorine and bromine groups introduced indicated that the number of hydroxyl and unsaturated groups in the molecules differed.

The behavior of the methoxyl to halogenation and of the hydroxyl to methylation in alkali and to halogenation in carbon tetrachloride or water suggested that the groups to which these radicals were attached represented at least three types.

The results obtained from the methylation and chlorination of lignin in wood showed that lignin is little changed during isolation by the modified sulfuric acid method.

Evidence is presented to show that lignin in the wood is combined with carbohydrates.

MADISON, WIS. RECEIVED OCTOBER 11, 1933