was obtained in the anti-R system, but an increase in the anti-Bg. Since the R_3' antigen had only three combining sites and bovine globulin would probably have many more, one might expect the R_3' system to be inhibited more easily by incomplete antibodies while the large number of combining sites on the globulin molecule could accomodate both incomplete and precipitating antibodies and result in an actual increase in precipitated protein. Interpretation must, however, await further investigation since it is obvious too little is known at present regarding the significance of the relative concentration of the various antibodies and mechanisms involving competition for antigenic combining sites.

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[CONTRIBUTION NO. 226 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY AND ENZYMOLOGY, FORDHAM UNIVERSITY]

Investigations on Lignin and Lignification. VI. The Comparative Evaluation of Native Lignins[1]

BY S. F. KUDZIN, ROBERT M. DEBAUN AND F. F. NORD

The native lignins of white Scots pine, oak, birch and maple and the native lignin of white Scots pine obtained after decay by *Lentinus lepideus* were compared by chemical and physical methods. The lignins were methylated with diazomethane, oxidized with nitrobenzene and alkali, subjected to paper chromatography and tested colorimetrically with the phosphotungstic–phosphomolybdic reagent for phenols. Infrared spectra of the softwood lignins and of the methylated softwood and hardwood lignins are presented. The lignins from sound and decayed white Scots pine contained the same number of methylatable groups, while the other lignins differed from the white Scots pine and from each other in this respect. None of the lignins yielded syringaldehyde upon oxidation, excepting that derived from maple wood. All the lignins responded similarly to the phenol reagent, except lignin from oak wood. The lignins from sound and from decayed white Scots pine were shown to be similar in every respect investigated.

Chemically prepared lignins from softwoods and hardwoods are known to be materially different, especially as regards methoxyl content. Although aspen native lignin[2] has been shown to differ from softwood native lignins, the native lignins of oak, birch and maple[3] possess many properties similar to those of softwood lignins.[4] Furthermore, the native lignins from sound and decayed softwoods also possess similar properties.[4c,d,e]

We have extended the comparison of the native lignins from sound white Scots pine and the native lignin from that wood after decay by *Lentinus lepideus* and of the native lignins of the hardwoods oak, birch and maple by means of methylation with diazomethane, infrared spectra of the methylated lignins, quantitative determination of the vanillin and syringaldehyde obtained on oxidation with nitrobenzene and alkali, paper chromatography, and colorimetric response to the phenol reagent.[5] The previously reported[4] spectra of the lignins from white Scots pine are interpreted with respect to the functional groups present.

Methylation with diazomethane is generally considered to be specific for phenolic, enolic and acidic hydroxyl groups. In addition to the purely comparative value, the oxidation of the hardwood lignins was undertaken to corroborate our previous suggestion[3] that oak and birch native lignins were devoid of syringyl groups, whereas the maple native lignin appeared to contain such structures. The lignins were subjected to paper chromatography in order to ascertain the homogeneity of the samples. Quantitative response to the phenol reagent was estimated as a relative measure of the groups reacting with this substance. A similar reagent[6] was used in the comparison of native lignin preparations obtained from cork.[7] Although the reagent is affected by phenolic hydroxyl groups, the chemistry of the reaction is not altogether clear.[8,9] The reagent is a phosphotungstic–phosphomolybdic acid of the 1:18 series according to the earlier nomenclature.[10]

Experimental

The native lignins studied were those previously reported.[3,4c]

Methylation.—A dioxane solution of diazomethane for the methylation of one gram of native lignin was prepared by adding one gram of nitrosomethylurea to a cooled mixture of dioxane (14 ml.) and 45% potassium hydroxide (2 ml.). The dioxane solution was then decanted and allowed to stand over pellets of potassium hydroxide for several hours. This solution was then added to a 10% solution of native lignin in dioxane, and the mixture was allowed to stand overnight, centrifuged and precipitated into ether. This procedure was repeated until a constant methoxyl value was obtained.

Oxidation of Native Lignin and Quantitative Determination of Vanillin and Syringaldehyde.—A chromatographic separation[11] was employed for these studies.

Paper Chromatography of the Native Lignins.—Dioxane solutions of the native lignins were placed in a front along the bottom of a piece of Schleicher and Schuell paper No. 696. The dioxane solutions were allowed to dry, and the paper rolled into a cylinder, held fast with paper clips and

(1) Presented at the *Lignin Round Table* held during the XIIth Internat. Congress of Chemistry, New York, N. Y., 1951. These data are taken from parts of the dissertations of S.F.K. and R.M.D. submitted to the Graduate School of Fordham University in partial fulfillment of the requirements of their degrees of Doctor of Philosophy, 1951. For paper No. V of this series see THIS JOURNAL, **73**, 1358 (1951).

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

(3) S. F. Kudzin and F. F. Nord, *ibid.*, **73**, 690 (1951).

(4) (a) F. E. Brauns, *ibid.*, **61**, 2120 (1939); (b) *J. Org. Chem.*, **10**, 211 (1945); (c) W. J. Schubert and F. F. Nord, THIS JOURNAL, **72**, 977 (1950); (d) *ibid.*, **72**, 3835 (1950); (e) F. F. Nord and W. J. Schubert, *Holzforschung*, **5**, 1 (1951).

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(11) J. E. Stone and M. J. Blundell, abstract of a paper presented before the Division of Cellulose Chemistry, A. C. S., Chicago, Ill., September, 1950. The present authors appreciate the courtesy of Dr. Stone enabling them to read the manuscript prior to publication.

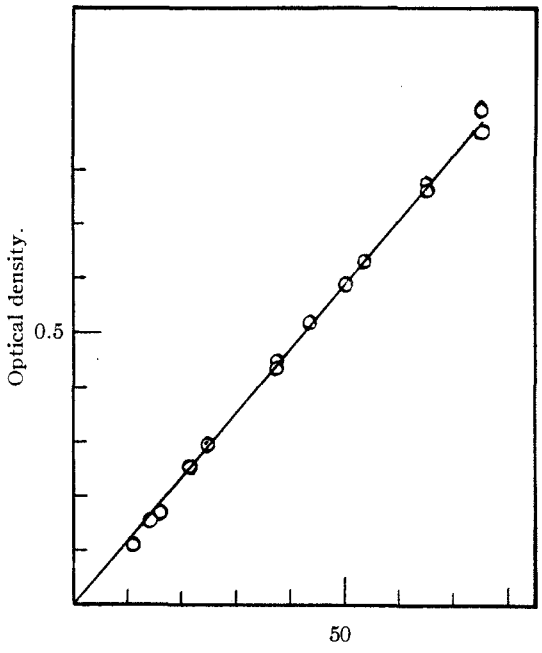

Fig. 1.—Calibration curve of phenol reagent method.

way, pink spots indicative of the lignin itself[12] could be observed. Also, a spray with a ten times diluted Folin phenol reagent, followed by a spray with a 15% sodium carbonate solution, applied to an identical strip of paper brought out any substance reacting with this reagent. Both colors were transient. Water was found to be the best solvent for resolving substances which reacted with either reagent, from those which reacted only with the phenol reagent. Dioxane, ethanol and methyl and ethyl acetate were also used as were their binary and ternary mixtures. On the basis of a purely visual observation the relative amounts of these materials could be estimated.

Colorimetry.—The phenol reaction was carried out quantitatively by using β-naphthol as a standard substance. The region of maximum absorption was above 700 mμ, as obtained with a Coleman spectrophotometer. The reaction was run on a quantitative basis with an Evelyn Photoelectric colorimeter, using a filter of 720 mμ. Two-tenths ml. of a dioxane solution of the substance to be tested, containing about 100 γ, was mixed with 0.8 ml. of 0.04 *N* NaOH. One ml. of Folin phenol reagent was then added, then 3.0 ml. of 15% sodium carbonate and finally 5.0 ml. of distilled water. After one hour the color intensity was measured. The blank was identical except that it contained pure dioxane instead of a dioxane solution. The results are reported as optical density at 720 mμ, divided by γ of the substance present, the whole multiplied by 1,000. The calibration curve with β-naphthol is presented in Fig. 1.

Infrared Absorption Spectra.—An experimental model of the Baird Double-Beam Recording Infrared Spectrophotometer was used. The wave length calibration of this instrument is within 0.04μ. The samples were mulled in mineral oil, and a wire mesh served as the compensating cell.

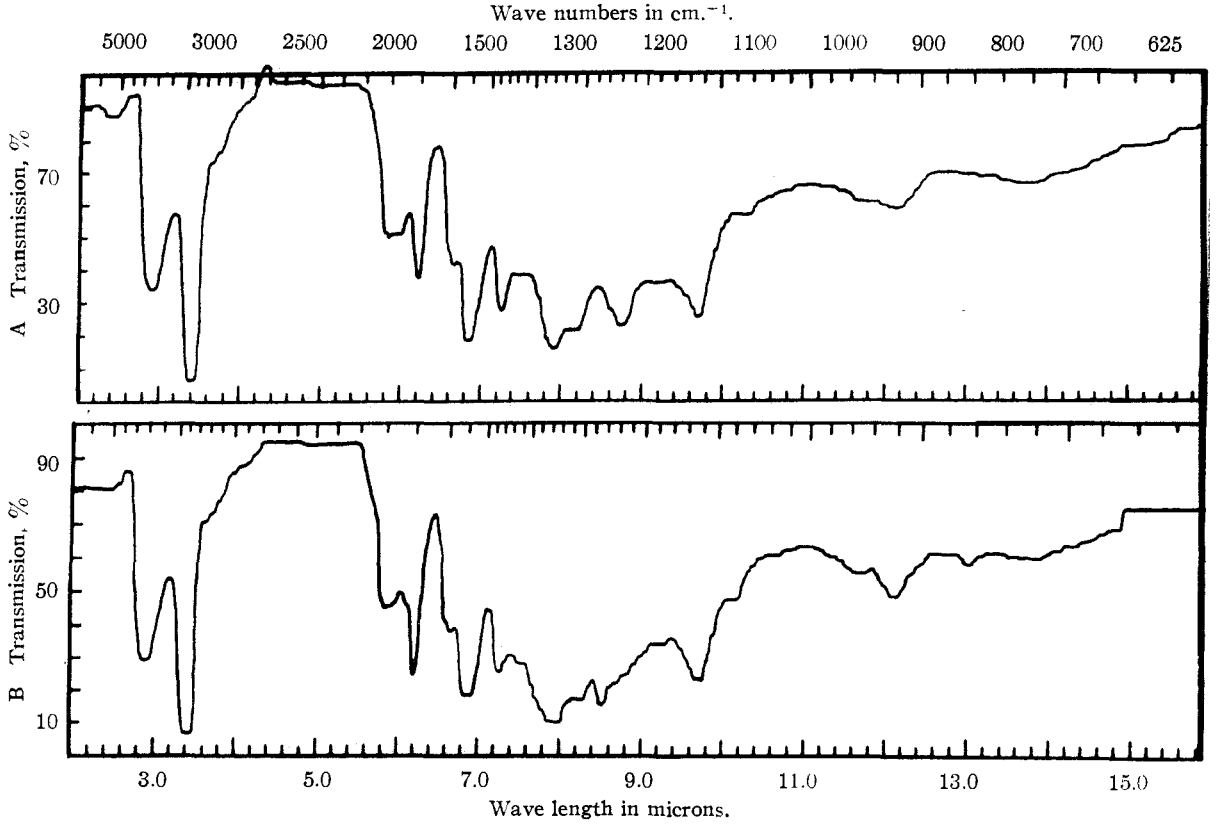

Fig. 2.—Infrared absorption spectra of white Scots pine lignins: A, lignin from sound wood; B, lignin from decayed wood.

placed upright in a transparent cylinder, containing about 50 ml. of developing solvent, the top of which was then covered with a glass plate and sealed shut with stopcock grease. After an appropriate time the paper was removed, dried in a current of air, and the colored fronts were developed. The colored spots could be obtained by spraying the paper with an 0.1 *M* solution of phloroglucinol in alcohol, followed by a spray with concentrated hydrochloric acid. In this

Results and Discussion

The infrared absorption spectra of the native lignins from sound white Scots pine and from that wood after decay by *Lentinus lepideus* are presented in Fig. 2. The infrared spectra of the methylated

(12) R. M. DeBaun and F. F. Nord, *TAPPI*, **34**, 71 (1951).

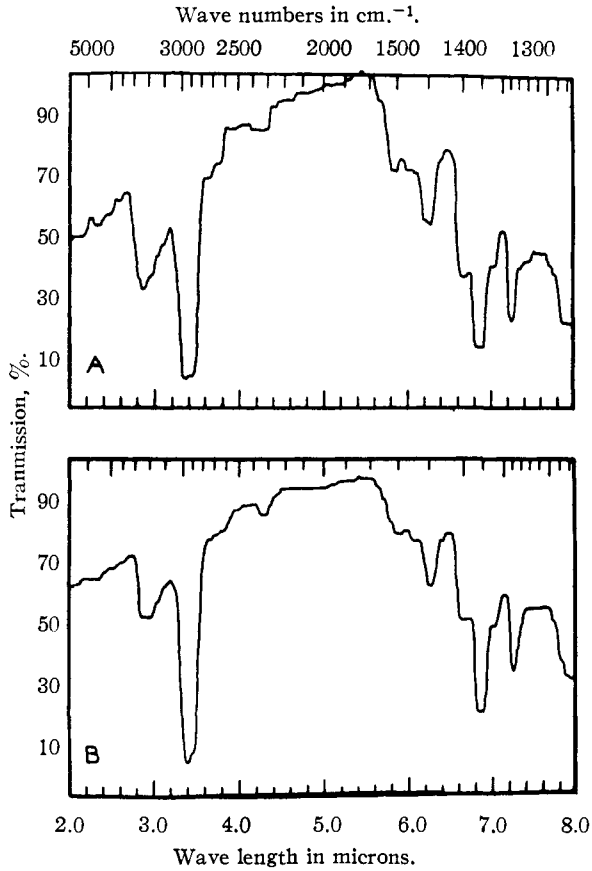

Fig. 3.—Infrared absorption spectra of methylated white Scots pine lignins: A, from sound wood; B, from decayed wood.

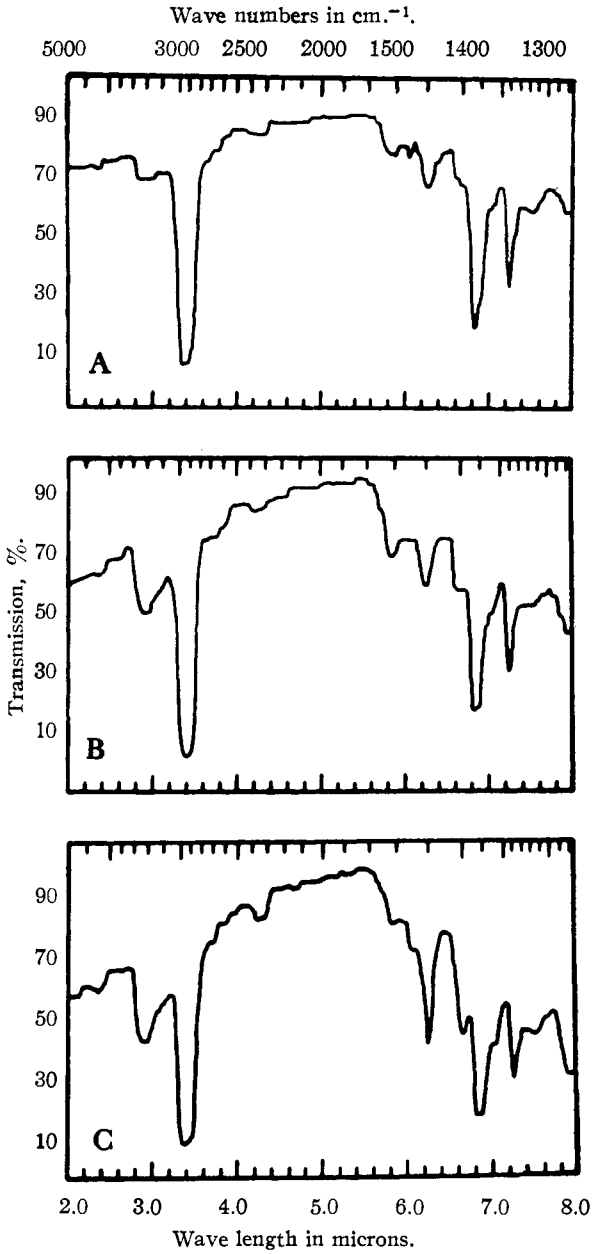

Fig. 4.—Infrared absorption spectra of methylated hardwood lignins: A, oak; B, birch; C, maple.

lignins are presented in Figs. 3 and 4. In Table I are recorded the calculated and observed methoxyl values of the methylated lignins for the number of groups introduced, the quantities of vanillin and syringaldehyde obtained on oxidation with nitrobenzene and alkali, the relative response of the lignins to the phenol reagent and the behavior of each lignin toward chromatography.

The structural similarity of the native and enzymatically liberated lignins of white Scots pine is apparent from the general appearance of their infrared curves (Fig. 2). The lack of distinct sharp bands is indicative of a high molecular weight, amorphous substance. Specifically, however, the band at 3450 cm.$^{-1}$ suggests bonded hydroxyl groups. A carbonyl group is represented by the band at 1710 cm.$^{-1}$. Phenyl ring skeletal vibrations with possible para substitution are evidenced at 1605 and 1510 cm.$^{-1}$. The bands at 1262 and 1220 cm.$^{-1}$ may be attributed in general to aromatic C–O–C, aromatic C–O and phenyl OH and

TABLE I

Native lignin	Unit mol. wt.[a]	Methylation: No. of groups introduced	Methylation: Methoxyl, % Calcd.	Methylation: Methoxyl, % Found	Oxidation yields, %: Vanillin	Oxidation yields, %: Syringaldehyde	Phenol[c] reagent	Chromatographic impurities[d]
White Scots pine	848	1	18.0	18.5	19.5	Nil	2.96	Trace
White Scots pine[e]	848	1	18.0	17.5	18.7	Nil	3.02	Trace
Oak	838	3	24.7	25.0	21.3	Nil	5.15	Trace
Birch	833	3–4	24.8–27.8	26.0	18.8	Nil	2.87	Trace
Maple	875[b]	2	24.0	23.6	17.2	4.5	2.90	Trace

[a] Molecular weight calculated on the basis of one carbonyl and four methoxyl groups per unit. [b] Molecular weight calculated on the basis of one carbonyl and five methoxyl groups per unit. [c] (Optical density at 720 mμ/γ of lignin) × 1000. [d] Indicates material moving with developing solvent and reacting with phenol reagent, but not with phloroglucinol-HCl. [e] Obtained from wood decayed by *Lentinus lepideus*.

CHO groups. Phenyl ring substitution is established at 820 and 760 cm.$^{-1}$.

Inasmuch as both the lignins from this wood possess only one methylatable group, which is undoubtedly phenolic, it is evident that the carbonyl group did not enolize during methylation.[4] The proportionate diminution of the 3450 cm.$^{-1}$ band with methylation, as is evidenced in Fig. 3, confirms the identification of its hydroxyl group origin. The close resemblance between the two softwood lignins, both before methylation (Fig. 2) and after methylation (Fig. 3), together with their comparable behavior upon methylation, oxidation and colorimetric assay, attest to the probable identity of these two preparations as previously indicated.[4c,d,e] This assertion is strengthened by the fact that both lignins behaved as essentially homogeneous under the conditions of our chromatographic procedure.

The infrared spectra of the hardwood native lignins[3] indicated a resemblance to the softwood native lignins in the type of functional groups present but a variance in the molecular quantities of these groups. This fact is substantiated by the divergence in the quantitative extent of methylation with diazomethane and by the varying results with the phenol reagent.

On the basis of analytical and spectroscopic data and the specificity of the Mäule test, it was suggested[3] that the native lignin fractions of oak and birch are related to the native lignin of softwood, whereas the maple wood yields a native lignin more characteristic of hardwoods. This relationship is borne out by the failure of oak and birch native lignins to yield syringaldehyde. On the basis of our methylation experiments in the case of the hardwood lignins, we cannot discount the possibility of an enolizable carbonyl group, since more than one group underwent methylation in each case. The proportionate decrease in intensity of the 3450 cm.$^{-1}$ band in the methylated products (Fig. 4) establishes its hydroxyl group origin. These lignins also behaved as essentially homogeneous materials in our chromatographic procedure.

Considering our findings with the oak and birch native lignins, which resemble softwood lignins very closely, the designation of a lignin as "softwood" or "hardwood" lignin, depending upon its source, is no longer justifiable. Since the differentiation between softwood and hardwood lignins was based upon the presence of only guaiacyl groups in the former and of both guaiacyl *and* syringyl groups in the latter, and since it has now been demonstrated that only guaiacyl groups comprise the building units of a portion of the lignins in certain hardwoods, the introduction of the use of a nomenclature based upon the presence of these characteristic groups rather than the source of the lignin would be advisable. Thus, since the lignins from softwoods possess only guaiacyl groups as a building unit, the term "guaiacyl softwood lignin," or simply "softwood lignin" would suffice. However, since the lignins of certain hardwoods 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."

These results amply support the contention that lignins must be studied with a view to diversity as well as similarity, as they can vary not only between hardwood and softwood but also from one species to another and within the same species.

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