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Native Lignin¹ I. Its Isolation and Methylation

BY F. E. BRAUNS

The experiments reported in the present paper deal with isolated native lignin. Inasmuch as the term "native lignin" has very often been misused in the lignin literature, its meaning in the present investigation is explained briefly. It is a general belief that lignin as it occurs in the plant material is insoluble in organic solvents and cannot be extracted by them without the use of a mineral acid as a catalyst. It is true that it occasionally has been noted that, on extraction of wood with alcohol, some lignin goes into solution. It seems that Klason² isolated a product by hot alcohol extraction which he considered to be lignin, but he did not even carry out a test for the presence of methoxyl groups in his product. Moreover, Freudenberg³ has observed that on extraction of wood with hot alcohol-benzene some low molecular members of the lignin series are removed along with the resins, but the quantity was so small that he discarded the fraction. On the other hand, Hägglund,⁴ in his "Holzchemie," says: "The native lignin of the wood is not soluble in organic solvents without the addition of acids." There is little doubt that the principal part of the lignin can be isolated from wood only by the use of strong mineral acid, cuprammonium hydroxide, or by organic solvents in the presence of mineral acids. In the method where alcohols are used without mineral acids,⁵ high temperatures (up to 185°) must be applied; the lignin obtained on distillation of the solvent is a black pitchlike product and can no longer be considered identical with native lignin.

Native or unaltered lignin can be obtained only if one can succeed in isolating it by the use of an indifferent solvent and without the application of an elevated temperature. The present investigation deals with the isolation of such a lignin. It has been found during the last fifteen years that a large number of lignin preparations are soluble in methyl or ethyl alcohol and that almost all, except

those prepared by extraction of the cellulose with mineral acids, are also soluble in dioxane. If lignin is to be extracted in an unchanged state, these solvents should be the most suitable ones. Furthermore, it has been found possible to extract a small amount (about 2 to 3%) of the lignin present in wood by using such solvents without the application of a high temperature. *The term "native lignin," therefore, means a lignin isolated in such a way that the solvent does not react with the lignin or alter it in any way.*

When sprucewood meal is allowed to stand for three to four days in 96% ethyl alcohol and is then filtered and the clear light brown filtrate evaporated under reduced pressure, a resinous residue is obtained which is washed with distilled water and ether. With the removal of the resins by the ether, the native lignin is obtained as a very finely divided light cream-colored powder, which is precipitated repeatedly from its solution in dioxane into ether until a constant methoxyl content is obtained. This powder gives the typical lignin color reactions: a deep purple with phloroglucinol and hydrochloric acid and a bluish-green with phenol and hydrochloric acid. The native lignin has a methoxyl content of 14.8%. This seems to be low, considering that the methoxyl content of lignin as it occurs in wood has always been assumed to be about 17.3%; furthermore, it is a well-known fact that the methoxyl content of sprucewood lignin, isolated according to Klason, Willstätter, or Freudenberg, varies between 14.5 and 17%, depending upon the conditions of preparation. A methoxyl content of about 17% has been calculated also for spruce lignin by Fuchs⁶ from the lignin and the methoxyl contents of the wood, on the obviously incorrect assumption that all the methoxyl in the wood is combined with the lignin. It has been shown by Ritter and his co-workers,⁷ and more recently by Hägglund and Sandelin,⁸ however, that 0.56% of the total methoxyl content (4.6%) in sprucewood is attached to carbohydrate material. When this part was taken into consideration, Hägglund⁴

(1) Presented in part at the Baltimore meeting of the American Chemical Society, April, 1939. This is the first of a series of papers on this subject; future papers will clarify many of the points raised herein.

(2) Klason, "Beiträge zur Kenntnis der chemischen Zusammensetzung des Fichtenholzes," Berlin, 1911, pp. 34-36.

(3) Freudenberg, *et al.*, *Ber.*, **69**, 1415 (1936).

(4) Hägglund, "Holzchemie," Leipzig, 1939, p. 205.

(5) Aronovsky and Gortner, *Ind. Eng. Chem.*, **28**, 1270 (1936).

(6) Fuchs, "Die Chemie des Lignins," Berlin, 1926, p. 76.

(7) Kurth and Ritter, *THIS JOURNAL*, **56**, 2720 (1934); Ritter and Barbour, *Ind. Eng. Chem., Anal. Ed.*, **7**, 238 (1935); Harris, Sherrard and Mitchell, *THIS JOURNAL*, **56**, 889 (1934).

(8) Hägglund and Sandelin, *Svensk Kem. Tid.*, **46**, 83 (1934).

(pp. 199, 299) calculated a methoxyl content of 14.8% for lignin. This is exactly the amount found during this investigation.

The first questions to be answered are, of course: "Is this native lignin really lignin, or is it some other methoxyl-containing constituent of the wood, and to what extent is this part of the lignin identical with the principal part of the lignin in wood?" The color reaction with phloroglucinol and hydrochloric acid is not decisive, for there are many other compounds in wood which give the same reaction. In a very recent article Wiechert⁹ claims to have shown that this coloration, at least as far as beech lignin is concerned, has no relation to the lignin. Without going into further details, it can be asserted positively that this phloroglucinol color reaction is typical at least of spruce lignin¹⁰; the mechanism of this reaction is the subject of an investigation now in progress.

There are two other typical lignin reactions—the reactions of lignin with phenol and with thioglycolic acid. The isolated native lignin condenses with phenol in the presence of hydrochloric acid with the formation of a phenol lignin having a methoxyl content of 11.5% which is identical with the same derivative prepared from Willstätter or Freudenberg lignin.¹¹ Inasmuch as Willstätter lignin is obtained in nearly quantitative yield and the two phenol lignins are apparently identical, it would seem that isolated native lignin has the same chemical properties as the total lignin in the wood. This is further shown by the preparation of the thioglycolic acid derivative of the isolated native lignin with a methoxyl content of 10.5%. This content is in agreement with that found by Holmberg¹² for a tetrathioglycolic acid lignin from sprucewood which is obtained in an almost quantitative yield, but about 1% lower than that found by Ahlm and Brauns.¹³ To what extent the phenol and the thioglycolic acid derivatives of the isolated native lignin are identical with corresponding lignin derivatives obtained directly from wood in a quantitative yield will be shown later. If it is possible to prove that this native lignin behaves chemically in a manner similar in every respect to the total lignin, it is obviously an ideal product for a chemical study of the lignin problem.

(9) Wiechert, *Papier-Fabr.*, **37**, 17, 30 (1939).

(10) Brauns and Brown, *Ind. Eng. Chem.*, **30**, 779 (1938).

(11) Buckland, Brauns and Hibbert, *Can. J. Research*, **13B**, 61 (1935).

(12) Holmberg, *Ing. Vetenskaps Akad. Handl.*, **103**, 64 (1930).

(13) Ahlm and Brauns, *THIS JOURNAL*, **61**, 277 (1939).

The first experiment carried out with the isolated native lignin was the action of 42% hydrochloric acid and of 72% sulfuric acid. In preliminary experiments it was found that in both cases about 5 to 8% of the lignin could not be recovered. Native lignin gives with supersaturated hydrochloric acid a bright emerald-green color similar to that observed at the beginning of the preparation of Willstätter spruce lignin; with native lignin the green color is much brighter (purer) and lasts much longer than with wood meal. A part of the lignin dissolves in the hydrochloric acid, and it can be recovered from the filtrate on evaporation.

With 72% sulfuric acid a bluish-green coloration appears at the beginning, lasts only a short time, and soon changes to black. From the clear, colorless filtrate of the sulfuric acid treatment, after removal of the acid with barium carbonate, a white, crystalline product is obtained. The ease with which it crystallizes and the fact that it is optically inactive indicate that it is not a carbohydrate, even though it reduces Fehling solution quite strongly. A further investigation of this crystalline product is in progress. The behavior of the native lignin after treatment with mineral acids resembles that of Willstätter and Klason lignins. The methoxyl contents of these lignins vary from 15.2 to 16%, the same range as that given in the literature for those lignins isolated directly from sprucewood by these methods in a quantitative yield.

The presence of free hydroxyl groups in the native lignin is shown by its acetylation and methylation. With pyridine and acetic anhydride an acetylated native lignin is obtained having a methoxyl content of 11.8%.

Methylation of native lignin with diazomethane in anhydrous dioxane solution gives a partially methylated, nearly white lignin, with a methoxyl content of 21.4%. However, when the methylation with diazomethane is carried out with a suspension of the dry product in anhydrous ether, the methylation seems to stop at about 18.3%, but on further methylation in dioxane solution a final methoxyl content of 21.4% is obtained. The product with 18.3% methoxyl is completely soluble in dilute sodium hydroxide solution, indicating that an acidic hydroxyl group in the lignin molecule is still free; if the lignin were in part completely methylated and in part completely unmethylated, only a portion would be alkali solu-

ble. It should be noted that this partially diazomethane-methylated native lignin still gives the bright purple color reaction with phloroglucinol and hydrochloric acid, whereas on complete methylation with diazomethane this color fails to appear.

A quantitative yield of a lignin with the same methoxyl content (21%) is also obtained when sprucewood meal is methylated with diazomethane and the lignin is isolated according to Klason with 72% sulfuric acid.¹⁰ When the diazomethane-methylated native lignin is treated with 72% sulfuric acid under the same conditions as those used in the Klason method, a partially methylated Klason lignin with a methoxyl content of 21.15% is obtained, which has the same appearance as that isolated directly from diazomethane-methylated sprucewood. The experiment with the methylated native lignin and 72% sulfuric acid has not been followed quantitatively; it is therefore possible, or even probable, that in this case, as in the experiment with the unmethylated native lignin, a small fraction is split off the lignin. A larger amount of the diazomethane-methylated product has been prepared, and an investigation of the fraction split off will soon be started.

A spruce lignin with a methoxyl content of about 21% is also obtained when Willstätter or Freudenberg lignin is methylated in cyclohexanol suspension with diazomethane.¹⁴ As these lignins are isolated in a nearly quantitative yield, it shows that the isolated native lignin, on methylation with diazomethane, behaves in the same way as the lignin which remains in the wood after extraction of the soluble native lignin.

The diazomethane-methylated native lignin still has some free hydroxyl groups, as acetylation with pyridine and acetic anhydride gives an acetylated, partially methylated native lignin with a methoxyl content of 18.9%.

When sprucewood meal is extracted with methanol, using 2% hydrochloric acid as a catalyst, so-called methanol lignin is obtained.¹⁵ By using other alcohols, such as ethyl, butyl, or amyl in the presence of hydrochloric acid, it is found that lignin forms condensation products with these alcohols. In the case of methanol, the reaction could not immediately be established,

since the lignin itself contains methoxyl groups. As a matter of fact, when Friedrich¹⁶ first prepared his so-called "primary lignin" by hydrolysis of wood with hydrochloric acid followed by extraction with ethyl alcohol, he believed that native spruce lignin had a methoxyl content of approximately 21%. Hägglund and Urban^{15c} later ascertained the presence of ethoxyl groups in Friedrich's "primary lignin."

It was therefore of interest to determine whether isolated native lignin would react with alcohols in the presence of hydrochloric acid. When native lignin is dissolved in methanol, a yellow-brown solution is obtained which, on addition of a solution of anhydrous hydrochloric acid in absolute methanol, immediately turns to a deep wine-red, indicating that a reaction takes place almost instantly. When the concentration of the hydrochloric acid is so chosen that a 2% methanol solution is used and the solution refluxed for four to five hours, about 35% of the lignin separates out as a resin, which is insoluble in dioxane. Its methoxyl content is 20.85%. From the methanol solution a methanol lignin with a methoxyl content of 21.5% is isolated. This methoxyl content agrees exactly with that of methanol lignin prepared under the same conditions directly from sprucewood.¹⁵ The large amount of polymerized methanol lignin formed is undoubtedly due to the relatively high concentration of hydrochloric acid, for when a concentration of only 0.5% is used the same change in color of the solution occurs, but all the lignin remains in solution; the methoxyl content of the isolated methanol lignin in this case is 20.9% and does not change when the treatment with 0.5% methanol-hydrochloric acid is repeated. This observation is in direct contradiction to an experiment recently reported by Hibbert and co-workers,¹⁷ in which it is claimed that "an acetylated, carefully purified oak lignin (8.7% OCH₃, 35% COCH₃) [is] reconverted into its primary building units by refluxing it with anhydrous ethanol containing 2% hydrogen chloride." The methoxyl content of this oak lignin, calculated on the acetyl-free lignin, is then about 13.5%, a rather low content for an oak lignin, for which a methoxyl content of 21.8% is given in the literature⁴ (p. 229). The discrepancy between the behavior of isolated native spruce lignin and

(14) Brauns and Hibbert, *Can. J. Research*, **13B**, 78 (1935).

(15) (a) Klason, *Tek. Tid., Avd. Kem.*, **23**, 55 (1893); (b) Holmberg and Runius, *Svensk Kem. Tid.*, **37**, 89 (1925); (c) Hägglund and Urban, *Cellulosechem.*, **8**, 69 (1927); **9**, 49 (1928); (d) Brauns and Hibbert, *Can. J. Research*, **13B**, 28 (1935).

(16) Friedrich and Diwald, *Monatsh.*, **46**, 31 (1925); Friedrich and Brüda, *ibid.*, **46**, 600 (1925).

(17) Peniston, McCarthy and Hibbert, *THIS JOURNAL*, **61**, 530 (1939).

Hibbert's acetylated oak lignin toward alcohol and hydrogen chloride can be explained only by the fact that the latter was decomposed in its preparation, as is shown by its low methoxyl content. The fact that a part of the native lignin becomes insoluble when treated with a 2% methanol-hydrogen chloride solution may also explain the assumption made by Hägglund⁴ (p. 190) that a condensation of the lignin takes place in the solid phase through the mineral acid and for this reason not all the lignin can be extracted in such a way from the wood.

The question now arises as to how methanol and alcohols in general react with lignin in the presence of mineral acids. It has generally been assumed that in this reaction an oxygen ring, such as the furan or pyran ring, is opened with the formation of a new alkoxy group and a new hydroxyl group. Hägglund and Urban,^{15c} using butyl and amyl alcohols, agree with the opinion of Holmberg and Runius^{15b} that these so-called alcohol lignins are probably acetals and that, on a basis of a molecular weight of approximately 400 for lignin, a half-acetal is formed⁴ (p. 190). It will be pointed out later that, on a basis of a molecular weight of 840, a true acetal is obtained. If the alcohol is bound in an acetal-like manner, it should be possible to split off these groups with acids. When methanol lignin from isolated native lignin is treated with 72% sulfuric acid according to Klason, the methoxyl content decreases from 20.9 to 16.4% (which is the methoxyl content of Klason lignin from sprucewood); in other words, those methoxyl groups introduced by the treatment with methanol and hydrochloric acid are split off again. This is an experimental support for Hägglund's and Holmberg's assumption that in the reaction of alcohols with lignin in the presence of acids acetal-like compounds are formed.

Methanol lignin prepared directly from sprucewood is soluble in sodium hydroxide solution^{15b} and can be acetylated or methylated. The same is true of methanol lignin from isolated native lignin. In both cases acetylated or partially methylated methanol lignins, which are insoluble in caustic soda solution, are obtained. The acetylated product has a methoxyl content of 16.9%, the diazomethane-methylated product 25.36%, and the corresponding derivative prepared directly from spruce^{15d} 24.9%. A partially methylated lignin with a methoxyl content of 24.4% is obtained when the diazomethane-

methylated native lignin is treated with methanol and hydrochloric acid. On remethylation of this product with diazomethane, the methoxyl content increases to 25.45% or to the same value as that of diazomethane-methylated methanol lignin. In other words, isolated native lignin gives partially methylated lignin derivatives with the same methoxyl content regardless of whether it is first methylated with diazomethane and then treated with methanol and hydrochloric acid, or whether this process is reversed.

On further methylation of the diazomethane-methylated native lignin and of the methanol native lignin, two different lignin derivatives fully methylated with regard to dimethyl sulfate are obtained, the first with a methoxyl content of 31.3% and the second with a methoxyl content of 33.0%.

Formulas for Lignin and its Derivatives

The results of these experiments show a far-reaching agreement with those of the corresponding products obtained in an almost quantitative yield directly from sprucewood. Based on these earlier results, an empirical formula (I) for the lignin building unit¹⁸ was proposed.¹⁹ As was later found by Hägglund^{19c} and Holmberg,^{19d} this formula is in good agreement with the experimental results of other lignin investigators, and the question arises as to how the experimental results obtained from isolated native lignin fit into this formula.

It must be recalled that this formula was developed on the assumption found at that time in the literature, namely, that "native lignin" has a methoxyl content of 17.3% and that one new methoxyl group is formed in the lignin molecule on reaction with methanol and hydrochloric acid; it was also based on the assumption that one methoxyl group was introduced into the lignin building unit on methylation with diazomethane. The assumptions, however, were proved to be in error (this indicates again how cautious one has to be in making assumptions in lignin chemistry) for the methoxyl content of the isolated native lignin is found to be only 14.8%, which is in

(18) The term "lignin building unit" was used by Brauns and Hibbert in 1933 for a unit with a molecular weight of approximately 840. In his recent publications, Hibbert uses the same term for one with a C₆ group only. In order to avoid confusion it would seem to be advisable to use the term "lignin building stone" for the C₆ groups as has already been done by Freudenberg ("Tannin, Cellulose, Lignin," Berlin, 1933, p. 134).

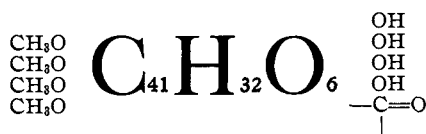
(19) (a) Brauns and Hibbert, *THIS JOURNAL*, **55**, 4720 (1933); (b) Sarkar, *J. Indian Chem. Soc.*, **12**, 547 (1935); (c) Hägglund, *Svensk Papperstidn.*, **39**, 347 (1936); (d) Holmberg, *Papier-Fabr.*, **36**, 218 (1938); (e) Freudenberg, *ibid.*, **37**, 28 (1939).

agreement with that calculated by Häggglund. In the methylation of the isolated native lignin either with diazomethane or with methanol and hydrochloric acid, two partially methylated lignin derivatives with *almost* the same methoxyl contents are obtained. They differ, however, from each other in that the diazomethane-methylated product is insoluble in sodium hydroxide, whereas the methanol derivative is soluble, and also in that their behaviors toward 72% sulfuric acid are dissimilar. With this reagent the methoxyl content remains unchanged in the case of diazomethane-methylated isolated native lignin (IV), whereas that of methanol lignin is decreased to 16.4%. On the other hand, when diazomethane-methylated isolated native lignin (IV) is treated with methanol and hydrochloric acid (VII) and methanol native lignin is methylated with diazomethane (VI), products with the same methoxyl contents are obtained. It is obvious that with diazomethane an acidic hydroxyl group is methylated which remains free in the methylation with methanol and hydrochloric acid. On the other hand, in both cases one hydroxyl group is methylated either by diazomethane or by methanol and hydrochloric acid. Finally, there is a third hydroxyl group in the native lignin which is only methylated by methanol and hydrochloric acid. In other words, three methoxyl groups are introduced into the lignin molecule when diazomethane and methanol-hydrochloric acid are both used, and two when only one of these reagents is used. The fact that with methanol-hydrochloric acid two methoxyl groups are introduced which are split off again with 72% sulfuric acid indicates that these groups are present in an acetal-like form as proposed by Holmberg. The presence of a carbonyl group in the isolated native lignin is proved by the reaction with phenylhydrazine whereby a hydrazone is formed.



Original formula for native lignin
derived from methanol lignin

I



Native lignin keto form

II



Native lignin enol form
III



Native lignin methylated with diazo-
methane
IV



Methanol native lignin
V



Methanol native lignin methylated
with diazomethane
VI



CH₂N₂-methylated native lignin
treated with MeOH-HCl

VII

To return now to the development of the empirical formula (I), it is evident that this formula, insofar as methanol lignin is concerned, remains unchanged. With regard to the native lignin, however, all that has to be changed is that one methanol molecule has to be taken out because the calculation was based on the entrance of only one methoxyl group on methylation with methanol and hydrochloric acid, whereas actually two enter the lignin building unit forming a dimethylacetal with the carbonyl group. This carbonyl group is easily enolizable. The empirical formula for native lignin would then be as given in Formula II in its keto form and in formula III in its enol form with a molecular weight of around 840.

In Table I the methoxyl contents of the various derivatives of native lignin and, for comparison,

TABLE I
NUMBER OF GROUPS

Lignin derivative	Mol. wt.	CH ₃ O	OH or acetyl	Calcd. MeO	Found MeO	MeO found for corresponding comp. by other investigators
1 Native lignin (N.L.)	838.3	4	4 OH	14.80	14.8	14.8 ^a
2 N.L. acetylated Keto form	1006.3	4	4 Ac	12.33
Enol form	1048.3	4	5 Ac	11.84	11.8	..
3 N.L. methylated with CH ₂ N ₂	866.3	6	3 OH	21.48	21.4	21.2 ^b
4 N.L. methylated with CH ₂ N ₂ and acetylated	992.3	6	3 Ac	18.76	18.9	..
5 Methanol N.L.	884.3	6	4 OH	21.00	20.9	21.6 ^c
6 Methanol N.L. acetylated	1052.3	6	4 Ac	17.7	16.92	17.8 ^c
7 Methanol N.L. methylated with CH ₂ N ₂	898.3	7	3 OH	24.17	25.36	24.8 ^c
8 Methanol N.L. methylated with CH ₂ N ₂ and acetylated	1024.3	7	3 Ac	21.20	22.7	21.4 ^c
9 N.L. methylated with CH ₂ N ₂ and treated with MeOH-HCl	898.3	7	3 OH	24.1	24.4	24.8 ^c
10 No. 3 methylated with Me ₂ SO ₄ + NaOH	908.3	9	...	30.74	31.3	30.3 ^d
11 No. 5 methylated with Me ₂ SO ₄ + NaOH	940.3	10	...	33.00	33.1	32.3 ^c
12 Phenylhydrazone of N.L.	928.3	4	4 OH	13.36	13.4	..

^a Calculated by Hägglund⁹ for native spruce lignin. ^b Diazomethane-methylated Willstätter lignin. ^c MeO content of derivatives of methanol lignin directly isolated from sprucewood.^{16d} ^d Willstätter lignin methylated with CH₂N₂ followed by Me₂SO₄ and NaOH.

those calculated on the basis of the formula are given.

It can be seen that in those cases (1, 2, 3, 4, and 11) where neither alkali nor acid is used, the values found for the methoxyl contents agree very well with those calculated, whereas in those cases where the native lignin has come into contact either with alkali, as in 9 and 10, or with acid (HCl), as in 5, 6, 7, and 8, the methoxyl contents vary somewhat. This discrepancy may be explained by the possibility that a methylene oxide group²⁰ has been split off with the formation of free phenolic hydroxyl groups. Such a reaction seems to be indicated especially in the treatment of diazomethane-methylated native lignin with methanol and hydrochloric acid. When native lignin, completely methylated with regard to diazomethane and containing methoxyl groups which are stable even to 72% sulfuric acid, is treated with methanol-hydrochloric acid, a partially methylated methanol native lignin (VI) is obtained with almost the calculated methoxyl content (24.4 instead of 24.1%). On remethylation of this product with diazomethane, the methoxyl content increases to 25.4% or to the same value as the diazomethane-methylated methanol lignin.

It is a well-known fact that lignin as it occurs in wood is dissolved when heated with a bisulfite

(20) The presence of a methylene oxide group in lignin has been established definitely by Freudenberg, *et al.* [*Ber.*, **72**, 217 (1939)] in contrast to Hibbert and co-workers [*ibid.*, **71**, 734 (1938)], who believe that the formaldehyde obtained in the distillation of lignin with mineral acids is derived from carbohydrate material in the lignin.

solution. When isolated native lignin is heated with sodium bisulfite solution containing 1 g. of sodium oxide and 4.5 g. of sulfur dioxide per liter for twelve to fourteen hours at 125°, it goes completely into solution, giving liginosulfonic acid. In a recent paper¹¹ it was shown that when sprucewood meal is treated with diazomethane until the lignin has a final methoxyl content of 21%, this wood can no longer be pulped with bisulfite solution. Similar results have been obtained by Hägglund and J. Holmberg²¹ and by Klingstedt.²² This has been explained by the fact that in the lignin molecule a hydroxyl group capable of methylation with diazomethane is present which is essential for rendering the lignin soluble in a bisulfite cook; by blocking this hydroxyl group with methoxyl, this reaction can no longer take place. Freudenberg, on the other hand, showed that, when sprucewood is methylated with diazomethane to a methoxyl content of from 18 to 19% for the lignin, the lignin in this wood can be dissolved completely by a bisulfite cook. How can this discrepancy be explained? It is shown in the present investigation that on methylation of native lignin with diazomethane two methoxyl groups are introduced in the lignin molecule having a molecular weight of 840. Since one of these hydroxyl groups is also methylated by methanol and hydrochloric acid, it is evident that this hydroxyl group is an enolic one and that the

(21) Hägglund and Holmberg, unpublished work. See ref. 4, p. 149.

(22) Private communication.

second group is a phenolic one. As already mentioned, on partial methylation of native lignin in ether suspension with diazomethane, only one hydroxyl group in the lignin building unit is methylated, and since the phenolic hydroxyl is more acidic than the enolic, it is very probable that the former is the first to be methylated. Such a partially diazomethane-methylated native lignin has a methoxyl content of 18.3% (that calculated for a native lignin with five methoxyl groups is 18.14%) and with its unchanged carbonyl group it should still be soluble in bisulfite solution. An experimental proof of this statement will be carried out soon. Since Freudenberg methylated his sprucewood to a methoxyl content for the lignin of 18 to 19% only, it is quite understandable how, in his methylated wood, the lignin is dissolved with bisulfite. This would mean that instead of a "phenolic or an enolic hydroxyl" group being responsible for the reaction of the lignin with bisulfite liquor,¹⁰ it is in reality an easily enolizable carbonyl group.

Since a partially methylated lignin with the same methoxyl content is obtained from diazomethane-methylated sprucewood, it follows that in wood the keto form of the lignin is either already present in its enolic form or it is slowly enolized. The long period of time required to reach a final methoxyl content in the diazomethane-methylation of sprucewood may indicate that the keto group is slowly enolized during the methylation.

Experimental²³

Isolation of Native Spruce Lignin.—Black sprucewood meal (100- to 150-mesh) is extracted first with cold water, then with ether, and finally with alcohol at room temperature in a percolator until the alcohol drains off colorless. The alcohol solution is distilled off under reduced pressure, whereupon a finely divided creamy precipitate separates out in addition to a resinous material. This is washed with water and ether until the material becomes solid and powdery. It is filtered, dissolved in dioxane, and precipitated by pouring the solution into distilled water. Should a colloidal solution be formed, the addition of a few grams of sodium sulfate will coagulate it. The precipitate is filtered again, dried and redissolved in dioxane to give a 10% solution and the centrifuged and filtered dioxane solution dropped slowly into absolute ether with vigorous stirring. The native lignin separates out as a fine powder. This precipitation process is repeated until the methoxyl content remains constant. The product is washed after each purification with ether and finally with petroleum ether. The lignin so obtained is a very light

cream-colored powder. It is soluble in 4% sodium hydroxide solution, methanol, ethanol, dioxane, and pyridine, and insoluble in water, ether, benzene, and petroleum ether. It reduces Fehling solution and when dissolved in dioxane with phloroglucinol-hydrochloric acid gives the typical purple color reaction.

Anal. C, 63.62; H, 6.20; MeO, 14.7; after another precipitation from dioxane into benzene, C, 63.89; H, 6.07; MeO, 14.9, 14.6; another preparation gave C, 63.85; H, 6.19; and MeO, 14.8.

Acetylation of Native Lignin.—One gram of native lignin is dissolved in 15 cc. of dry pyridine, 12 cc. of acetic anhydride is added and the mixture allowed to stand at room temperature for two days. It is then poured over cracked ice and the creamy colored precipitate filtered. It is washed well with distilled water and dried in a desiccator over sulfuric acid and sodium hydroxide. It is then dissolved in dioxane, the solution centrifuged and filtered, and the product precipitated as a light cream-colored powder by pouring the solution drop by drop into dry ether. After washing with ether and petroleum ether it is dried. Its solubilities in organic solvents are the same as those of native lignin but it is no longer soluble in cold sodium hydroxide. *Anal.* Found: MeO, 11.9 and 11.7.

Methylation of Native Spruce Lignin with Diazomethane in Dioxane.—Twenty-five grams of native lignin is dissolved in 100 cc. of dioxane, and diazomethane prepared from 20 cc. of nitrosomethylurethan is passed into the solution. A vigorous nitrogen evolution takes place. After standing overnight a sample of the product is precipitated by dropping 5 cc. of the centrifuged solution into dry ether. The product is washed as usual and has a methoxyl content of 20.9%. After two more methylations, each with diazomethane from 10 cc. of nitrosomethylurethan, the methoxyl content is constant. The diazomethane-methylated native lignin is an almost white powder. It still reduces Fehling solution but no longer gives the color reaction with phloroglucinol and hydrochloric acid. It is soluble in alcohol, dioxane, acetone, and pyridine but insoluble in benzene, ether, petroleum ether and alkali solutions. *Anal.* Found: MeO, 21.4, 21.54, and 21.4.

Acetylation of Native Spruce Lignin Premethylated with Diazomethane.—Diazomethane-methylated native lignin is acetylated as described for acetylated native lignin. It is isolated as a cream-colored powder.

Methylation of Native Spruce Lignin Premethylated with Diazomethane with Dimethyl Sulfate and Sodium Hydroxide.—Diazomethane-methylated native spruce lignin (2.5 g.) is dissolved in 25 cc. of acetone and methylated twice at 20° with 25 cc. of dimethyl sulfate and 35 cc. of sodium hydroxide solution (30%) keeping the mixture only slightly alkaline. After eight hours of stirring, the acetone is distilled off under reduced pressure, whereupon the methylated lignin separates as an orange-colored solid material. This is filtered, thoroughly washed with distilled water, and dried in a desiccator. It is then redissolved in dry acetone, centrifuged, and a part of the solution dropped into dry ether with vigorous stirring, whereupon the methylated lignin is precipitated as a cream-colored powder. It is washed with ether and petroleum ether and dried. For the

(23) Many of the analyses were made by Dr. M. A. Buchanan of The Institute of Paper Chemistry.

analysis it is dried in an Abderhalden drier at 100° over phosphorus pentoxide at 10 mm. for several hours. It has a methoxyl content of 30.2%. It is remethylated as before and has then a final methoxyl content of 31.3%. In the final purification the product is precipitated once from its acetone solution into distilled water and twice from its dioxane solution into ether.

Phenylhydrazone from Native Spruce Lignin.—To a solution of 0.5 g. of native spruce lignin in 5 cc. of dioxane, 1 g. of phenylhydrazine is added and the mixture heated for two hours on a water-bath at 80°. It is allowed to stand overnight at room temperature, centrifuged, and the clear deep red solution precipitated into dry ether with vigorous stirring. It separates out as a lemon-yellow product and after another precipitation has a methoxyl content of 13.5%, which remains practically unchanged after a second treatment with phenylhydrazine.

Methanol Spruce Lignin from Isolated Native Lignin.—Ten grams of native spruce lignin is dissolved in 200 cc. of absolute methanol, and 50 cc. of methanol containing 1.25 g. of hydrochloric acid are added. The original yellow-brown color of the solution turns immediately to a cherry-red color. The mixture is refluxed for two and one-half hours, the methanol solution distilled off under reduced pressure, and the reddish-brown residue taken up in 100 cc. of methanol-acetone mixture (1:1). This solution is filtered and dropped into 2 liters of distilled water with vigorous stirring, whereupon the methanol lignin separates as a light brown flaky precipitate. It is filtered, washed well with distilled water, and dried. The yield is almost quantitative. It is twice precipitated from its dioxane solution into dry ether and shows then the same properties as the methanol lignin directly prepared from sprucewood.¹⁸ The acetylation of methanol spruce lignin from isolated native lignin and its methylation with diazomethane and with dimethyl sulfate and sodium hydroxide are carried out in the same way as that described for the isolated native spruce lignin. The methoxyl contents are given in the table.

Treatment with Methanol and Hydrochloric Acid of Isolated Native Spruce Lignin Premethylated with Diazomethane.—This reaction is carried out under the same conditions as those observed in the preparation of methanol spruce lignin from isolated native lignin. The product so obtained has almost the same methoxyl content (24.4%) as the corresponding derivative prepared directly from

sprucewood (24.7%), but on remethylation of the former with diazomethane the methoxyl content increases to 25.4% or the same as the diazomethane-methylated methanol lignin from native lignin.

Summary

1. An unchanged native lignin has been isolated from black spruce by an extraction with indifferent organic solvents without the use of acids or alkali. It has a methoxyl content of 14.8%.

2. Methylation of this lignin with diazomethane indicates that two methoxyl groups are introduced into the lignin building unit of a molecular weight of 840. One of these methoxyl groups is attached to a phenolic hydroxyl group, the second to an enolic hydroxyl group.

3. In the reaction between methanol and native lignin in the presence of 0.5% anhydrous hydrochloric acid, a methanol lignin is formed combining two new methoxyl groups in an acetal-like form to a carbonyl group.

4. The presence of a carbonyl group has been proved by the formation of a phenylhydrazone.

5. A molecular size of the lignin building unit of approximately 840 has been confirmed.¹⁸

6. The presence of a phenolic hydroxyl group in the lignin building unit has been shown by methylation with diazomethane of the original lignin and of the methanol lignin, and by the solubility of the native lignin and its methanol derivative in sodium hydroxide solution, from which it is reprecipitated by carbon dioxide.

7. The isolated native lignin is completely soluble in bisulfite but it becomes insoluble in this solvent when fully methylated with respect to diazomethane, by which the enolized carbonyl group is methylated.