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Infrared Spectra of Lignin and Related Compounds. II. Conifer Lignin and Model Compounds[1,2]

HERBERT L. HERGERT

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The infrared spectra of conifer lignin model compounds and degradation products were determined, and characteristic frequencies of carbonyl groups, ethylenic double bonds, aromatic rings, and hydroxyl groups are presented. These results are applied to lignins isolated from five different coniferous genera. The functional groups occurring in the lignin molecule are discussed and related to the mode of isolation.

Absorption spectrophotometry has been used increasingly as a supplement to purely chemical methods in order to elucidate the structure of gymnosperm and angiosperm lignin. While ultraviolet studies of the lignin molecule have been valuable,[3,4] the information provided is limited to aromatic ring substituents and conjugated groups. On the other hand, infrared spectra, which are potentially capable of yielding information concerning the lignin side chain, as well as aromatic and conjugated substituents, have been mainly used for characterization purposes.[5] In the present investigation, the infrared spectra of a large number of lignin products have been measured in order to determine whether differences exist between native (solvent-soluble) lignin[6] and mildly prepared whole wood lignin, whether lignin structure varies with genera, and to measure the changes introduced into the basic structure of the lignin molecule by commercially important isolation procedures such as the sulfite and kraft processes. In general, the procedure followed was to determine the effect, on the characteristic group frequencies of model compounds and low molecular weight lignin degradation products, of acetylation, methylation, treatment with alcoholic hydrogen chloride, *i.e.*, "ethanolysis," conversion to a sodium salt or phenolate, and reduction with sodium borohydride or lithium aluminum hydride. After assignments of frequencies to certain functional groups were made, comparisons of lignins isolated and treated by the same methods were then carried out.

EXPERIMENTAL

Spectra. The infrared instrument used in this work was a Model 21 Perkin-Elmer spectrophotometer equipped with sodium chloride optics and linear in wave number. Spectra were obtained as paraffin (Nujol) mulls, potassium bromide wafers, or films deposited from chloroform or dioxane. Liquid samples were run between salt plates separated by a lead spacer.

Compounds. Vanillin, vanillic acid, and related compounds were prepared in this laboratory by alkaline hydrolysis or copper oxide oxidation[7] of lignosulfonates derived from western hemlock wood. Acetylvanilloyl [1-(4-hydroxy-3-methoxyphenyl)-1,2-propanedione], α-hydroxy propiovanillone [2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone], α-ethoxy propioveratrone [2-ethoxy-1-(3,4-dimethoxyphenyl)-1-propanone], guaiacyl acetone [1-(4-hydroxy-3-methoxyphenyl)-2-propanone], β-hydroxy coniferyl alcohol[8] [3-hydroxy-1(3,4-dimethoxyphenyl)-2-propanone], and 1-ethoxy-1-(4-acetoxy-3-methoxyphenyl)propanone-2 were obtained from Dr. J. A. F. Gardner. The latter compound was subsequently deacetylated by the procedure of Kulka and Hibbert.[9] Dehydrodiconiferyl alcohol and pinoresinol were obtained from Prof. H. Erdtman. Coniferin was isolated from western hemlock cambium.[10] Vanilloylformic acid[11] was obtained from Dr. D. W. Glennie. Apocynol [1-hydroxy-1-(4-hydroxy-3-methoxyphenyl)ethane] and methylated derivatives were obtained from Dr. M. Cronyn.[12] The hydroxyl, carbonyl, and aromatic stretching frequencies of this series of compounds are presented in Table I.

Lignin products. Native lignins were prepared according to Brauns' procedure[6] from the wood of Douglas fir (*Pseudotsuga menziesii*), Pacific silver fir (*Abies amabilis*), longleaf pine (*Pinus palustris*), western red cedar (*Thuja plicata*), and western hemlock (*Tsuga heterophylla*). Further purification was effected by repeated precipitation of dioxane solutions of native lignins into chloroform or by adsorption chromatography.[13] Extractive-free longleaf pine and hemlock wood were ground in a vibratory ball mill, constructed according to plans furnished by National Bureau of Standards,[14] to give milled wood lignin[15] in yields of 12 and 16%, respectively.

(1) Presented in part at the Wood Chemistry Symposium, Northwest Regional Meeting of the American Chemical Society, Seattle, Wash., June 12, 1956.

(2) Part I of this series: H. L. Hergert and E. F. Kurth, *J. Am. Chem. Soc.*, **75**, 1622 (1953).

(3) O. Goldschmid, *J. Am. Chem. Soc.*, **75**, 3780 (1953); *Anal. Chem.*, **26**, 1421 (1954).

(4) G. Aulin-Erdtman and L. Hegborn, *Svensk Papperstidn.*, **60**, 671 (1957); K. Freudenberg and G. Schuhmacher, *Sitzber. heidelberg. Akad. Wiss.*, 127 (1956).

(5) (a) E. Jones, *Tappi*, **32**, 167 (1949); (b) S. Kudzin, R. Debaun, and F. Nord, *J. Am. Chem. Soc.*, **74**, 4615 (1953); (c) G. DeStevens and F. Nord, *J. Am. Chem. Soc.*, **73**, 4622 (1953).

(6) (a) F. Brauns, The Chemistry of Lignin, Academic Press, New York, 1952, p. 51; (b) *J. Am. Chem. Soc.*, **61**, 2120 (1939).

(7) I. A. Pearl, *J. Am. Chem. Soc.*, **72**, 2309 (1950); I. A. Pearl and E. Dickey, *J. Am. Chem. Soc.*, **74**, 614 (1952); I. A. Pearl and D. L. Beyer, *J. Am. Chem. Soc.*, **76**, 2224 (1954); *Tappi*, **39**, 171 (1956).

(8) J. A. F. Gardner, *Can. J. Chem.*, **32**, 532 (1954).

(9) M. Kulka and H. Hibbert, *J. Am. Chem. Soc.*, **65**, 1185 (1943).

(10) O. Goldschmid and H. L. Hergert, unpublished work.

(11) D. W. Glennie, H. Techlenberg, E. T. Keaville, and J. L. McCarthy, *J. Am. Chem. Soc.*, **77**, 2409 (1955).

(12) M. Cronyn, Paper presented at the Northwest Regional Meeting of the American Chemical Society (June 1957).

(13) P. Enslin, *J. Sci. Food Agr.*, **4**, 328 (1953).

TABLE I

HYDROXYL, CARBONYL, AND AROMATIC RING STRETCHING FREQUENCIES OF LIGNIN MODEL COMPOUNDS AND DEGRADATION PRODUCTS

Compound	Frequency (cm.$^{-1}$) OH	C=O	Aromatic Ring
"Ethanolysis" Products			
Acetylvanilloyl[a]	3330	1700, 1649	1596,[d] 1587, 1502
α-Hydroxy propiovanillone[a]	3430, 3395,[e] 3140	1664	1596, 1587, 1512
α-Ethoxy propioveratrone[a]	—	1671	1590, 1582, 1509
α-Ethoxy propioveratrone[b]	3450[e]	1677	1596, 1589, 1517
Guaiacyl acetone[c]	3410	1705	1598, 1511
1-Ethoxy-1-guaiacyl propanone-2[a]	3429	1710	1600, 1512
1-Ethoxy-1-guaiacyl propanone-2 acetate[b]	—	1768, 1715	1602, 1512
Aldehydes			
Vanillin[a]	3145	1663	1595,[d] 1588, 1509
Vanillin acetate[a]	—	1752, 1699, 1688, 1675	1598, 1508
Vanillin-sodium salt[a]	3450,[d] 3230	1685,[e] 1655, 1638[e]	1582, 1548,[e] 1502
Veratraldehyde[a]	—	1696, 1684, 1672	1597, 1587, 1512
5-Formyl vanillin[a]	—	1683, 1650	1591, 1474
5-Carboxyl vanillin[a]	—	1678, 1655[d]	1578, 1483
Dehydrodivanillin[a]	3250	1673	1603, 1585, 1502
3,4-Dihydroxybenzaldehyde[a]	3320, 3230	1654, 1646	1596, 1537
4-Hydroxy benzaldehyde[a]	3140	1662	1594, 1515
Syringaldehyde[a]	3250	1668	1604, 1585, 1512
Benzaldehyde[c]	—	1702	1599, 1587
Coniferyl aldehyde[a]	3135	1652	1594, 1578, 1514
3,4-Dimethoxy cinnamaldehyde[18]	—	1661	1588, 1575
Cinnamaldehyde[c]	—	1672	1600, 1570, 1490
Ketones			
Acetovanillone[a]	3290	1653	1600, 1572, 1513
Acetovanillone acetate[a]	—	1768, 1680	1595, 1584, 1510
Acetovanillone-sodium salt[a]	3240	1639	1576, 1538, 1509
Acetoveratrone[c]	—	1672	1596,[d] 1590, 1513
p-Hydroxy acetophenone[a]	3160	1645	1600, 1585, 1514
p-Hydroxy acetophenone[b]	3200	1655	1605, 1575, 1515
p-Hydroxy propiophenone[a]	3180	1648	1600, 1568, 1510
Acetophenone[c]	—	1685	1599, 1482
β-Hydroxy coniferyl alcohol[a]	3435, 3355	1709	1599, 1505
β-Hydroxy coniferyl alcohol[b]	3450, 3370	1714	1605, 1513
Acids and Esters			
Vanillic acid[a]	3480	1677	1599, 1583, 1525
Vanillic acid acetate[a]	—	1760, 1686	1602, 1594,[d] 1509
Sodium vanillate[a]	3330	1550	1600, 1518
Methyl vanillate[a]	3540	1699	1600, 1515
5-Formyl vanillic acid[a]	3490	1672, 1651	1579, 1485
5-Carboxyl vanillic acid[a]	3410, 3210	1682,[d] 1655	1600, 1581, 1488
Vanilloyl formic acid[a]	3490	1735, 1625	1580, 1517
3,4-Dimethoxy benzoic acid[a]	—	1672	1590, 1518
Conidendrin[b]	3420	1754	1610, 1600, 1507, 1580

[a] Paraffin mull. [b] Kbr, pellet. [c] l, Liquid film. [d] Shoulder. [e] Weak.

Dioxane lignin was prepared from extractive-free wood samples previously used to prepare native lignins as follows: Twenty-gram samples of pulverized wood were placed in alundum thimbles and extracted for 2 hr. in a glass Soxhlet extractor with 250 cc. of dioxane and 2.5 cc. of concentrated hydrochloric acid. The extract was concentrated to 15 cc. *in vacuo* and poured into 500 cc. water. The water-insoluble lignin was dissolved in dioxane and precipitated into ether. Yield, 60–72% of the Klason lignin content of the wood. Methoxyl content varied from 14.7–15.2%.

Kraft lignin was prepared by bubbling carbon dioxide into the filtrate from a conventional kraft cook of western hemlock wood. The precipitate was filtered off, washed, and dried. Analyses indicated 2.3% sodium, 0.9% sulfur, 13.3% methoxyl, and an average diffusion coefficient of 23.1 mm.^{2}/day, *i.e.*, an apparent molecular weight[16] of 1870. The product was subsequently converted to the free acid by suspension in dilute sulfuric acid. After filtration, washing, and drying, the product was further purified by dissolving it in dry dioxane, filtering, and then precipitating it into dry ether. The product contained 0.05% sodium and 13.4% methoxyl. Similar products were prepared from pine and Douglas fir.

Calcium lignosulfonate was prepared from the filtrate of a conventional calcium-base sulfite cook of western hemlock according to the procedure of Gray and Crosby.[17] It con-

(14) F. Forziati, W. Stone, J. Rowen, and W. Appel, *J. Research Natl. Bur. Standards*, **45**, 109 (1950).

(15) A. Bjorkman, *Svensk Papperstidn.*, **59**, 477 (1956).

(16) V. Felicetta, A Markham, Q. Peniston, and J. McCarthy, *J. Am. Chem. Soc.*, **71**, 2879 (1949).

(17) K. Gray and H. Crosby, U. S. Patent **2,801,994** (August 6, 1957).

tained 8.6% methoxyl, 4.69% calcium, and 5.2% sulfur. The free lignosulfonic acid was prepared by treating a 5% aqueous solution of the calcium lignosulfonate with a large excess of Amberlite IRC 120 resin. The solution was evaporated to 25% solids *in vacuo* and poured into dioxane to give a tan-colored precipitate which was washed with acetone, ether, and hexane. After drying in a vacuum desiccator over phosphorus pentoxide for 8 hr., the infrared spectrum was immediately determined. A desulfonated product was prepared by suspending 10 g. calcium lignosulfonate in 100 cc. 10% sodium hydroxide solution for 1 hr. at 175°. After cooling, the mixture was acidified with sulfuric acid and the precipitate purified similarly to kraft lignin. The product contained less than 0.2% sulfur.

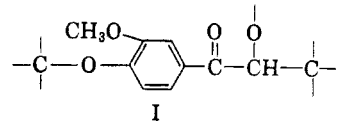
Acetate derivatives were prepared by treatment with acetic anhydride and pyridine. Phenolic hydroxyl groups were methylated in lignin products by refluxing 1 g. lignin in a mixture of 100 cc. dry acetone, 10 g. anhydrous potassium carbonate, and 3 g. dimethyl sulfate for 3 hr. Reduced lignins were prepared by lithium aluminum hydride reduction in dry tetrahydrofuran (3 hr.) or sodium borohydride in 50% methanol (4 hr.). Native lignin (1 g.) was alkylated[6,8] by refluxing for 3 hr. in 100 cc. absolute ethanol and 0.4 g. hydrogen chloride. The product was recovered by precipitation into ether. Ethanol–hydrochloric acid lignin was prepared by refluxing extractive-free wood with 0.5% ethanolic hydrogen chloride for 8 to 24 hr. The product was worked up in the same way as dioxane–hydrochloric acid lignin.

RESULTS

Native lignins. The infrared spectra of native lignins from five different coniferous genera were compared and found to be similar but not identical. It was concluded that the lignins differed in structure and/or in their content of extraneous matter. The most readily apparent difference is in the 1650–1775 cm.$^{-1}$ region. Silver fir, western red cedar, and hemlock native lignins have an absorption band with varying intensity at 1749, 1760, and 1751 cm.$^{-1}$, respectively, which is absent in the spectra of pine and Douglas fir native lignins. This band is not removed upon reduction with sodium borohydride, but disappears upon lithium aluminum hydride reduction, so it was attributed to the presence of a five-membered ring lactone carbonyl group. Examination of these native lignins by two dimensional paper chromatography indicated the presence of several extraneous compounds, one of which was identified as α-hydroxy matairesinol.[18,19] These compounds are relatively insoluble in ether and tend to be occluded with lignin during the precipitation into ether. Since they are somewhat more soluble in chloroform, repeated precipitations of a dioxane solution of hemlock native lignin into chloroform markedly decreased the 1751 cm.$^{-1}$ absorption band, and the presence of extraneous compounds on chromatograms.

The Douglas fir and hemlock native lignins further differed from the other native lignins and mildly prepared whole wood lignins in the relatively greater height of the 1600 cm.$^{-1}$ aromatic ring stretching band as compared with the 1510 cm.$^{-1}$ band. In phenolic extractives such as tannins, flavanones, etc., which contain a phloroglucinol and catechol nucleus, the 1600 cm.$^{-1}$ band is more intense than the 1500 cm.$^{-1}$ band, so this suggested that similar materials might be present in these native lignins as impurities and would be reflected in a higher phenolic hydroxyl content. This has been substantiated by an appreciably higher phenolic hydroxyl content of hemlock native lignin (3.8%) compared with longleaf pine or spruce native lignin (2.5-3.3%) as determined by ultraviolet alkaline difference spectra.[3] Furthermore, a leucoanthocyanin test[20] was positive. In the case of Douglas fir native lignin, a peak in the alkaline ultraviolet spectrum at 3300 Å and an infrared band at 1640 cm.$^{-1}$ indicated that the product was contaminated with traces of taxifolin glucoside[21] and/or taxifolin degradation products.

All of the native lignins show a moderately strong absorption at 1660 cm.$^{-1}$ and a very weak band at 1712 cm.$^{-1}$, both of which disappear upon reduction with sodium borohydride and must, therefore, be due to aldehyde or ketone groups. Methylation does not shift the frequency of either band. Acetylation shifts the frequency of the original 1660 cm.$^{-1}$ band to 1670 cm.$^{-1}$, but does not affect the frequency of the 1712 cm.$^{-1}$ band. The 1660 cm.$^{-1}$ band is unaffected by formation of a sodium salt of the native lignin, but the 1712 cm.$^{-1}$ band disappears and is replaced by a new band at 1575 cm.$^{-1}$ Since the 1710 cm.$^{-1}$ band reappears upon acidification, but is not now removable by sodium borohydride reduction, an aliphatic carboxyl group has evidently been formed. Examination of carbonyl frequencies of model compounds (Table I) leads to the conclusion that the 1660 cm.$^{-1}$ band originates from a ketone carbonyl alpha to an aromatic ring, with the *para-* position etherified and with an oxygen atom (as a hydroxyl group or etherified) in the two-position (I). The model for



I

this is α-ethoxy propioveratrone (Figure 1A). The slight shift obtained upon acetylation is attributed to the removal of intermolecular hydrogen bonds (which tend to lower carbonyl frequencies in the solid state) by acetylation of hydroxyl groups present in adjacent molecules. Alternatively, the slight shift may be due to acetylation of an adjacent hydroxyl group.[22] The aldehyde carbonyl frequency

(18) K. Freudenberg and L. Knof, *Chem. Ber.*, **90**, 2857 (1957).

(19) H. Hergert, unpublished work.

(20) W. Pigman, E. Anderson, R. Fischer, M. Buchanon, and B. L. Browning, *Tappi*, **36**, 4 (1953); W. E. Hillis, *J. Soc. Leather Trades Chem.*, **38**, 91 (1954).

(21) H. L. Hergert and O. Goldschmid, *J. Org. Chem.*, **23**, 700 (1958).

(22) As an example of this, acetylation of phenacyl alcohol shifts the frequency from 1690 cm.$^{-1}$ to 1698 cm.$^{-1}$

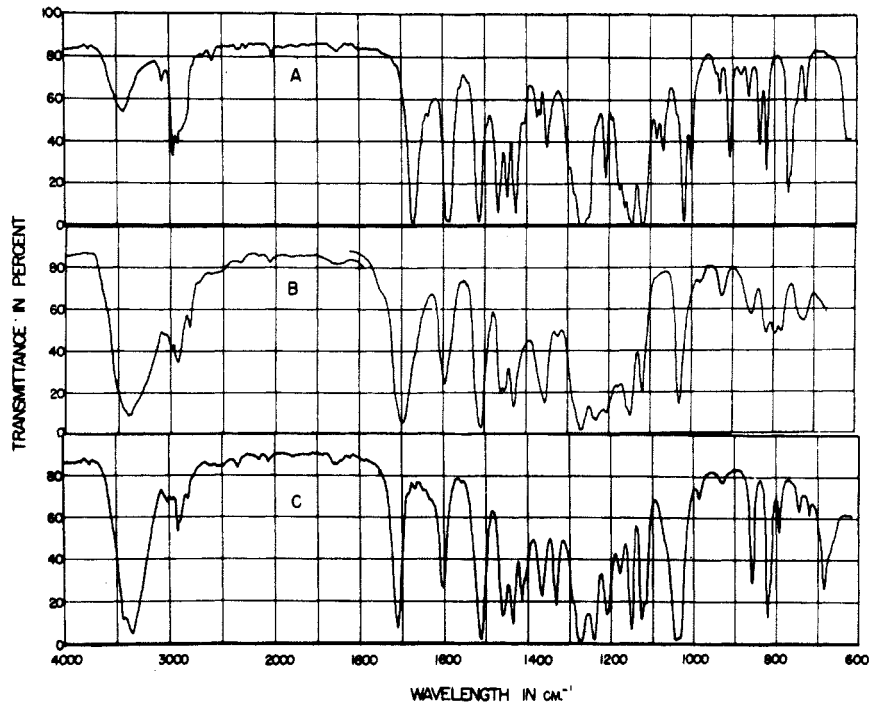


Fig. 1. Infrared spectra of: A. α-Ethoxy propioveratrone (wafer). B. Guaiacyl acetone (liquid). C. β-Hydroxy coniferyl alcohol (wafer)

of an etherified coniferylaldehyde also occurs in the 1660–1670 cm.$^{-1}$ region. Colorimetric studies[23] indicate that no more than one in twenty-five to thirty-five lignin monomeric units is a coniferylaldehyde group. Consequently, intensity considerations alone suggest that the 1660 cm.$^{-1}$ band in native lignin could originate only in a very small part from coniferylaldehyde groups. The 1712 cm.$^{-1}$ band must originate from a nonconjugated ketone carbonyl group. The model for this is guaiacyl acetone (Figure 1B) or the keto form of β-hydroxy coniferyl alcohol (Figure 1C). Treatment of these two compounds with alkali, as in the alkaline treatment of lignin, results in the destruction of the 1712 cm.$^{-1}$ ketone carbonyl and the formation of a carboxyl group, probably through oxidative cleavage. Comparison of the relative heights of the carbonyl stretching bands in the model compounds with those of lignin indicates the presence of about one conjugated α-carbonyl group per five monomers, *i.e.*, 0.2 CO per OCH_3, and about one nonconjugated carbonyl group per twenty-five monomeric units.

The spectra of the various native lignins contained only two distinct aromatic stretching bands, 1510–1515 and 1595–1603 cm.$^{-1}$, typical of an unconjugated guaiacyl nucleus. A very weak "shoulder" is also discernible at 1580–1585 cm.$^{-1}$ Since this band usually appears only in conjugated aromatic compounds, this band can be attributed to a vibration of the rings which are conjugated with an α-carbonyl group (structure I). A relatively strong band at 1150 cm.$^{-1}$, believed to originate from a 1:2:4 substituted aromatic ring,[24] occurs in all the guaiacyl compounds in Table I and also occurs in the native lignins. Moderately strong bands at 858 and 817 cm.$^{-1}$ due to monohydrogen and two adjacent ring hydrogens out-of-plane deformations, respectively, were observed in the native lignin and almost all of the guaiacyl model compound spectra.

An α,β- double bond conjugated with an aromatic ring is the only type of ethylenic double bond likely to be encountered in the lignin molecule. Measurement of model compounds containing aromatic conjugated *trans* double bonds (Table II)

TABLE II

ABSORPTION BANDS ASSOCIATED WITH *Trans*- DOUBLE BONDS CONJUGATED WITH AN AROMATIC RING

Compound	C=C Stretching	=CH Deformation
Cinnamic acid	1626	980
3-Methoxy-4-hydroxy cinnamic acid	1620	972
3-Methoxy-4-acetoxy cinnamic acid	1626	985
3,4-Dimethoxy cinnamic acid	1625	980
3,4-Dihydroxy cinnamic acid	1620	975
3,4-Diacetoxy cinnamic acid	1626	988
Cinnamaldehyde	1618	968
3-Methoxy-4-hydroxy cinnamaldehyde	1612	962
Isoeugenol	1618	962
Coniferyl alcohol	~1612	965
Coniferin	~1610	960
Dehydrodiconiferyl alcohol	~1610	965
Guaiacyl glycerol β-coniferyl ether	~1608	965

(23) E. Adler, K. J. Bjorkvist, and S. Haggroth, *Acta Chem. Scand.*, **2**, 93 (1948); E. Adler, *Ind. Eng. Chem.*, **49**, 1377 (1957).

(24) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, Methuen and Co., London, 323 pp. (1954).

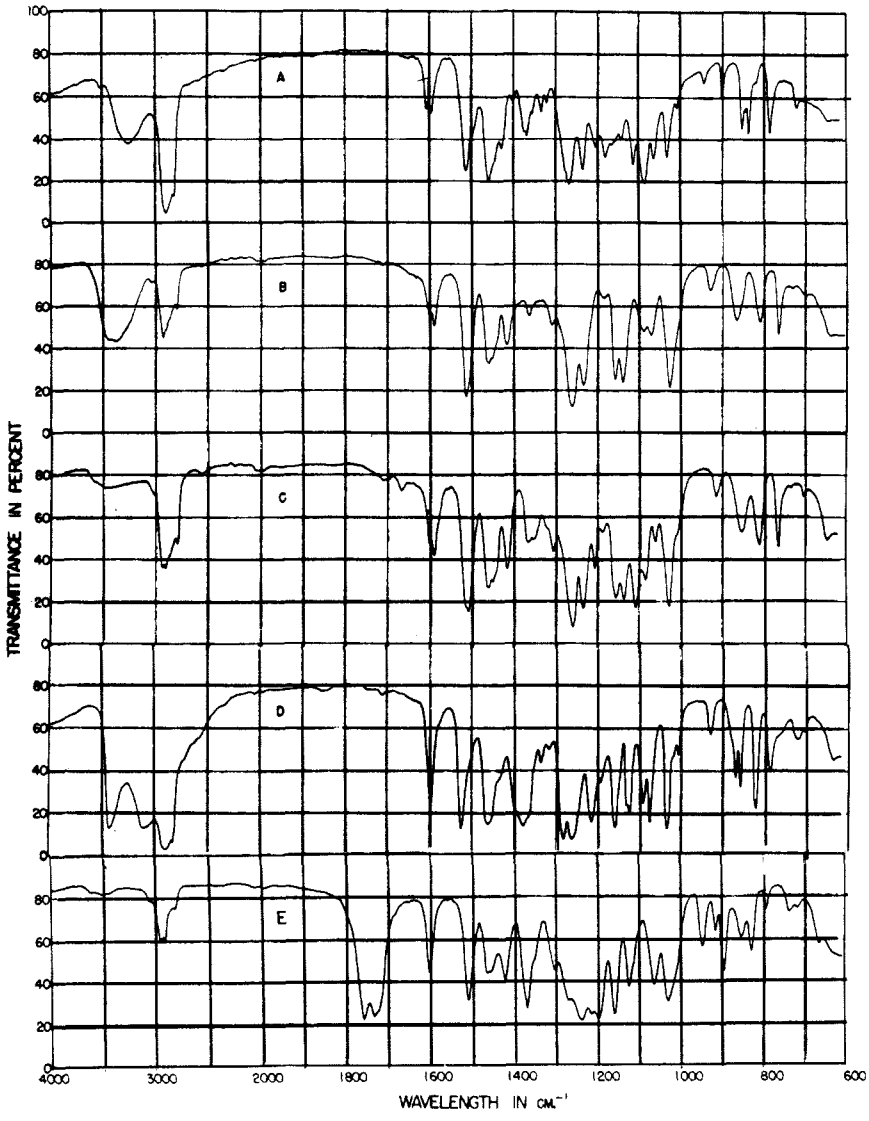


Fig. 2. Infrared spectra of: A. 1-Ethoxy-1-(4-hydroxy-3-methoxyphenyl)ethane (mull). B. 1-Hydroxy-1-(3,4-dimethoxyphenyl)ethane (liquid). C. 1-Methoxy - 1 - (3,4 - dimethoxyphenyl)ethane. D. 1-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-ethane (apocynol) (mull). E. Apocynol diacetate (film)

indicated that the C = C stretching vibration occurs in the range of 1608–1626 cm.$^{-1}$, while the C—H out-of-plane deformation absorbs at 960–988 cm.$^{-1}$ The absence of an absorption band at 1610–1625 cm.$^{-1}$ and the extremely weak band at 970 cm.$^{-1}$ suggests that very few double bonds (probably less than one for each twenty monomers) are present in the native lignin molecule.

Bands at 3400 (shoulder at 3190), 1220, 1087, and 1043 cm.$^{-1}$ are interpreted as arising from hydroxyl group vibrations. The O—H stretching band at 3400 cm.$^{-1}$ is broad, indicating hydrogen bonding. Phenolic hydroxyl groups give rise to a 1200–1225 cm.$^{-1}$ absorption in guaiacyl compounds which is attributed to the aryl C—O stretching mode and is thus assigned in lignin. The intensity of this band is lowered upon methylation of the lignin phenolic groups as is the 1200 cm.$^{-1}$ band in vanillyl alcohol and α-hydroxy propiovanillone upon methylation. Similar methylation of apocynol causes the band to disappear entirely.

The O—H deformation or C—O stretching band was found to occur at about 1000 cm.$^{-1}$ for benzyl alcohols, 1040 cm.$^{-1}$ for primary alcohol groups, and 1075–1090 cm.$^{-1}$ for secondary hydroxyl groups in the model compounds studied. Since aliphatic ethers also absorb at 1085–1120 cm.$^{-1}$ and the C—O deformation of the methoxyl groups occurs at 1030 cm.$^{-1}$,[25] assignment of the absorption bands with certainty in this area of the lignin spectrum is difficult. Comparison of the spectral effect of acetylation and methylation of lignin with model compounds led to the conclusion that the both aliphatic ether linkages and secondary hydroxyl groups are present in the native lignin molecule and are responsible for the 1082–1087 cm.$^{-1}$ absorption band. The presence of both phenolic and aliphatic hydroxyl groups was further confirmed by the presence of strong 1760 and 1740 cm.$^{-1}$ ester carbonyl stretching bands in the acetate spectra. Methoxyl group and aromatic-aliphatic ether ab-

(25) L. H. Briggs, L. D. Colebrook, H. M. Fales, and W. C. Wildman, *Anal. Chem.*, 29, 904 (1957).

TABLE III

INFRARED FREQUENCY ASSIGNMENTS (TENTATIVE) OF NATIVE LIGNIN AND SEVERAL GUAIACYL MODEL COMPOUNDS

Assignment	Frequency (Cm.$^{-1}$) 1-Hydroxy 1-guaiacyl ethane (Apocynol)	β-Guaiacyl-glycerol coniferyl ether[a]	β-Hydroxy coniferyl alcohol	Southern pine native lignin	Western hemlock native lignin
O—H stretching (H-bonded)	3420	3390	3440	3400	3420
	3100		3360	3150[c]	3160[c]
C—H stretching (methoxyl groups and side-chain CH)	[b]	2920	2920	2920	2920
	[b]	~2850	2820	2850	2875, 2820[c]
C=O stretching					
Aliphatic ketone			1712	1712	1712
p-Substituted aryl ketone				1660	1655
C=C stretching		1653[d]			
C=C skeletal vibrations (aromatic ring)	1597	1608, 1587	1602, 1597	1606	1607
	1525	1515	1512	1512	1512
C—H deformation (asymmetric)	[b]	1462	1461	1462	1462
Unassigned (present in all guaiacyl compounds examined)	[b]	1414	1435, 1415	1423	1432
C—H deformation (symmetric)	1365	1364	1362	1365	1365
C—O stretching, aromatic (methoxyl)	1263, 1282	1270	1270	1270	1268
C—O stretching, aromatic (phenol)	1216	1222	1235, 1212	1220	1232, 1215
Unassigned (methoxyl group)	1192	~1185	1178	~1190	~1185
Unassigned (1:2:4 substitution)	1160	1159	1152	1153	1155[c]
Unassigned	1132	1131	1127	1135	1145
Unassigned (aromatic ether)	1124	~1120[c]	1118	~1125[c]	1120
C—O deformation (aliphatic ether or secondary hydroxyl)	1092, 1076	1085		1087	1082
C—O deformation (primary hydroxyl)		1040[c] (?)	1042[c]	1043	1043[c]
C—O deformation (methoxyl group)	1035	1031	1034	1031	1031
Unassigned	1010[d]		987[d]		990[d]
=CH out-of-plane deformation (*trans*)		965		970[d]	968[d]
Unassigned (possibly OH out-of-plane deformation)	931		~930		~930
C—H out-of-plane deformation					
(One H, aromatic ring)	860, 872	857	858	858	857
(Two H, aromatic ring)	825	814	825, 816[c]	817	815

[a] K. Freudenberg and W. Eisenhut, *Chem. Ber.*, **88**, 626 (1955). [b] Band obscured by Nujol. [c] Shoulder. [d] Very weak.

sorptions occurred at 2920, 2850, 1462, 1365, 1270, 1190, 1125, and 1031 cm.$^{-1}$, identical with guaiacyl model compounds. The band assignments of longleaf pine and purified western hemlock native lignin, which appeared to be relatively free of extraneous constituents, and several lignin model compounds, which contain functional groups believed to be present in lignin, are summarized in Table III.

Milled wood lignin. Bjorkman's recently discovered procedure[15] for lignin isolation from extractive-free wood appears to give a product structurally less altered than any other procedure devised to date. The infrared spectra of pine- and hemlock-milled wood lignins were compared with each other and with corresponding native lignins. Although no major differences in wave lengths of the absorption bands were observed, differences in the relative intensities of the bands were apparent, especially in the 1000–1250 cm.$^{-1}$ region. Since all of these lignin samples were isolated by the same procedure, the spectral differences must be interpreted as indicating structural differences in the lignin molecule which are dependent upon genera. The spectral region in which these differences are most conspicuous involves ether linkages and hydroxyl groups, so it may be concluded that lignins from different genera differ in molecular structure. If Freudenberg's hypothesis is correct, *viz.*, that lignin polymerization proceeds through a number of different dimeric products[26] which vary mainly in ether linkages and hydroxyl groups, then the structural differences in lignins from different sources would involve different proportions of the dimers in the polymer.

The milled wood lignins shows a relatively strong band at 1660 cm.$^{-1}$ and a weaker band at 1710–1715 cm.$^{-1}$ Both of these bands are stronger than those in the corresponding native lignins. They appear to be due to the same type of carbonyl groups, *i.e.*, an unconjugated ketone alpha to an aromatic ring with the *para-* position etherified, since the characteristic shifts upon acetylation, reduction, and treatment with base are identical in both cases. Differences in ring substitution were suggested by the relative heights of the 815 and 850–860 cm.$^{-1}$ C—H out-of-plane deformation bands. The 860 cm.$^{-1}$ band, which indicates one free ring hydrogen atom, is stronger than the 815 cm.$^{-1}$ band (two adjacent ring hydrogens) in the milled wood lignins while the reverse was observed with native lignins. This may indicate a much

(26) K. Freudenberg, *Angew. Chem.*, **68**, 81 (1956).

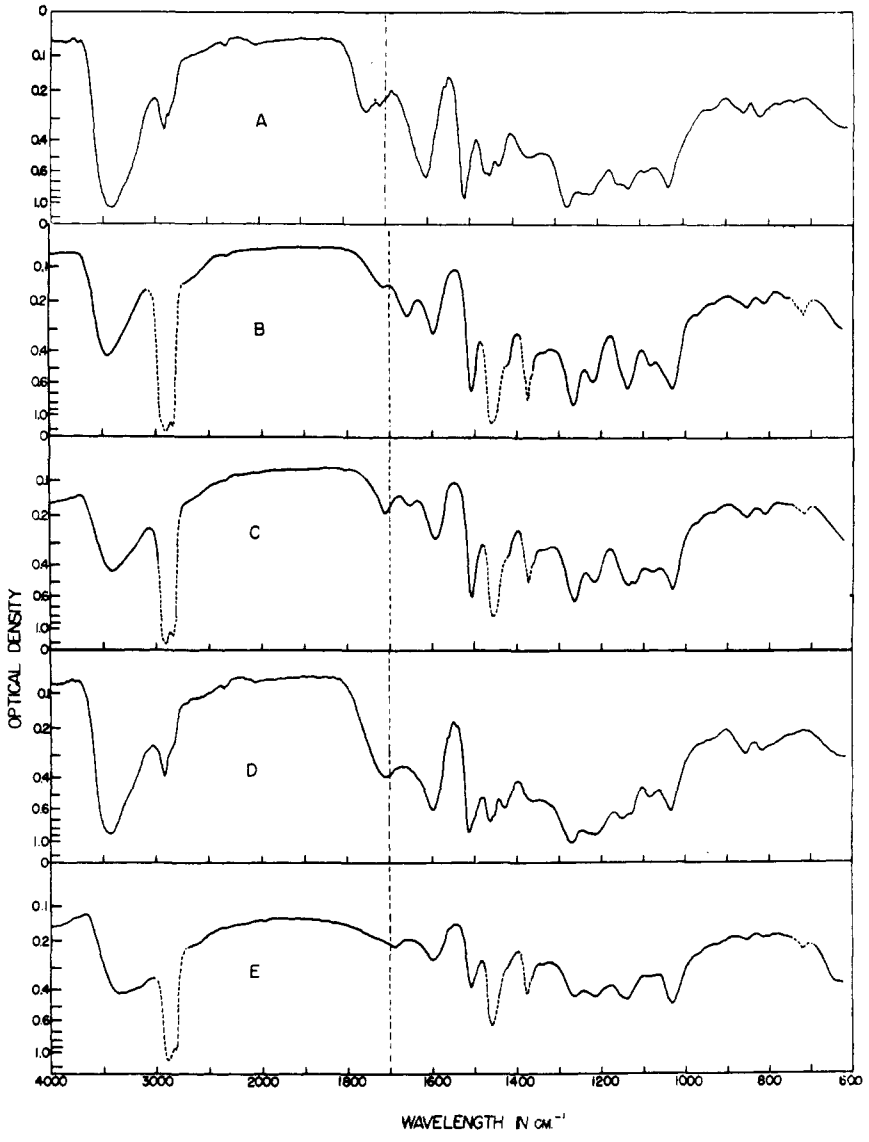


Fig. 3. Infrared spectra of western hemlock lignin: A. Native lignin (wafer). B. Milled wood lignin (mull). C. Dioxane lignin (mull). D. Kraft lignin (wafer). E. Lignosulfonic acid (mull). Dotted lines indicate bands due to paraffin in mulls

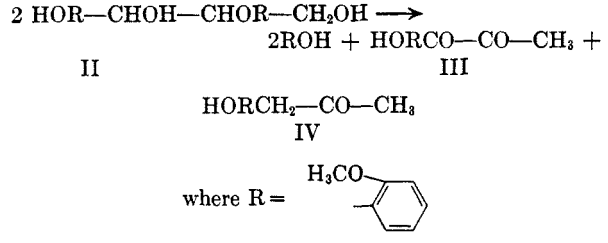
higher degree of substitution in the 5-position of wood lignin as compared with native lignins.

Further differences between milled wood and native lignins are apparent from a comparison of the spectra of the acetate derivatives (determined as potassium bromide wafers of equal concentration). The total hydroxyl content, as evidenced by the intensity of the acetate ester carbonyl stretching bands, was markedly higher in native lignin than milled wood lignin. The ratio of aliphatic to phenolic hydroxyl groups (as indicated by the aliphatic ester band, 1735–1740 cm.$^{-1}$, and phenolic ester band, 1760 cm.$^{-1}$) was higher in the milled wood lignin than the native lignin. The 1085 cm.$^{-1}$ band, which appears to indicate the presence of aliphatic ether linkages in the acetate derivative, is markedly higher in the milled wood lignins than in the native lignins. This suggests that some of the oxygen atoms, appearing as hydroxyl groups in native lignin, are bound in aliphatic ether linkages in whole wood lignin.

Dioxane lignin. One of the disadvantages of the use of milled wood lignin for structural studies is that preparation is exceedingly slow and results in very small amounts of material. Both of these difficulties may be circumvented by the use of dioxane–hydrochloric acid as an extraction medium.[27] The spectra of dioxane lignins from five coniferous genera were similar but not identical. Although this provided further evidence for the variation of lignin structure with genera, there was indication of structural rearrangement which had taken place during isolation. The 1710 cm.$^{-1}$ band was appreciably stronger than the corresponding band in native or milled wood lignin. It is due to an unconjugated ketone carbonyl group which is apparently formed during the isolation process. Treatment of native or milled wood lignin with dioxane–hydro-

(27) (a) O. Engel and E. Wedekind, *Ber.*, **69B**, 2434 (1936); (b) H. Kiefer and E. Kurth, *Tappi*, **36**, 14 (1953); (c) E. Adler, J. M. Pepper, and E. Eriksoo, *Ind. Eng. Chem.*, **49**, 1391 (1957).

chloric acid similarly results in an intensified 1710 cm.$^{-1}$ band, while dioxane–hydrochloric acid treatment of native lignin reduced with lithium aluminum hydride results in the formation of both unconjugated and conjugated carbonyl groups. Further differences in dioxane lignin and milled wood lignin are indicated by the higher phenolic hydroxyl content of dioxane lignin, as evidenced by a more intense 1760 cm.$^{-1}$ phenolic acetate carbonyl stretching band in the acetate derivative, and differences in the 1125–1150 cm.$^{-1}$ bands, which are related to aromatic aliphatic ether linkages. All of these changes are paralleled in the treatment[27c] of the guaiacyl ether of guaiacyl glycerol (II) with dioxane–hydrochloric acid in which guaiacol is liberated and vanilloyl acetyl (III) and guaiacyl acetone are formed by dehydration and rearrangement.



This strongly points to the presence of similarly substituted guaiacyl glycerol units in lignin. If dioxane–hydrochloric acid treatment of wood is extended from 2 to 12 or 24 hr., additional changes are found in the 1000–1250 cm.$^{-1}$ area of the spectrum. This suggests that further extensive rearrangements have taken place, and strongly indicates that short extraction periods, *i.e.*, 2 hr., should be used in the dioxane–hydrochloric acid procedure if relatively unchanged lignin is desired.

Alcoholysis of lignin. When lignin is isolated from wood with methanol or ethanol and hydrochloric acid, or when native lignin is treated with these same reagents, the alkoxyl content is appreciably increased. Brauns concluded that the methoxyl or ethoxyl groups introduced in the lignin molecule were attached to a carbonyl group, probably in an acetal linkage. As proof of this, he cited[28] the infrared study of Jones,[5a] in which it was stated that the absorption band in the spectrum of native lignin at 1663 cm.$^{-1}$, which was attributed to an aldehyde or ketone group, was absent in the spectrum of native lignin methylated with methanol–hydrochloric acid. In direct opposition to this, Adler and Gierer,[29] who believe that alkylation with methanol–hydrochloric acid involves a reaction with benzyl alcohol groups, observed that the carbonyl band in the infrared spectrum of native lignin did not disappear upon methylation with methanol–hydrochloric acid. During the present study, the spectra of native lignin treated with ethanol–hydrochloric acid and of ethanol–hydrochloric acid wood lignin were determined. Both products showed absorption bands at about 1650 cm.$^{-1}$ and 1710–1715 cm.$^{-1}$, in agreement with the work of Adler and Gierer. The unconjugated carbonyl band (1710 cm.$^{-1}$) is appreciably stronger than in the original lignin, while the 1650 cm.$^{-1}$ band is slightly less intense. Treatment of reduced native lignin with methanol–hydrochloric acid results in a product which also shows bands at these same frequencies. Other differences in the spectra, *i.e.*, in the 1000–1200 cm.$^{-1}$ and 750–900 cm.$^{-1}$ regions indicate that considerable structural rearrangements have taken place. These are interpreted as hydrolysis to liberate phenolic hydroxyl groups and dehydration to form carbonyl groups, as in dioxane lignin, and methylation of secondary (benzyl) alcohol groups. In view of this, it appears unwarranted to draw conclusions concerning the structure of wood lignin which are based exclusively on the study of alcoholysis lignin.

Kraft lignin. The spectra of kraft lignins differed depending upon whether they were precipitated with carbon dioxide or mineral acid from the alkaline pulping liquor. Chemical analyses of the product precipitated with carbon dioxide showed it to contain 2.3% sodium, and the spectrum had an absorption band at 1580–1590 cm.$^{-1}$ typical of carboxylate ion.[30] Absorption at 1660–1720 cm.$^{-1}$ was absent. Upon subsequent acidification with sulfuric acid, the sodium content was reduced to a negligible value, and the spectrum (Figure 3D) now showed a moderately strong band at about 1713 cm.$^{-1}$, which was assigned to the stretching frequency of a nonconjugated carboxyl group. Kraft lignins also contain a conjugated ketone carbonyl group which shows an absorption at about 1650 cm.$^{-1}$; however, this functional group appears to be conjugated through the aromatic ring to a *para*-phenolic hydroxyl rather than to a *para*-ether linkage as in the original wood lignin. This is evidenced through a shift of the band to about 1635 cm.$^{-1}$ upon conversion of the lignin to a sodium salt.

The remainder of the spectrum of acidified kraft lignin closely resembled the dioxane lignin spectrum. Comparison of spectra (potassium bromide pellets of equal concentration) indicated the kraft lignin to have slightly higher intensities of the 1600 cm.$^{-1}$ and 1220 cm.$^{-1}$ bands and weaker intensities for the 1515, 1125, 1085, and 1030–1035 cm.$^{-1}$ bands. These differences are interpreted as indicating that the kraft process causes an increase in phenolic hydroxyl content through ether cleavage and loss of methoxyl group and, possibly, some loss of aliphatic hydroxyl groups either through dehy-

(28) Ref. 6a, pp. 230–231.

(29) E. Adler and J. Gierer, *Acta Chem. Scand.*, 9, 84 (1955).

(30) Infrared evidence of carboxyl groups in kraft lignin has also been suggested by J. J. Lindberg, *Finska Kemistsamfundets Medd.*, 64, 23 (1955).

dration or mercaptan or sulfide formation. Unfortunately, the carbon-sulfur vibrations are relatively weak and occur at 600–700 cm.$^{-1}$ region, which was not readily accessible with the equipment available in this laboratory.

Lignosulfonates. The spectral shift observed upon conversion of sodium and calcium lignosulfonates to free lignosulfonic acids by ion exchange indicated the presence of carboxyl groups in lignosulfonates, although the amount appears to be less than that present in kraft lignins. Unconjugated carbonyl groups are absent and conjugated carbonyl groups are present in relatively small amount, except in lignosulfonates which have been treated with alkali. In the latter case, an appreciable content of ketone carbonyl groups conjugated with a free *para*-hydroxyl group is indicated.

The absorption bands in the lignosulfonic acid spectrum, as compared to dioxane–hydrochloric acid lignin, are rounded or less well defined. The 815 and 860 cm.$^{-1}$ bands are very weak. This strongly suggests higher molecular weights (increases in molecular weight usually produce a more diffuse spectrum), structural rearrangement and/or condensation. Colthup[31] suggests the following series of absorption bands for sulfonic acids and salts: 1260–1150 cm.$^{-1}$ (strong), 1080–1010 cm.$^{-1}$ (medium) and 600–700 cm.$^{-1}$ (medium). A strong but broad band at 1200–1210 cm.$^{-1}$, a band at 1040 cm.$^{-1}$ (evidenced by markedly increased absorption at this wave length as compared with dioxane–hydrochloric acid lignin), and a medium intensity band at 650 cm.$^{-1}$ were observed in the lignosulfonic acid. The band at 1200–1210 cm.$^{-1}$ is somewhat more pronounced in the lignosulfonate salt. Both the 1210 and 1040 cm.$^{-1}$ bands occur at the same frequency as absorption bands already present in the unsulfonated lignin molecule, so they are not particularly valuable for diagnostic studies. The 650 cm.$^{-1}$ band does not occur in unsulfonated lignin. Desulfonation of a sodium lignosulfonate by treatment with sodium hydroxide at elevated temperatures results in a loss of the 650 cm.$^{-1}$ band and a marked decrease in the 1040 and 1210 cm.$^{-1}$ absorptions. The spectrum of the desulfonated lignosulfonate, though containing bands of approximately the same wave lengths, is readily distinguishable from the dioxane lignin spectrum. Considerable structural alteration has apparently occurred in the desulfonation process, including increase in phenolic hydroxyl content and loss of methoxyl groups.

Although considerable information about the structure of conifer lignin has been gained by a study of their infrared spectra, the work reported here also indicates that elucidation of lignin structure may be even more difficult than hitherto suspected. Thus, not only are native lignins not identical with whole wood lignin, but lignin structure appears to vary with genera. Further difficulties are involved in the fact that many previous investigators have attempted to apply their results on lignins isolated by processes such as alcoholysis, sulfonation, etc., to lignin as it exists in wood. Infrared spectra show, however, that many of these lignins are rearranged during isolation; consequently structural studies based on them are of limited value for this purpose. Since generic differences in lignins are apparent from the spectra, it is proposed that future work should be devoted to functional group analyses of lignins isolated by the same process from various genera, and that the enzyme systems and cambial constituents (lignin intermediates) of various genera and species be compared. In this way many past discrepancies in the lignin literature are likely to be clarified.

(31) N. Colthup, *J. Opt. Soc. America*, **40**, 397 (1950).

SHELTON, WASHINGTON

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

The Reaction of Saccharin with Amines. *N*-Substituted-3-Amino-1,2-benzisothiazole-1,1-dioxides

CALVERT W. WHITEHEAD AND JOHN J. TRAVERSO

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Saccharin reacts with excess amines to produce *N*-substituted 3-amino-1,2-benzisothiazole-1,1-dioxides. Under the same conditions *N*-methylsaccharin produces *N*-substituted *o*-methylsulfamylbenzamides. Saccharin with one equivalent of amine produces *N*-substituted *o*-sulfamylbenzamides. The reaction products of hydrazine hydrate with saccharin and *N*-methylsaccharin have been assigned structures based on the similarity of their infrared spectra to that of benzoic acid hydrazide.

A number of 3-amino-1,2-benzisothiazole-1,1-dioxides were prepared for pharmacological evaluation as diuretic or hypoglycemic agents. As in the case of arylamines,[1] refluxing saccharin with alkyl- and aralkylamines boiling at least at 130° for eight hours gave crystalline 3-amino-1,2-benzisothiazole-1,1-dioxides. Derivatives of lower boiling amines

(1) A. Mannessier-Mameli, *Gazz. chim. ital.*, **65**, 51 (1935); *Chem. Abstr.*, **29**, 3996 (1935).