[CONTRIBUTION NO. 205 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY AND ENZYMOLOGY, FORDHAM UNIVERSITY]

Investigations on Lignin and Lignification. IV. Studies on Hardwood Lignin*

BY S. F. KUDZIN AND F. F. NORD

Hardwood native lignin has been isolated from oak, birch and maple. Chemical analytical, ultraviolet and infrared absorption data on the native lignins were compared with those of softwood native lignin and were found to be in close agreement. Results of the Mäule test and of the oxidative isolation of vanillin from the lignins further illustrated the similarities between oak and birch native lignins and other softwood lignins. Maple native lignin was characterized as hardwood lignin. An approach to the solution of the difficulties in the identification of hardwood lignin is discussed.

In previous communications of this series,[1a,b,c,d] the enzymatic liberation of the residual lignin from native lignin-free white Scots pine by the fungal decay of the cellulose has been reported. Extraction of the wood with 95% ethyl alcohol after 15 months of decay resulted in the isolation of 22.7% of a lignin which was identical in every respect with the native lignin fraction of the softwood. These experiments have shown conclusively that native lignin, independent of the extent of its extractability, represents the total lignin of softwood. They proved also that it is possible to obtain lignin without a partial or complete loss of its characteristic functional groups.

In view of our findings with softwood lignin, it was of interest to extend these investigations to several typical hardwoods, oak, birch and maple[2] being chosen for our study. It has been claimed that hardwood lignin differs from softwood lignin in that the former contains a higher (*ca.* 20%) methoxyl content due to the presence of guaiacyl and syringyl nuclei, whereas only guaiacyl nuclei are found in the latter, possessing a correspondingly lower (*ca.* 15%) methoxyl content. It was therefore to be expected that the attempt to isolate native lignin from our hardwoods might result in a compound of high methoxyl content, as was indeed obtained by Brauns from aspen wood.[3] A report on the isolation and properties of native lignin of oak, birch and maple comprises the subject matter of this paper.

Experimental

Isolation of Native Lignin.—A kg. sample of finely ground wood (60–100 mesh) was extracted with 95% ethyl alcohol at room temperature, in a percolator-type extractor,[1d] until the extract no longer responded to the phloroglucinol-HCl test[4] for lignin. The alcohol was then distilled off under reduced pressure, and the resulting dark brown material was triturated with water and ether three times each. A 10% dioxane solution of the powder thus obtained was then slowly filtered into 30–40 times its volume of ice-water. This procedure resulted in the formation of a colloidal dispersion. Addition of a saturated solution of sodium sulfate caused the coagulation of a light brown substance which was filtered, dissolved in dioxane, and reprecipitated into ether. The resulting lignin was precipitated successively into water and ether until the methoxyl content remained constant. The yields of lignin amounted to 1.0–1.5% of the wood.

The phenylhydrazone and acetate derivatives were also prepared.

Oxidation of Native Lignin.—Two-gram samples of native lignin were subjected to alkaline oxidation with nitrobenzene.[5a,b] Vanillin was isolated as a condensation product with *m*-nitrobenzhydrazide, melting at 212°. An authentic sample of vanillin was condensed likewise with *m*-nitrobenzhydrazide and the product did not depress the melting point when mixed with a sample of the substance isolated from the oxidation reaction.

Sulfuric Acid Lignin.—Sulfuric acid lignin was prepared in a customary way.[6]

Ultraviolet Absorption Spectra.—A Beckman quartz spectrophotometer was used to determine the absorption curves of our samples. The solutions were prepared by dissolving 3 to 5 mg. of native lignin per 100 ml. of solvent (90 parts of purified dioxane to 10 parts of distilled water).

Infrared Absorption Spectra.—An experimental model of the Baird Double-Beam Recording Infrared Spectrophotometer was used.[7] The wave length calibration of this instrument is within 0.04 μ. The samples were mulled in mineral oil and run against a wire mesh as a blank.

Results and Discussion

All three woods responded to the Mäule test,[8] exhibiting a brilliant red color when treated successively with potassium permanganate, dilute hydrochloric acid and ammonium hydroxide, and each yielded a "Klason lignin" with a methoxyl content of about 20%. However, the native lignin fractions of these woods differed appreciably from the native aspen lignin. Thus, the methoxyl contents for oak and birch were characteristically those found in softwood lignins, being 14.8 and 14.9%, respectively. This similarity extended to their phenylhydrazone and acetate derivatives, and was further verified by the ultraviolet absorption spectra. The native lignins were soluble in ethyl alcohol, methyl alcohol, pyridine, dilute sodium hydroxide and acetone, and insoluble in glacial acetic acid, water, ether, petroleum ether and benzene. In the case of maple, the methoxyl contents were somewhat higher, but the ultraviolet absorption curve strongly resembles that characteristic of softwood native lignin.

In the infrared absorption curves, however, some differences are evident. The 1725 cm.$^{-1}$ band, present in all the lignins investigated, differs in intensity being weakest in maple lignin. Absorption bands at this point are attributed in general to ester or acid carbonyl groups. The band at 1668 cm.$^{-1}$ is in the region attributed to the aldehyde or ketone carbonyl. The aromatic nature of lignin is indicated by the bands at 1600 and

---

* Presented at the Symposium on Lignin held during the meeting of the Amer. Chem. Society, Chicago, Ill., September, 1950.

[1] (a) W. J. Schubert and F. F. Nord, THIS JOURNAL, **72**, 977 (1950); (b,c) W. J. Schubert and F. F. Nord, *ibid.*, **72**, 3835, 5337 (1950); (d) F. F. Nord and W. J. Schubert, *Holzforschung*, **5**, No. 1 (1951).

[2] Samples of these hardwoods were obtained from the Composition Materials Co., New York, N. Y.

[3] M. A. Buchanan, F. E. Brauns and R. L. Leaf, Jr., THIS JOURNAL, **71**, 1297 (1949).

[4] J. Wiesner, *Sitzungsber. Akad. Wiss. Wien*, **A77**, 60 (1878).

[5] (a) F. Schimmel and Co., German Patent No. 693,350 (1940); (b) K. Freundenberg and W. Lautsch, *Naturwiss.*, **27**, 227 (1939).

[6] G. J. Ritter, R. M. Seborg and R. L. Mitchell, *Ind. Eng. Chem., Anal. Ed.*, **4**, 202 (1932).

[7] We wish to thank Drs. Carl C. Clark and James D. Hardy of the Department of Physiology, Cornell University Medical School, for their courtesy and the facilities placed at our disposal.

[8] C. Mäule, *Beiträge wiss. Bot.*, **4**, 166 (1900).

TABLE I

COMPARISON OF HARDWOOD AND SOFTWOOD NATIVE LIGNINS

	Analyses, %										
	Native lignin (N. L.)			Klason lignin of N. L.			Klason lignin of the wood			Phenylhydrazone of N. L.	Acetate of N. L.
Wood species	C	H	MeO	C	H	MeO	C	H	MeO	MeO	MeO
Oak	58.6	5.3	14.8	59.2	5.0	14.7	60.6	5.2	20.5	13.7	10.2
Birch	61.4	5.5	14.9	61.0	5.3	14.8	61.0	5.1	20.0	13.1	10.2
Maple	61.0	5.6	17.4	60.8	5.4	17.6	60.8	5.3	20.8	15.5	12.8
White Scots pine[1a]	64.0	6.3	15.3	..	...	..	63.4	5.8	14.4	13.3	10.1

1510 cm.$^{-1}$. The 1430 cm.$^{-1}$ band establishes the presence of aliphatic groupings in the lignin complex. A difference in relative intensity at 1325 cm.$^{-1}$ should also be pointed out. Such variations are more evident at 1265, 1255 and 1220 cm.$^{-1}$, which are in the spectral region of aromatic or unsaturated C–O, and also at 1140, 1163, 1155 and 1125 cm.$^{-1}$. These differences possibly are due to different atomic environments in the lignin complex, and since bands in this region arise from vibrations of the molecule as a whole, interpretation cannot be specific. Pronounced bands at 890 and 870 cm.$^{-1}$ are found in the maple lignin curve. Jones,[9] using a film technique by evaporation of a dioxane solution of native spruce lignin, reported bands in the same region even after prolonged drying. Dioxane is known to absorb in this part of the spectrum, but in our case at least, using mineral oil mulls, it can be safely stated that the bands are characteristic of the lignin studied. These bands are suggestive of trisubstituted phenyl groups and the higher methoxyl content of maple lignin lends support to the spectral evidence of a higher degree of substitution. Due to the absorption of mineral oil, the bands at 2945, 1460, 1380 and 725 cm.$^{-1}$ cannot be interpreted with respect to the lignin molecule. In general it may be said that our absorption spectra resemble those obtained with other softwood native lignin preparations in position of the absorption maxima but differ in the relative intensity of the bands. These differences could suggest a variance in the molecular quantity of functional groups in the lignins investigated.

These results are recorded in Table I and in Figs. 1 and 2.

The native lignins on treatment with 72% sulfuric acid under the conditions of the lignin determination, gave approximately 90% of a "Klason lignin" whose composition in each case corresponded with that of the native lignin itself.

The possibility that native lignin might be contaminated with phenolic bodies, tannins and other extractives has been considered by other workers.[10] However, non-durable woods such as maple and birch, contain only colorless extractives, in small quantities.[11] It is improbable therefore that our lignin preparations from these woods were contaminated with tannin material. On the other hand, durable woods are known to contain tannin, but it has been demonstrated that oak wood tannin is soluble in water,[12a,b,13] and hence, it would have been removed in the course of purification of the native lignin.

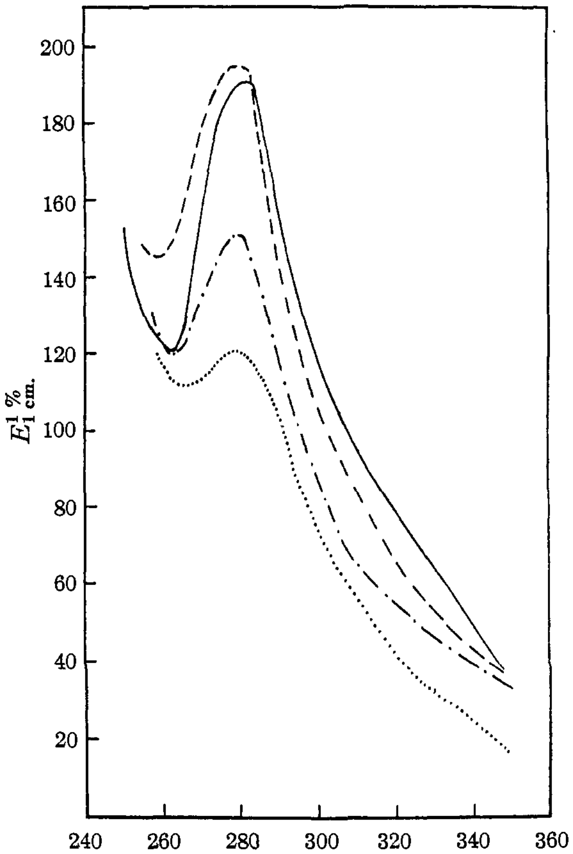


Fig. 1.—Ultraviolet absorption spectra of native lignins: —, lignin from white Scots pine; ---, lignin from oak; -·-·-·, lignin from maple; ......, lignin from birch.

From the methoxyl contents of oak and birch native lignins, the presence of only guaiacyl nuclei might be expected. However, the somewhat higher methoxyl content of maple lignin suggested the possibility that syringyl groups might be present also. Consequently, in order to establish further whether our lignins were those characteristic of softwoods or hardwoods, an alkaline oxidation of the native lignins was carried out in an attempt to determine the presence of only guaiacyl, or of guaiacyl and syringyl groups. From each of the three lignins studied vanillin was isolated as the *m*-nitrobenzhydrazide derivative. The isolation of

---

(9) E. J. Jones, Dissertation, Institute of Paper Chemistry, Appleton, Wisconsin, 1949.

(10) "Nature of the Chemical Components of Wood," TAPPI Monograph Series No. 6, p. 132, 1948.

(11) E. Kurth, in Wise's "Wood Chemistry," Reinhold Publishing Corp., New York, N. Y., 1944, p. 425.

(12) (a) J. Löwe, *Z. anal. Chem.*, **13**, 46 (1874); (b) J. Löwe, *ibid.*, **20**, 208 (1881).

(13) C. Böttinger, *Ber.*, **20**, 761 (1887).

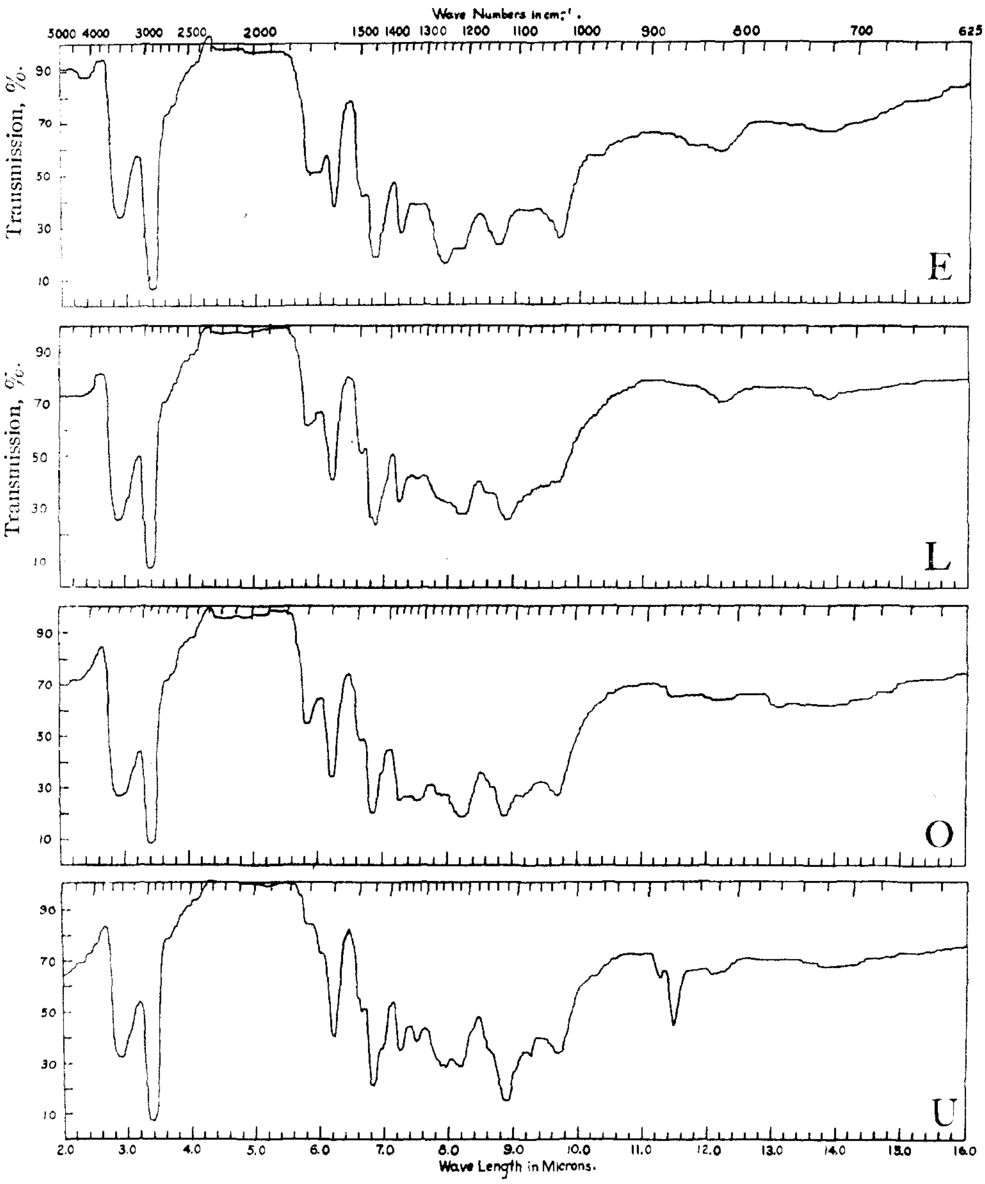


Fig. 2.—Infrared absorption spectra of native lignins: E, lignin from white Scots pine; L, lignin from birch; O, lignin from oak; U, lignin from maple.

any syringyl derivative by the oxidation procedure employed was not realized. However, it will be recalled that the Mäule test, used to distinguish softwoods from hardwoods, is based on the presence of syringyl groups in the lignin of the latter and consequently results in a red coloration of the lignified material. Softwoods, on the other hand, give only an indefinite yellow color. This test when applied to our native lignins imparted a yellow color to the oak and birch lignins, but colored the maple lignin red. Thus it can be recognized that the native lignin fractions of oak and birch woods are closely related to the native lignin of softwood, whereas maple wood yields a native lignin characteristic of hardwoods. These findings are suggestive for consideration of the relationship between the native and the residual lignin in hardwoods:

(1) The native lignin (that fraction extractable with alcohol) and the residual lignin (that portion non-extractable with alcohol) are essentially different, the former conceivably representing a transient phase in the process of lignification, originating from the carbohydrate of the cell and arising from some immediate precursor. This might serve as a substrate for an enzymatic conversion into the residual lignin, identifiable by its higher methoxyl content:

$$\text{carbohydrate} \xrightarrow{\text{degradation}} \begin{array}{c}\text{low molecular}\\ \text{weight product}\end{array} \xrightarrow{\text{synthesis}} \begin{array}{c}\text{native lignin}\\ \text{(15\% MeO)}\end{array} \longrightarrow \begin{array}{c}\text{residual lignin}\\ \text{(20\% MeO)}\end{array}$$

(2) The native and residual lignins are essentially the same, the former existing in a readily available state, whereas the latter seems to be surrounded more firmly by surfacial association with cellulose.[14a,b] The latter case represents the true

---

(14) (a) K. Hess and K. E. Heumann, *Ber.*, **75**, 1813 (1942); (b) K. Hess, *Holzforschung*, **2**, 46 (1948).

state in the conifers as was demonstrated by comparative studies on the native, and the enzymatically liberated lignins of white Scots pine. Should this condition prevail for the hardwoods studied then it must be concluded that the high methoxyl contents of the "Klason lignins" obtained from the woods are artifacts resulting from the vigorous action of the reagents applied during the isolation.

A study of the lignin liberated by means of fungal decay of native lignin-free hardwood should reveal which one of the above two possibilities actually prevails. If the lignin so liberated possesses a characteristically high methoxyl content and contains syringyl as well as guaiacyl nuclei, the first condition should represent the true state of the lignin. If, on the other hand, the enzymatically liberated lignin resembles the native lignin portion and contains only guaiacyl groups, then the conclusion must be drawn that there exists no essential difference between these hardwood lignins and softwood lignin.

Such enzymatic studies are at present in progress.

Acknowledgment.—This work was carried out with the aid of a grant from the Office of Naval Research.

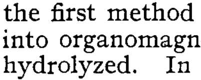
NEW YORK 58, N. Y. RECEIVED JUNE 19, 1950

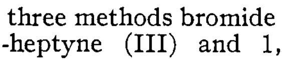
[CONTRIBUTION NO. 768 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

The Propargylic Rearrangement. II. The Dehalogenation of Propargylic Bromides

BY JOHN H. WOTIZ

Propargylic bromides were dehalogenated in three different ways: by treating their Grignard reagent with water, by means of the zinc–copper alcohol reagent, or by the action of lithium aluminum hydride. The product was always a mixture of acetylenic and allenic hydrocarbons. Possible reaction mechanisms are discussed.

In a recent paper[1] it was shown that primary propargylic bromides, $R—C\equiv C—CH_2Br$, can be converted in high yields *via* nitriles or by the malonic ester synthesis into acetylenic acids. However, the same bromides when converted into Grignard reagents on carbonation[2] yielded a mixture of acetylenic and allenic acids. The present study was undertaken to determine whether the formation of an organometallic compound is necessary for the formation of rearranged (allenic) products. Such a possible explanation was suggested by Hennion and Sheehan[3] who dehalogenated *n*-propylethynylcarbinyl chloride, $C_3H_7—CHCl—C\equiv CH$, and obtained 1,2-hexadiene in 71% yields. A small amount of the isomeric 1-hexyne was found. The authors pictured the reaction mechanism as going through an intermediate organozinc compound,  $C_3H_7—\underset{ZnCl}{\underset{|}{CH}}—C\equiv CH$, which in the presence of alcohol yielded the allenic product.

Two isomeric propargylic bromides, 1-bromo-2-heptyne (I), $C_4H_9—C\equiv C—CH_2Br$, and 3-bromo-1-heptyne (II),  $C_4H_9—\underset{Br}{\underset{|}{CH}}—C\equiv CH$, were dehalogenated by three different methods to yield mixtures of acetylenic and allenic hydrocarbons. In the first method (A) the bromides were converted into organomagnesium bromides[2] which were then hydrolyzed. In the second method (B) the zinc–copper–alcohol reagent described by Hennion[3] was used. In the third method (C) the halogen was removed by the action of lithium aluminum hydride.[4] In all three methods bromide I produced a mixture of 2-heptyne (III) and 1,2-heptadiene (IV), and bromide II, a mixture of 1-heptyne (V) and 1,2-heptadiene (IV). The yields were found to depend on the method of preparation. The findings of Hennion[3] that 3-halo-1-alkynes yield mainly 1,2-dienes when dehalogenated with zinc–copper–alcohol were substantiated. However, the isomeric 1-halo-2-alkyne with the reagent yielded only one half as much allenic as acetylenic product. Individual experiments are listed in Table I, and the yields are calculated from the amount of hydrocarbons distilling over the listed boiling range. Despite the use of high efficiency fractionating columns, imperfect separation took place. This was apparent from the refractive index and infrared spectrum determinations of individual distillate fractions. The product ratios III to IV and V to IV in Table I are estimated from the refractive index and infrared spectral data. The tabulation of constants of possible products is given in Table II. In Fig. 1 are given the infrared spectra of two fractions of reaction products in the dehalogenation of II by method B, to illustrate how the products were qualitatively and quantitatively analyzed. A linear variation in refractive indices of mixtures of isomeric hydrocarbons was assumed in order to obtain a semi-quantitative estimation of composition. The semi-quantitative analysis on the basis of the infrared spectrum utilized the extinction coefficient for pure IV, which was estimated for the typical allenic band near 1950 cm.$^{-1}$. This value allows an estimation of this compound in mixtures by the use of Beer's law. Usually the minor component was determined spectroscopically and the other one by difference. Thus the fractions in Fig. 1 boiling at 97 and 103°, contain 10 and 0% of V, on the basis of absorption, and 20 and 1%, respectively, on the basis of mixed refractive indices.

Discussion of Results

The infrared spectrograms of samples in each individual experiment listed in Table I, show con-

---

[1] Newman and Wotiz, THIS JOURNAL, **71**, 1292 (1949).

[2] Wotiz, *ibid.*, **72**, 1639 (1950).

[3] Hennion and Sheehan, *ibid.*, **71**, 1964 (1949).

[4] Trevoy and Brown, *ibid.*, **71**, 1675 (1949).