[CONTRIBUTION NO. 227 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY AND ENZYMOLOGY, FORDHAM UNIVERSITY]

# Investigations on Lignin and Lignification. VII. Characterization of Enzymatically Liberated Hardwood Lignins

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The enzymatically liberated lignins of oak, birch and maple woods, obtained by alcoholic extraction following a 10 month period of decay by the brown rot mold, *Daedalea quercina*, were compared with the respective native lignin fractions of these woods. The comparative studies included elementary and methoxyl analyses of the lignins proper, the methoxyl analyses of their acetate, phenylhydrazone and diazomethane-methylated derivatives, the yields of vanillin and syringaldehyde obtained by oxidation with alkali and nitrobenzene, and the ultraviolet and infrared absorption spectra of the lignins proper. The native and enzymatically liberated lignins of each wood were shown to be identical in all respects studied. A nomenclature based upon the presence of the guaiacyl and the syringyl grouping in lignin, rather than upon the source of the lignin, is proposed.

That the native lignin fractions of certain conifers most probably represent the total lignin of these woods has been demonstrated by subjecting the native lignin-free woods to the cellulolytic action of certain molds, extracting the lignins so liberated with ethyl alcohol at room temperature, and then comparing the enzymatically liberated products with the native lignins.[2a,b,c,d] It was thus shown that in a limited period of time 22.7% of the extra-native, *i.e.*, residual, lignin of white Scots pine could be enzymatically freed, and that this lignin was probably identical with the native lignin obtained from the same wood.

We have now extended our investigations to the lignin of the hardwood species. Thus far, it has been found that certain hardwoods possess native lignin fractions which closely resemble softwood native lignin.[2d,e] For example, oak and birch woods have yielded native lignin fractions of low methoxyl content, and devoid of syringyl groups. That the native lignins of hardwoods differ from one species to another is evidenced by the high methoxyl content of, and the presence of syringyl groups in, the native lignin of maple.

Having characterized oak, birch and maple native lignins, it was decided to study the residual lignins of these woods by subjecting the native lignin-free woods to the cellulolytic action of the brown rot fungus, *Daedalea quercina*, an organism well-known as the principal attacker of oak wood.[3]

## Experimental

The experimental procedures employed in this investigation have been reported in previous communications of this series.[2a,d,e] The species of wood investigated were oak, birch and maple. Native lignin-free samples of these woods were sterilized and then inoculated with mycelial spore suspensions[2a] of the brown rot organism, *Daedalea quercina*. The extent of decay was followed by periodic analyses for lignin and cellulose.[2d] After a 10-month period of growth, the wood samples were freed of the mycelium and extracted for lignin with ethyl alcohol.[2a] The acetate, phenylhydrazone and diazomethane-methylated derivatives were prepared.[2d] Vanillin and syringaldehyde were obtained by oxidizing lignin with alkali and nitrobenzene.[2e] The ultraviolet and infrared absorption spectra[2d] of the lignins proper were also determined.

## Results and Discussion

The yields of native lignin obtained from oak, birch and maple woods amounted to 1.0–1.5% of the wood weight.[2d] A 10-month period of decay of these woods, freed of their native lignin fractions, resulted in an increase in the per cent. of lignin with a concomitant decrease in the per cent. of cellulose, as indicated in Table I.

TABLE I

EFFECT OF THE ACTION OF *Daedalea quercina* ON THE COMPOSITION OF OAK, BIRCH AND MAPLE

| | Period of decay, mos. | Holocellulose, % | Lignin, % |
|---|---|---|---|
| Oak | 0 | 80.7 | 20.0 |
| | 10 | 68.1 | 32.5 |
| Birch | 0 | 79.0 | 21.6 |
| | 10 | 64.8 | 35.0 |
| Maple | 0 | 76.8 | 22.9 |
| | 10 | 66.2 | 33.7 |

When the partially decayed wood samples were extracted with ethyl alcohol, the yields of enzymatically liberated lignin amounted to 3.2% of the wood weight from oak, 3.0% from birch and 2.8% from maple.

It was now of importance to determine whether the lignins isolated from the decayed woods were

TABLE II

COMPARISON OF NATIVE AND ENZYMATICALLY LIBERATED HARDWOOD LIGNINS

| | Oak | | Birch | | Maple | |
|---|---|---|---|---|---|---|
| | N. L.[a] | E. L.[b] | N. L. | E. L. | N. L. | E. L. |
| C, % | 58.6 | 58.4 | 61.4 | 61.6 | 61.0 | 61.3 |
| H, % | 5.3 | 5.2 | 5.5 | 5.6 | 5.6 | 5.5 |
| MeO, % | 14.8 | 14.6 | 14.9 | 14.8 | 17.4 | 17.8 |
| Phenylhydrazone MeO, % | 13.7 | 13.6 | 13.1 | 13.4 | 15.5 | 15.8 |
| Acetate MeO, % | 10.2 | 10.3 | 10.2 | 10.4 | 12.8 | 12.9 |
| Diazomethane-methylated lignin MeO, % | 25.0 | 24.8 | 26.0 | 25.8 | 23.6 | 23.9 |
| Oxidized lignin Vanillin, % | 21.3 | 20.9 | 18.6 | 19.1 | 17.2 | 16.9 |
| Syringaldehyde, % | Nil | Nil | Nil | Nil | 4.5 | 4.2 |

[a] Native lignin. [b] Enzymatically liberated lignin.

(1) The data recorded have been abridged from a part of the dissertation submitted by S. F. K. to the Graduate School of Fordham University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1951. Presented at the Lignin Symposium held during the XIIth International Congress of Chemistry, New York, 1951.

(2) (a) W. J. Schubert and F. F. Nord, THIS JOURNAL, **72**, 977 (1950); (b) W. J. Schubert and F. F. Nord, *ibid.*, **72**, 3835 (1950); (c) F. F. Nord and W. J. Schubert, *Holzforschung*, **5**, 1 (1951); (d) S. F. Kudzin and F. F. Nord, THIS JOURNAL, **73**, 690 (1951); (e) S. F. Kudzin, R. M. DeBaun and F. F. Nord, *ibid.*, **73**, 4615 (1951).

(3) K. St. G. Cartwright and W. P. K. Findlay, "Decay of Timber and Its Prevention," His Majesty's Stationery Office, London, 1946, p. 176.

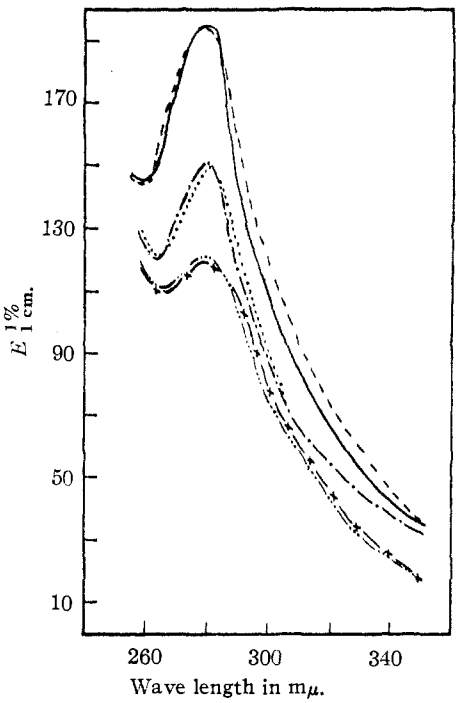


Fig. 1.—Ultraviolet absorption spectra of native and enzymatically liberated hardwood lignins: ——, oak native lignin, N. L.; - - - - - - -, oak lignin enzymatically liberated, E. L.; —·—, maple, N. L.; . . . . . ., maple, E. L.; —· · —, birch, N. L.; —x—x—, birch, E. L.

and syringaldehyde obtained upon oxidation with alkali and nitrobenzene. The results of these comparisons are recorded in Table II.

The ultraviolet and infrared absorption spectra of the native and enzymatically liberated lignins are presented in Figs. 1, 2, 3 and 4.

Although from the above data, it is evident that the native lignins of oak, birch and maple woods are most probably identical with the respective lignins obtained after partial decay of these woods by *Daedalea quercina*, it must not be assumed that the total residual lignin of these woods is identical with their respective native lignin fractions. On the contrary, the opposite must be true for, undoubtedly, there exists in oak and birch woods a lignin fraction containing syringyl groups as evidenced by the high methoxyl content of the sulfuric acid lignins of these woods, by the red coloration exhibited by the woods in the Mäule test, and by the fact that the lignosulfonic acids of oak and birch when degraded with hot aqueous alkali yield syringaldehyde as well as vanillin.[4a,b,c] In the case of maple, the higher methoxyl content of its sulfuric acid lignin[2d] as compared to the native lignin methoxyl content indicates another lignin unit possessing a higher percentage of syringyl groups than that present in the native and enzymatically liberated lignins.

On the basis of our findings with oak and birch native lignins which resemble softwood lignins very closely, it was necessary to introduce a lignin

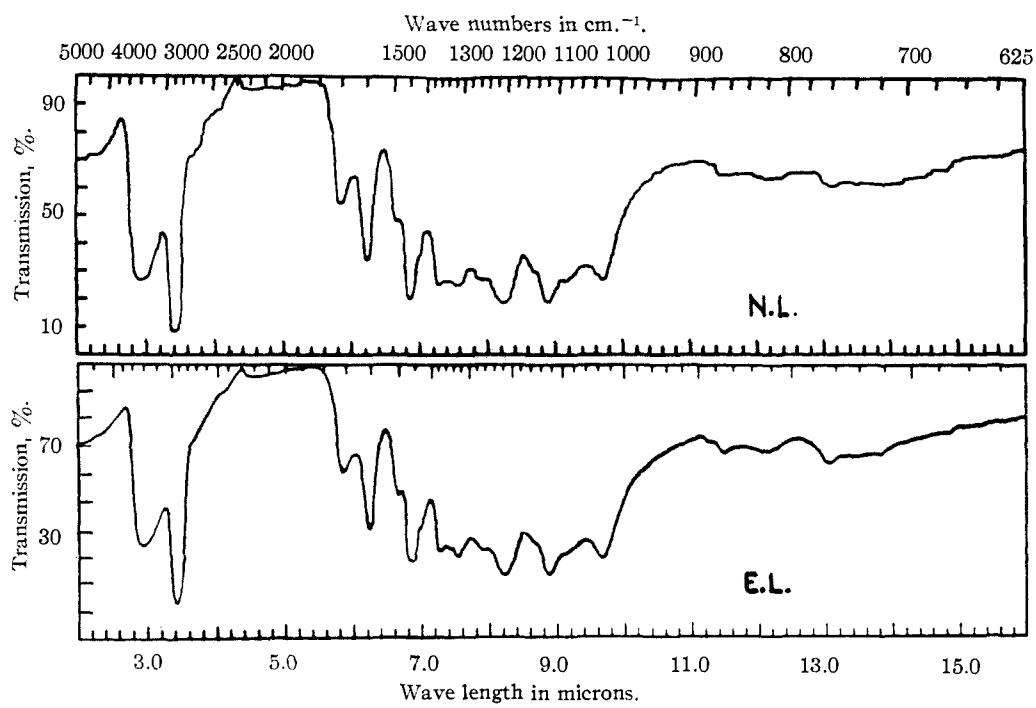


Fig. 2.—Infrared absorption spectra of oak native and enzymatically liberated lignins.

similar to or different from the native lignins isolated from the sound woods. Consequently, the enzymatically liberated lignins were compared with the native lignins as to their elementary and methoxyl analyses, the methoxyl analyses of their acetate, phenylhydrazone and diazomethane-methylated derivatives, and the yields of vanillin

nomenclature based upon the presence of the characteristic guaiacyl and syringyl groups rather than upon the source of the lignin.[2e] It was proposed that the lignins from softwoods, inasmuch as they

(4) (a) W. L. Hawkins, G. F. Wright and H. Hibbert, THIS JOURNAL, **69**, 2447 (1937); (b) F. Leger and H. Hibbert, *ibid.*, **60**, 565 (1938); (c) F. Leger and H. Hibbert, *Can. J. Research*, **16B**, 68 (1938).

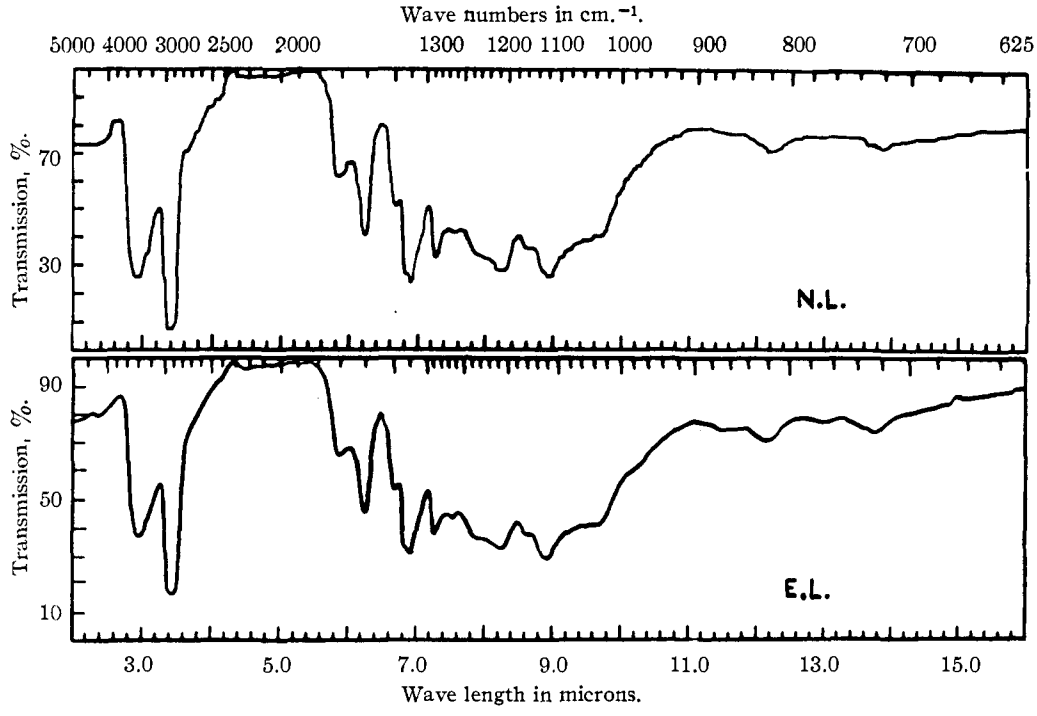


Fig. 3.—Infrared absorption spectra of birch native and enzymatically liberated lignins.

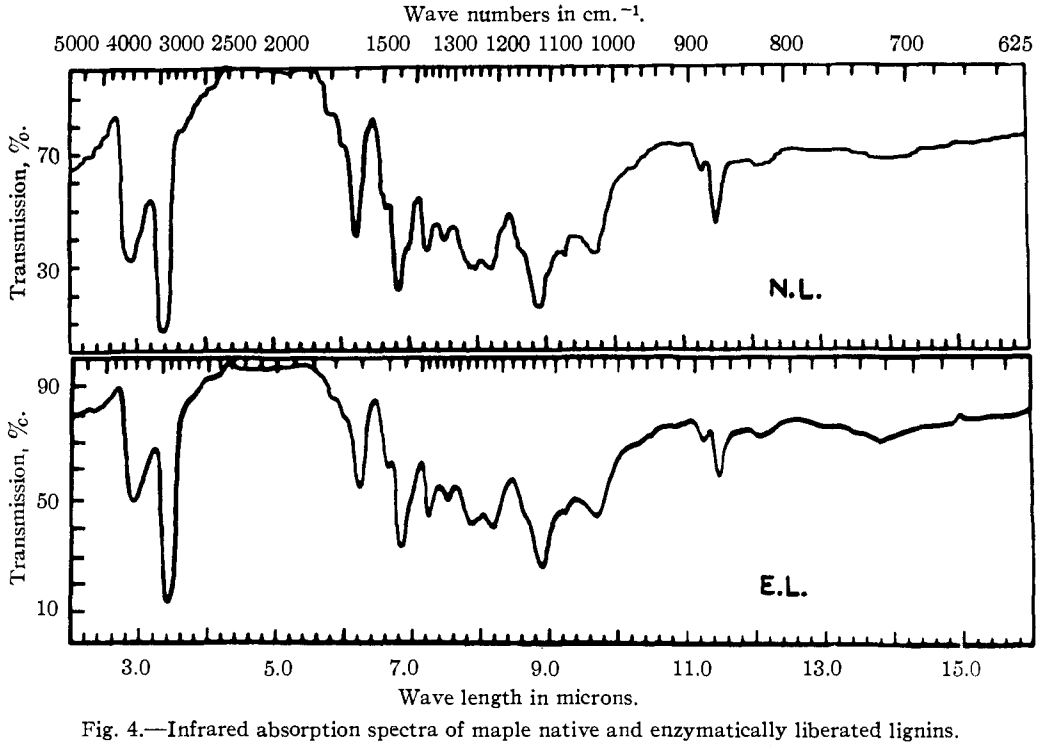


Fig. 4.—Infrared absorption spectra of maple native and enzymatically liberated lignins.

possess only guaiacyl groups in their building unit, should be designated by the term "guaiacyl softwood lignin," or simply as "softwood lignins." However, since the lignins of some hardwoods (*e.g.*, oak and birch) are composed not only of fractions containing both guaiacyl and syringyl groups, but also of fractions containing only guaiacyl groups, the former should be designated as "guaiacyl-syringyl hardwood lignin" and the latter as "guaiacyl hardwood lignin."

The possibility should not be overlooked of the existence of a "syringyl lignin" in hardwoods. However, as yet, the fractionation procedures to which lignin has been subjected are not sufficiently specific to isolate the building units of "syringyl lignin," if it exists at all. Such a unit might be obtained by a continuation of the enzymatic decay of hardwoods like oak and birch until all of the "guaiacyl lignin" has been liberated. That the "guaiacyl lignin" would be preferentially freed from

cellulosic incrustation is evidenced by increased yields of this lignin by the action of *Daedalea quercina* on oak and birch. Therefore, further decay of the "guaiacyl lignin"-free woods should liberate the syringyl containing fraction or fractions either as "guaiacyl-syringyl lignin" and/or as "syringyl lignin."

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# Investigations on Lignin and Lignification. VIII.[1] Isolation and Characterization of Bagasse Native Lignin

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Bagasse native lignin has been isolated and characterized with respect to chemical composition, solubilities in various solvents, color tests, the acetate and phenylhydrazone derivatives and ultraviolet and infrared absorption spectra. Lignin liberated by the action of the cellulolytic mold, *Poria vaillantii*, was also isolated and found to be identical with the native lignin in all respects examined. Enzymatic decay increased the yield of native lignin by eight times. Ultraviolet spectroscopic studies reveal that bagasse native lignin probably contains a carbonyl or ethylenic double bond conjugated with a dioxy phenyl ring. The oxidation of this lignin yielded vanillin and syringaldehyde in a ratio of 1:0.75. Bagasse native lignin is classified as a guaiacyl-syringyl lignin.

Wood-destroying molds are often classified as either "brown rot" or "white rot" fungi, the former assimilating mainly the carbohydrate portion of the wood, while the latter utilize lignin as their substrate. Consequently, a degradation of cellulose by "brown rot" molds should liberate the incrusted lignin, thus making it more accessible to isolation by the use of an inert solvent. That this is actually the case was recently shown when molds of this type grew on white Scots pine. The native lignins isolated before and after enzymatic attack were found to be identical.[2]

Previous investigations on lignin from bagasse, the supporting fiber of the annual plant *Saccharum officinarum*, employed specimens which had been extracted with solvents containing either alkali[3] or dilute nitric acid.[4] In consequence, studies on such specimens would be only of limited value in determining the chemical nature of the lignin as it exists *in situ*. The present report characterizes bagasse "native lignin" and the additional lignin liberated from the incrustants by the action of the "brown rot" fungus *Poria vaillantii*. Since the lignins were extracted with neutral ethanol at room temperature, they probably suffered no chemical change during isolation. Nord and co-workers[5a,b] gave references to similar isolations of lignin fractions from various woods and discussed the significance of certain intermediates in the mechanism of lignification.

## Experimental

**Isolation of Native Lignin.**—The lignin employed for comparative purposes was obtained essentially by the method of Brauns.[6] Air-dry virgin bagasse, ground to 40 mesh, was extracted thoroughly with cold water and with ether. It was then extracted at room temperature in a percolator-type extractor[5b] with 95% ethyl alcohol until the extract no longer gave the phloroglucinol-hydrochloric acid color reaction. Upon removal of the alcohol by distillation at reduced pressure, a resinous material remained. This was washed well with cold water and with ether. The resulting powder was dried, dissolved in dioxane, centrifuged, filtered and precipitated into thirty times its volume of ice-cold distilled water. The precipitate was dried, redissolved in dioxane and reprecipitated into thirty times its volume of ether. This procedure was repeated until a constant methoxyl value was obtained. The lignin so isolated and purified was a light tan colored, electrostatic powder. The yield amounted to 0.4% on a moisture-free basis.

The acetate and phenylhydrazone derivatives were also prepared.[6]

**Sterilization and Inoculation of Bagasse Samples.**—Ten-gram samples of ground bagasse were weighed into five Fernbach-type culture flasks, and to each was added 30 ml. of the following nutrient medium

| | |
|---|---|
| Neopeptone | 1.0 g. |
| $KH_2PO_4$ | 1.5 g. |
| $MgSO_4 \cdot 7H_2O$ | 0.5 g. |
| Thiamine hydrochloride | 2.0 mg. |
| Tap water to | 1 liter |

The flasks were plugged with cotton and sterilized by Tyndallization. After cooling, each flask was inoculated with a 5-ml. spore-mycelial suspension of *Poria vaillantii*[7] which had been previously sown on a medium containing the above nutrients plus glucose and agar. This organism was used because preliminary experiments indicated that it assimilated the carbohydrates in bagasse at a faster rate than some other wood-destroying molds.

The inoculated flasks were incubated in the dark at 27–28° for eight months. The decayed bagasse was analyzed periodically for its relative cellulose and lignin content.

**Analytical Methods.**—After separation of the fungal mycelia from the decayed bagasse, the latter was collected and dried. Cellulose was determined according to an earlier method[8] and lignin by the standard method.[9]

**Isolation of Enzymatically Liberated Bagasse Lignin.**—The enzymatically liberated lignin was extracted from the decayed bagasse in the same manner as indicated above under "Isolation of Native Lignin."

(1) Presented at the Lignin Round Table held during the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., 1951.

(2) W. J. Schubert and F. F. Nord, THIS JOURNAL, **72**, 977, 3835 (1950).

(3) Y. Hachihama and H. Saegusa, *J. Soc. Chem. Ind. Japan*, **37**, suppl. binding 771–2 (1934).

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(5) (a) S. F. Kudzin and F. F. Nord, *ibid.*, **73**, 680 (1951); (b) F. F. Nord and W. J. Schubert, *Holzforschung*, **5**, 1 (1951); (c) S. F. Kudzin, R. M. DeBaum and F. F. Nord, THIS JOURNAL, **73**, 4615 (1951).

(6) F. E. Brauns, *ibid.*, **61**, 2120 (1939).

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(9) E. C. Sherrard and E. E. Harris, *Ind. Eng. Chem.*, **24**, 103 (1923).