TABLE IV

ACETYLATION OF COMPOUNDS RELATED TO LIGNIN

Compound	Wt., g.	C_5H_5N	Ac_2O	Purification procedure	Product	Yield, g.
Vanillic acid (Ih)	1.00	7.5	4	Et_2O extract washed with dil. HCl, H_2O, $NaHCO_3$ soln., H_2O, and dried	White crystals, m.p. 141–143° (Ie)	0.64
Apocynol (IIIb)	0.317	5	4	Same as Ih	Thick oil, $n^{25}D$ 1.5020 (IIId)	0.301
1-(3,4-Dimethoxyphenyl)-ethanol (IIIf)	0.629	7.5	6	Same as Ib	Thin oil, $n^{25}D$ 1.5133 (IIIg)	0.263
Coniferyl benzoate (IIb)	1.0	15	12	Solvent evap. off and pptd. from Et_2O and pentane	Thick sirup. *Anal.* (IIc). Calcd.: C, 69.93; H, 5.56; OMe, 9.51 Found: C, 69.78; H, 5.48; OMe, 9.37	
1-(3,4-Dimethoxyphenyl)-2-(2-methoxy-4-propylphenoxy)-ethanol (VIIb)	0.3	4.5	3.6	Recrystd. from H_2O, EtOH dried, 0.1 mm., 65°, overnight	White solid, m.p. 87.4–88.0° (VIIc)	0.303
1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-propane-1,3-diol (VIId)	0.336	5	4	Same as Ih	Thick oil. *Anal.* (VIIe). Calcd.: C, 63.14; H, 6.26; OMe, 22.25. Found: C, 63.08; H, 6.46; OMe, 22.22	0.19
Pinoresinol (VIIIa)	0.302	5	4	Recrystd. from AcOH and petr. ether, dried P_2O_5, 0.3 mm., 65°, overnight	White crystals, m.p. 165.4–166.9° (VIIIc)	0.195
Dehydrodisoeugenol (IXa)	0.313	5	4	Recrystd. from H_2O, EtOH, dried P_2O_5, 0.3 mm., 65°, overnight	White crystals, mp. 112.7–113.7° (IXb)	0.237
Dehydrodiconiferyl alcohol (IXc)	0.046	0.6	0.5	Same as Ih, dried 100°, 0.1 mm., overnight	Thick sirup, n.m.r. shows no unexpected signals (IXd)	0.035

1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-ethanol (VIa) was prepared by the sodium borohydride reduction of ω-(2-methoxyphenoxy)-acetoveratrone (Va) in 87% crude yield; m.p. 131–131.5° after recrystallization from 95% ethanol and drying at 65° and 0.1 mm. for several hours.

Anal. Calcd. for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62. Found: C, 67.07; H, 6.65.

Acetylations.—The compounds listed in Table IV were acetylated by mixing the amounts of reactants specified in an erlenmeyer flask equipped with a glass stopper, allowing the mixture to stand at room temperature 24 to 48 hr., hydrolyzing the products over cracked ice, and purifying them as described.

Nuclear Magnetic Resonance Spectra.—All n.m.r. spectra were obtained using a Varian Associates Model HR-60, equipped with a probe, V-4331-A. Calibrations were performed by the conventional side-band technique. For most of the work redrawn standard 5-mm. o.d. Pyrex glass tubing was used to make the sample tubes. Each tube was charged with about a 60-mg. sample dissolved in 540 mg. of $CDCl_3$ containing about 1% hexamethyldisiloxane as internal standard. Some samples which were not easily soluble in the solvent at room temperature were placed in the tubes dry and then the proper amount of solvent was added. The tubes were degassed and sealed and examined at room temperature except when it was necessary to warm them gently to encourage solution.

Acknowledgment.—The authors express their appreciation to Professors Holger Erdtman, Karl Freudenberg, and Karl Kratzl, Mr. John C. Pew, Dr. S. W. Schubert, and Dr. G. Merril Andrus for their gifts of model compounds used in this study; to Professor K. V. Sarkanen for his helpful suggestions regarding this manuscript; to Mr. Tharwat Saleh for the acetylation and n.m.r. work on compound IXa; and to the Northwest Pulp and Paper Association and Institute of Forest Products for financial assistance.

[CONTRIBUTION FROM PULP MILLS RESEARCH AND THE INSTITUTE OF FOREST PRODUCTS, DEPARTMENTS OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF WASHINGTON, SEATTLE 5, WASH.]

Lignin. XIII.[1] The High Resolution Nuclear Magnetic Resonance Spectroscopy of Protons in Acetylated Lignins

BY CHARLES H. LUDWIG, BERNARD J. NIST, AND JOSEPH L. MCCARTHY

RECEIVED JULY 18, 1963

The n.m.r. spectra of protons in acetylated derivatives of dioxane acidolysis lignin, Bjorkman milled wood lignin, and Brauns native lignins from gymnosperm woods have been studied using a procedure for relating signals within certain ranges of chemical shifts to characteristic protons in lignin model compounds. Integration of the spectra allows semiquantitative estimates to be made on the number of condensed aromatic systems, free benzylic hydroxyls, aliphatic and aromatic hydroxyls, and total aliphatic hydrogens in the parent lignin preparations. The results essentially substantiate earlier analytical estimates and are unique in providing a reliable measure of condensed aromatic ring systems.

The n.m.r. spectra of model compounds related to lignins have been discussed in a previous publication.[1] This paper reports the application of the information gained in the model compound study to the interpretation of the more complex and diffuse lignin spectra.

(1) Lignin. XII: C. H Ludwig, B. J. Nist, and J. L. McCarthy, *J. Am. Chem. Soc.*, **86**, 1186 (1964).

The gymnosperm lignin preparations, Table I, after acetylation were all found to be sufficiently soluble in deuteriochloroform for n.m.r. determinations. The same solvent and general conditions were used for these lignin studies as for the study of model compounds in order to justify direct comparisons between the chemi-

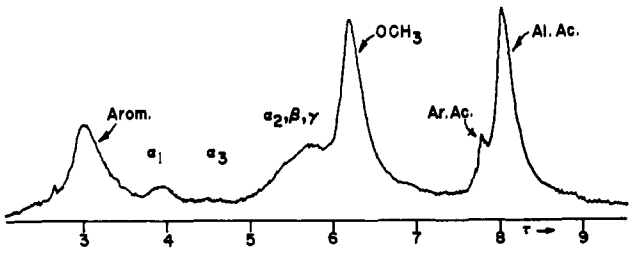

Fig. 1.—Acetylated milled wood spruce lignin.

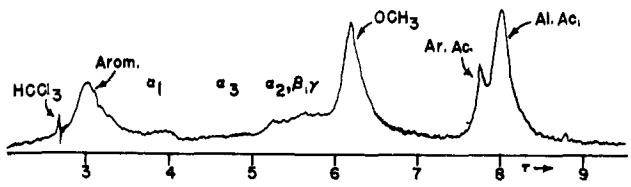

Fig. 2.—Acetylated dioxane acidolysis western hemlock lignin.

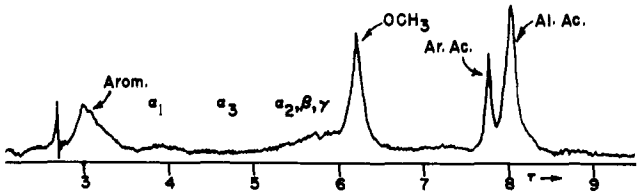

Fig. 3.—Acetylated native spruce lignin.

cal shifts of proton signals in the lignin and model compound spectra.

TABLE I

LIGNIN PREPARATIONS USED IN N.M.R. STUDIES

Lignin preparations	Empirical formulas
Acetylated Bjorkman milled wood lignin, spruce[a]	$C_9H_{7.7}O_{2.4}(OMe)_{0.97}(Ac)_{1.15}$
Acetylated dioxane acidolysis lignin, west. hemlock	$C_9H_{6.7}O_{2.4}(OMe)_{0.94}(Ac)_{1.37}$
Acetylated Brauns native lignin, spruce[b]	$C_9H_{7.0}O_{2.6}(OMe)_{0.85}(Ac)_{1.36}$
Acetylated Brauns native lignin, west. hemlock[b]	$C_9H_{6.4}O_{2.7}(OMe)_{0.88}(Ac)_{1.54}$
Acetylated methanol Brauns native lignin[b]	
Acetylated diazomethane methylated Brauns native lignin[b]	

[a] Provided by Prof. E. Adler. [b] Provided by Dr. F. E Brauns.

An alternative solvent for n.m.r. studies applicable only to water-soluble lignin derivatives is deuterium oxide. While two monomeric lignosulfonates dissolved in this solvent gave sharply resolved n.m.r. spectra,[2] all attempts to study higher molecular weight lignin sulfonates under varying conditions of temperature and dilution yielded spectra which were featureless. Apparently coulombic repulsion of the sulfonate groups combine with solvation effects to give the lignin molecules a rigid, solid-like character decreasing their random motion with a consequent increase in spin–spin relaxation times. This creates very broad and low lying n.m.r. signals that are useless for high resolution work.

The Qualitative Interpretation of Lignin Spectra.—The n.m.r. spectra of the acetylated lignins are shown in Fig. 1 through 6. Typical τ-values found for protons in model compounds are listed in the order of increased shielding in Table II. The τ-values for protons found

(2) S. W. Schubert, G. M. Andrus, C. H. Ludwig, and J. L. McCarthy, unpublished results.

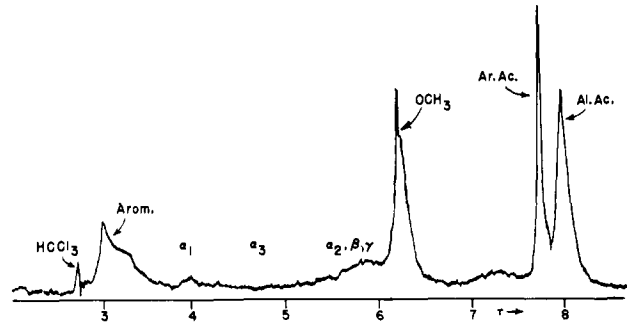

Fig. 4.—Acetylated Brauns native lignin from western hemlock.

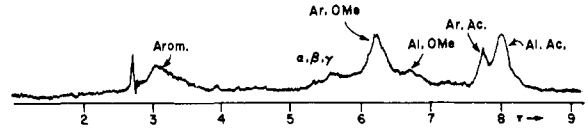

Fig. 5.—Acetylated methanol Brauns native lignin.

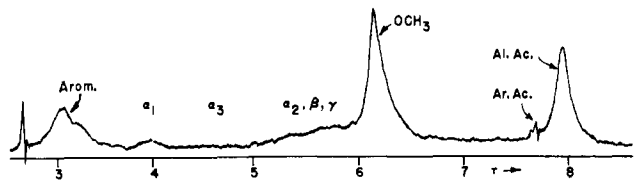

Fig. 6.—Acetylated diazomethane methylated Brauns native lignin.

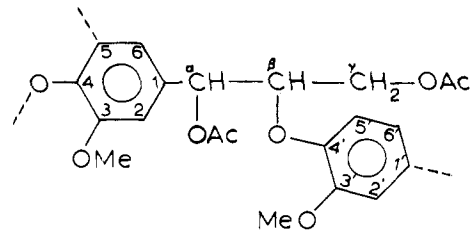

Fig. 7.—The β-aryl ether type linkage.

in monomeric model compounds show that the maxima in the lignin spectra at 3.0, 6.2, 7.7, and 8.0 are from the aromatic, methoxyl, aromatic acetoxyl, and aliphatic acetoxyl protons, respectively. The τ-values for signals from protons peculiar to dimeric models allow addi-

TABLE II

RANGES OF τ-VALUES FOR CHEMICAL SHIFTS OF SIGNAL FROM PROTONS FOUND IN MODEL COMPOUNDS ADJUSTED FOR LIGNIN PREPARATIONS

Range no.	Proton types	Chemical shifts from models[a]	Chemical shifts adjusted for lignin preparations
1	Carboxylic and aldehydic	−1.50 to 0.52	−1.50 to 2.00
2	Aromatic	2.30 to 3.33	2.00 to 3.72
	ortho to α-carbonyl	2.30 to 2.68	
	all others	2.80 to 3.33	
	α-Vinyl	3.28 to 3.53	
3	β-Vinyl	3.68 to 4.14	3.72 to 4.26
	α, as in Fig. 7	3.90 to 4.06	
4	α, as in Fig. 9	4.42 to 4.35	4.26 to 4.82
5	Methoxyl	6.10 to 6.30	4.82 to 7.50
	α, β, and γ other than those in ranges 3, 4, and 8	5.10 to 7.28	
6	Aromatic acetoxyl except those *ortho* to a biphenyl linkage	7.71 to 7.73	7.50 to 7.81
7	Aromatic acetoxyl, *ortho* to biphenyl link	7.87	7.81 to 8.42
	Aliphatic acetoxyl	7.91 to 8.02	
8	Highly shielded aliphatic	8.09 to 9.22	8.42 to 9.62

Reference 1.

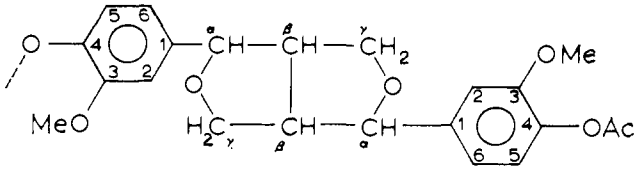

Fig. 8.—The pinoresinol type linkage.

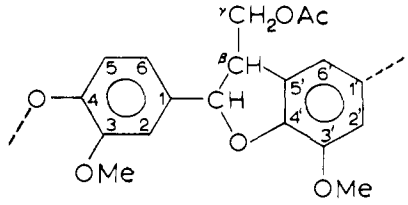

Fig. 9.—The phenyl coumaran type linkage.

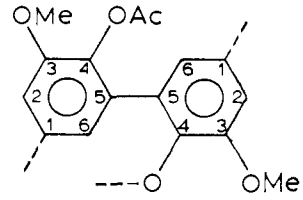

Fig. 10.—The 5,5′-biphenyl type linkage.

and to spin–spin splitting effects. This broadening, estimated to be of the order of 0.6 to 0.8 p.p.m., has been used with the apparent boundaries of signals in the lignin spectra, to adjust the chemical shifts from model compound spectra, and set the boundaries for ranges of τ-values for typical protons, shown in column 2 of Table II. These ranges will be discussed later in connection with the quantitative interpretation of lignin spectra

The spectra of the Brauns native lignins, Fig. 3 and 4, are considerably more sharply defined than those of the milled wood and dioxane lignins, Fig. 1 and 2. This may be explained by the relatively low molecular weight of the former lignins, which makes for greater mobility of the molecules in solution. It is also apparent that the signal intensity at 8.0, which arises primarily from the aliphatic acetoxyl protons, is comparable to the intensity of the methoxyl proton signal at 6.2 in each of the four lignin spectra, Fig. 1 through 4. The sizes of the aromatic acetoxyl peaks at 7.7, on the other hand, vary markedly from a relatively small signal in the Bjorkman lignin, Fig. 1, to a much larger one in Brauns lignin from western hemlock, Fig. 4, indicating comparable differences in numbers of aromatic acetoxyl protons in these preparations.

The spectrum of the methanol Brauns lignin, Fig. 5, shows a decrease in the primarily aliphatic acetoxyl

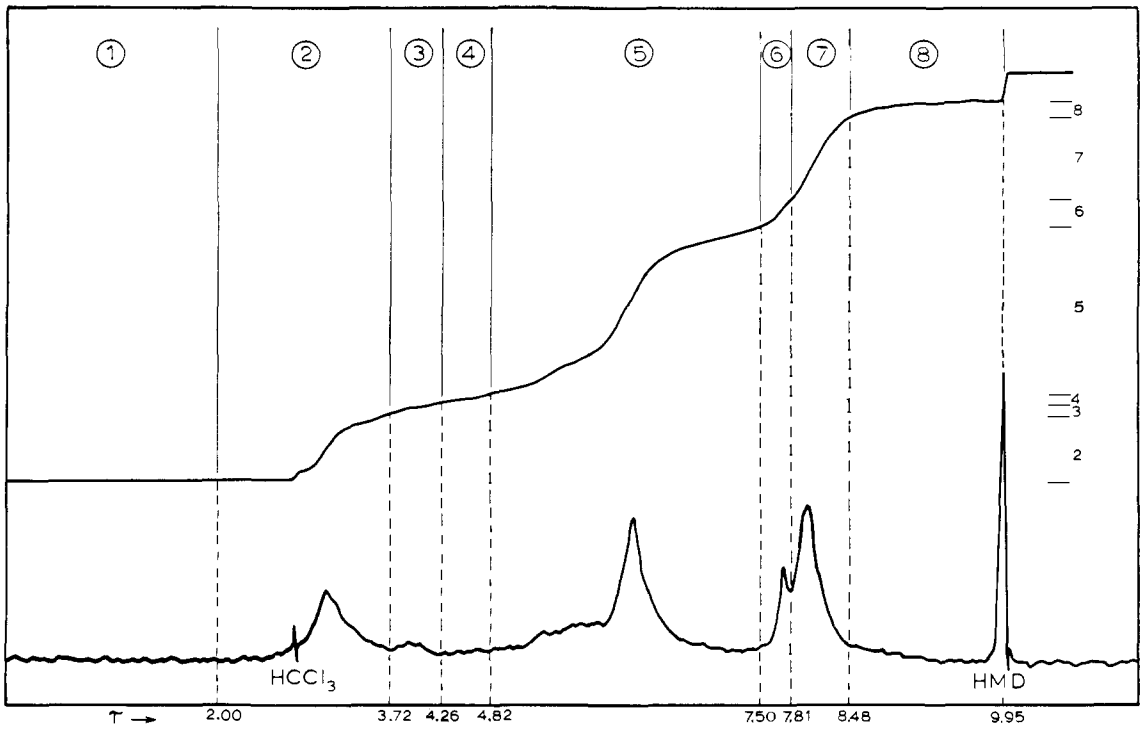

Figure 11.

tional assignments. The smaller maximum found at 3.9 in each of the lignin spectra except the Braun methanol lignin, Fig. 5, is assigned primarily to protons attached to benzylic carbons to which an acetoxyl group is bonded (*e.g.*, Fig. 7). Other protons bonded directly to side chain carbons such as those in Fig. 7 through 10 are responsible for the low lying signal in the vicinity of the methoxyl proton signal. The aromatic acetoxyl groups *ortho* to biphenyl type linkages, as in Fig. 10, may account for part of the predominantly aliphatic acetoxyl proton signal at 8.0.

Signal broadening, which is obvious from the appearance of the lignin spectra, is thought to be due to a tendency toward rigidity caused by cross linking and large rings in the macromolecular structure of the lignins

signal at 7.9 with the concurrent appearance of an additional signal just above the aromatic methoxyl signal at about 6.6 where aliphatic methoxyl signals are expected.[3] Also there is no noticeable maximum at 3.9 indicating the absence of benzylic acetoxyl groups from this derivative. This gives direct physical evidence confirming the prediction made by Adler[4] from comparison with similar reactions on model compounds that the acid methanolysis of lignin results in the formation of methyl ether groups at benzylic positions.

(3) N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "NMR Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962, p. 13.

(4) E. Adler, "Chinoide Strukturen und Benzylalkokol gruppierunger in der Chemie und Biochemie des Lignins," Fourth International Congress of Biochemistry, Vol. II, "Biochemistry of Wood," Pergamon Press, New York, N. Y., 1958, pp. 148–150.

TABLE III

THE PERCENTAGES OF TOTAL SIGNAL FROM PROTONS IN ACETYLATED LIGNINS WHICH FALL WITHIN VARIOUS RANGES OF τ-VALUES

Lignin	Range no.	2	3	4		5		6	7	8
	τ-Values in p.p.m.	2.00–3.72	3.72–4.26	4.26–4.82		4.82–7.50		7.50–7.81	7.81–8.42	8.42–9.62
	Proton type	Arom.	α_1		OMe	OMe	α_2,β,γ	Arom.	Aliph.	
	Run	α-vin.	β-vin.	α^3	α_2,β,γ	(calcd.)	(diff.)	Ac.	Ac.	?
Bjorkman (spruce)	1	19.1	3.3	2.2	45.0			5.7	23.4	1.4
	2	18.9	2.6	2.2	45.0			5.8	23.0	2.0
	3	18.9	2.6	2.0	45.1			6.4	23.4	1.5
	4	17.6	2.5	1.6	46.1			5.7	24.6	1.9
	Average	18.6	2.8	2.0	45.4	(20.2)	(25.2)	5.9	23.6	1.7
Dioxane (western hemlock)	1	17.2	2.9	2.4	44.3			7.6	21.7	3.9
	2	17.2	3.1	2.3	43.9			8.1	22.6	2.8
	3	17.2	3.5	2.2	44.3			7.6	22.2	3.0
	Average	17.2	3.2	2.3	44.5	(20.7)	(23.8)	7.8	22.2	3.4
Fract. A	1	17.3	2.6	2.8	45.1			7.8	21.1	3.3
	2	17.4	2.7	2.7	44.7			7.8	21.4	3.8
	Average	17.4	2.7	2.7	44.7			7.8	21.2	3.6
Fract. B	1	17.9	2.8	2.6	45.7			8.0	21.4	1.6
	2	18.2	3.0	2.4	45.2			7.7	22.2	1.3
	Average	18.0	2.9	2.5	45.5			7.8	21.8	1.5
Fract. C	1	17.4	2.4	1.5	43.8			7.6	24.2	3.2
	2	17.3	2.5	1.3	43.3			8.1	24.1	3.4
	Average	17.4	2.4	1.4	43.6			7.8	24.2	3.3
Weighted average, fract. A, B, and C		17.6	2.7	2.3	44.5	(20.7)	(23.8)	7.8	22.6	2.7
Average, nine runs		17.4	2.9	2.2	44.5	(20.7)	(23.8)	7.8	22.5	3.0
Brauns (spruce)	1	17.5	3.2	1.9	42.4			8.7	23.7	2.6
	2	17.3	2.8	1.5	42.8			9.0	24.1	2.5
	Average	17.4	3.0	1.7	42.6	(19.3)	(23.3)	8.8	23.9	2.6
Brauns (western hemlock)	1	17.0	2.8	1.4	40.4			13.6	23.6	1.2
	2	17.0	2.6	1.4	40.7			13.6	23.3	1.4
	Average	17.0	2.7	1.4	40.6	(19.1)	(21.4)	13.6	23.4	1.3

The spectrum of the diazomethane methylated Brauns lignin, Fig. 6, shows an enlarged aromatic methoxyl signal compared with the aliphatic acetoxyl signal and only a very small aromatic acetoxyl proton peak, indicating that practically all of the aromatic hydroxyl groups had been etherified by the diazomethane so that they were no longer free to be acetylated. This shows the usefulness of n.m.r. in demonstrating the completeness of the etherification of phenolic hydroxyl groups in lignins.

Quantitative Estimations from N.m.r. Spectra.—The n.m.r. spectra of protons in organic compounds can usually be integrated electronically with a high degree of precision. However, the complexity of lignin spectra makes it necessary to use the procedure illustrated in Fig. 11 to determine the percentages of total signal strength found within selected ranges of τ-values. This procedure has been repeated for two or more runs for each lignin preparation with the results given in Table III. No detectable signal was noted in range 1 for any of the preparations so this range is not included in Table III. Signal strengths in range 2 are corrected for the presence of protons of the residual chloroform found in the solvent.

Since the signal for the methoxyl protons in these preparations occurs in range 5 along with the signals from aliphatic side chain protons, the percentage of signals due to methoxyl protons is calculated from empirical formulas for each lignin and then subtracted from the total signal in range 5 to arrive at an estimate for the side chain protons.

The total number of protons per C_9 monomeric unit in these lignins is available from the empirical formulas in Table II. From these values and the percentages given in Table III, values are determined for the average number of protons per C_9 signaling within each range as is shown in Table IV.

Discussion

The estimation of the relative numbers of "condensed" and "uncondensed" aromatic systems present in lignins remains one of the outstanding questions regarding this natural product. Prior to the use of n.m.r. the only procedure reported for making this type of estimation is the one used by Leopold,[5] who calculated the number of uncondensed units present in lignins from the amount of vanillin he obtained from them by nitrobenzene oxidation.

Protons attached to aromatic nuclei in lignin model compounds all give n.m.r. signals within range 2, Table II, τ 2.00 to 3.72. Vinyl protons on α-carbons may cause unpredictable interference in the estimation of aromatic protons in this range. According to Lindgren[6] and Marton[7] there are about 0.06 conjugated vinyl group in Bjorkman lignin per C_9 unit. Table IV shows that there are a total of about 2.56 protons per C_9 unit in Bjorkman lignin which signal in range 2. By subtracting 0.06 α-vinyl protons from this figure we find that there are an average number of

(5) B. Leopold, *Svensk Kem. Tidskr.*, **64**, 18 (1952).
(6) B. O. Lindgren and H. Mikawa, *Acta Chem. Scand.*, **11**, 826 (1957).
(7) J. Marton and E. Adler, *ibid.*, **15**, 370 (1961).

TABLE IV

PROTONS PER C_9 STRUCTURAL UNIT IN ACETYLATED LIGNINS AS DETERMINED BY THE INTEGRATION OF N.M.R. SPECTRA

Range	τ-Values	Protons known to give signals within range	Lignins: Bjorkman spruce	Dioxane west. hem.	Brauns spruce	Brauns west. hem.
1	−1.50–2.00	Carboxylic and aldehydic	[a]	[a]	[a]	[a]
2	2.00–3.72	Aromatic and α-vinyl	2.56	2.36	2.38	2.32
		α-Vinyl (calcd.)	0.06	0.06	0.06	0.06
		Net aromatic	2.50	2.30	2.32	2.26
3	3.72–4.26	Total	0.39	0.39	0.41	0.37
		β-Vinyl	0.06	0.06	0.06	0.06
		α, as in Fig. 7	0.33	0.33	0.35	0.31
4	4.26–4.82	α, as in Fig. 9	0.28	0.30	0.23	0.19
5[b]		All other α, β, and γ found in models	3.55	3.24	3.19	2.91
3, 4, and 5[b]	4.82–7.50	Total α, β, and γ	4.22	3.93	3.83	3.65
		Less β-vinyl	0.06	0.06	0.06	0.06
		Net α, β, and γ	4.16	3.87	3.78	3.50
5[c]		Methoxy, calcd.	2.82	2.82	2.64	2.62
6	7.50–7.81	Acetoxyl, aromatic except as in Fig. 10	0.83	1.06	1.20	1.86
7	7.81–8.42	Acetoxyl, aliphatic plus aromatic as in Fig. 10	3.34	3.06	3.27	3.21
6 and 7	7.50–8.42	Acetoxyl, total	4.17	4.12	4.47	5.07
8	8.42–9.62	Aliphatic, highly shielded	0.24	0.41	0.37	0.18
		Total protons per C_9 structural unit	14.01	13.64	13.69	13.66

[a] Insufficient signal to be reliably detected. [b] Total signal in range 5, less signal calculated for methoxyl protons. [c] Calculated from empirical formulas.

about 2.50 protons per C_9 unit attached to aromatic nuclei. Since the large signal in range 2 no doubt overlaps more into range 3 than *vice versa*, this number represents a minimum and indicates that about 50 to 60% of the monomeric units in Bjorkman lignin are "uncondensed," having three protons attached to each aromatic nucleus and the remaining 40 to 50% are "condensed" having only two protons per nucleus.

The dioxane lignin on the other hand, appears to contain fewer aromatic protons, Table IV. Similar reasoning to that above leads to the conclusion that there are about 60 to 70% "condensed" aromatic systems present in this lignin. A possible explanation for this is that the mild acidolysis used in the extraction of this lignin may have caused a condensation involving the aromatic rings.

The Brauns lignins also contain fewer protons which register in range 2 than the Bjorkman lignin. It thus appears that there may be a larger proportion of "condensed" units in Brauns lignins than in Bjorkman lignin. As yet no explanation for this is readily apparent.

The number of protons estimated per C_9 unit in ranges 6 and 7 are of particular interest because from them an estimate of the numbers of aromatic and aliphatic acetoxyl (and, by inference, hydroxyl groups in the parent preparations) may be made. Protons on all aromatic acetoxyl groups in model compounds give signals in range 6 except for those *ortho* to a biphenyl linkage as in Fig. 10. The reason for this exception has been previously discussed.[1] These latter aromatic acetoxyl protons give signals in range 7 along with the aliphatic acetoxyl protons. Three acetoxyl protons are equivalent to one hydroxyl proton in the parent lignin so the number of hydroxyl groups per C_9 unit in the parent preparations may be estimated. The fact that these ranges are well removed from other major signals in the n.m.r. spectra which were studied makes signal overlapping less of a problem and thus increases the reliability of the values found. Table V shows the number of aromatic hydroxyl groups per methoxyl found by n.m.r. for these lignins as compared with the number found for similar lignins by potentiometric titration.[8] The comparisons show that the n.m.r. results are in good agreement with the results from the potentiometric titration.

TABLE V

AROMATIC HYDROXYL ESTIMATIONS BY NUCLEAR MAGNETIC RESONANCE AND BY POTENTIOMETRIC TITRATION[a]

Lignin	Phenolic hydroxyl estimations: N.m.r.[b]	Titration[c]
Dioxane[e]	0.37	0.41
Bjorkman milled wood[d]	.29	.33
Brauns native	.45[d]	.55 to 0.7
	.70[e]	

[a] All values in terms of aromatic hydroxyl groups per methoxyl. [b] Range 6, acetoxyl protons, Table IV, divided by three and adjusted to aromatic OH per OMe. [c] Ref. 9. [d] Spruce. [e] Western hemlock.

Our lignin model compound studies[1] show that all of the protons attached directly to saturated side chain carbons in lignin-like linkages or chain endings give signals in ranges 3, 4, and 5. The only serious interference with these signals comes from methoxyl proton signals which register in range 5. Fortunately the percentage of total signal due to methoxyl groups is conveniently available by calculation from conventional analyses. This allows the net signal strength due to side chain protons to be calculated by difference for each lignin spectrum as shown in Table III.

A probable minor interference in range 3 by signals from β-vinyl protons estimated to be present to the extent of about 0.06 per C_9 unit in Bjorkman lignin is subtracted from this range as is shown in Table IV.

It is interesting to note that the sum of the number of protons signaling in ranges 3, 4, and 5 is 4.0 ± 0.2 for each of the first three lignins listed in Table IV. This is in accord with modern biosynthetic theory that there should be about four side chain protons per C_9 monomeric unit in lignins.

(8) F. E. Brauns, "The Chemistry of Lignin," Academic Press, Inc., New York, N. Y., 1960, p. 249.

TABLE VI

ACETYLATIONS OF LIGNIN PREPARATIONS

Lignin preparations	Analyses C	Analyses H	Analyses OMe	Lignin used, g.	Yield, g.	Analyses of products C	Analyses of products H	Analyses of products OMe	Analyses of products Ac
Dioxane, west. hemlock	64.75	5.81	15.43	2.0	2.22	63.00	5.63	12.26	29.56
						63.12	5.79	11.92	24.33
Bjorkman, spruce	...	..	15.53	0.103	0.105	...	..	12.05	22.39
Brauns, spruce	64.15	6.20	14.64	.506	.538	62.09	5.65	11.20	24.08
Brauns, west. hemlock	62.84	6.29	14.96	.501	.565	61.70	5.49	10.83	26.54
Brauns, methanol	65.01	6.05	19.35	.192	.195	63.52	5.53	15.31	19.75
Brauns, diazomethane	61.69	6.27	18.25	.303	.066	...	..	...	...

The results for ranges 3 and 4 are of special interest because in the model compounds studied only one type of proton attached to a saturated carbon was found to signal in each of these ranges. In range 3, Table IV, the number of protons per C_9 unit (after correcting for vinyl protons) is about 0.3 for each of the lignins. Protons attached to α-carbons bonded to acetoxyl groups (*e.g.*, see Fig. 7) are the only type found in model compounds which signal in this range. Following reasoning similar to that used in discussing the aromatic protons above, the value of about 0.3 proton per C_9 unit represents an upper limit of the number of this type of proton present in these lignins. This also means that there are the same number of benzylic acetoxyl groups present and by inference benzylic hydroxyl groups in the nonacetylated parent lignins.

The only signals from acetylated model compounds found in range 4 are from protons on an α-carbon in the phenylcoumaran ring system, Fig. 9. As a consequence of this, the integral in this range indicates an upper limit to the amount of this type of linkage which may be present in these lignins. The fact that the larger molecular weight Bjorkman and dioxane lignins each have on the order of 0.3 proton per C_9 in range 4 while the lower molecular weight Brauns lignins contain about 0.2 indicates that line broadening from signals in range 5 may be a factor adding to the total signal in range 4. Thus there are somewhat fewer than 0.2 phenylcoumaran unit per C_9 in these lignins.

The presence of from 0.2 to 0.4 proton per C_9 in range 8, τ 8.42 to 9.62, is ofs ignificance because no protons attached to model compounds studied by n.m.r.[1] which are expected to occur in lignins were found in this range. The types of protons which are known to signal in this range[1,2] are bonded to methyl or methylene carbons which are not attached directly to oxygen functions, carbonyl groups, aromatic systems, or other deshielding groups. Consequently, it is apparent that some such groups are present in lignins. That these signals are not simply due to line broadening effects from the acetoxy protons is borne out by the presence of similar signals in this range in n.m.r. spectra of methanol lignin from Eucalyptus in which there are no acetoxyl protons.[9] Just what the nature of these protons is and how they fit into the structure of lignins is open to speculation.

Several attempts were made carefully to determine whether any signal could be detected in range 1, τ -1.50 to 2.00; however, in none of the lignin spectra was any detectable signal noted. From this it appears that carboxylic and aldehydic protons are not present to the extent of more than 0.1 per C_9 unit, since a larger number of protons than this would be within the limits of detection by the spectrometer and integrator.

It was also noted that for these lignins there was no signal detected below about τ 2.5 in range 2 where protons which are attached to an aromatic nucleus and *ortho* to an α-carbonyl are expected to register (Table II). This leads one to conclude that there are fewer than 0.1 α-carbonyl group per C_9 unit in these lignins and is in good agreement with Marton and Adler[7] who have estimated that there are about 0.06 of these groups per C_9 in Bjorkman lignin. In the case of the dioxane lignin the n.m.r. evidence indicates that no more than 0.1 α-carbonyl group per C_9 unit is formed by the mild acidolysis which was used.

The integration of n.m.r. spectra of lignins is uniquely well adapted to the study of similarities and differences among lignins from different sources and isolated by different techniques. The differences between the Bjorkman lignin and the dioxane lignin, we have explained above as probably being the result of effects of the acidolysis used in the isolation of the dioxane lignin. However, it may be possible that some of these differences are related to the fact that the Bjorkman lignin is from spruce wood whereas the dioxane lignin is from western hemlock. In the case of the Brauns lignins significant quantitative differences are noted between the integrated spectrum from spruce lignin and that from western hemlock lignin. The most obvious difference is that in range 6 indicating that there are about half again as many aromatic acetoxyl protons in western hemlock as in the spruce Brauns lignin. Smaller differences between spectra of these two preparations which are evident in other ranges (Table IV) confirm the fact that gymnosperm Brauns lignins differ from one species to another.

Conclusion

The small amount of highly shielded aliphatic protons signal in range 8 is not readily accounted for by proton signals in the model compound spectra studied, and some of the interpretations given in this paper may need to be modified if linkages, other than those represented in the present models, are shown to occur in lignin. For instance, Pearl[10] has found evidence supporting the presence of an α–α' linkage in lignins, and Freudenberg[11] has suggested the presence of a tetralin type linkage in which α–6′ and β–β' links are present. Other inaccuracies in present interpretations may arise from the somewhat arbitrary setting of the boundaries for the τ-value ranges summarized in Table II.

A segment of a gymnosperm lignin molecule is represented speculatively in Fig. 12 and is drawn so as to be in agreement with this n.m.r. and earlier studies, although necessarily arbitrary in many ways. Features of it which are supported by n.m.r. spectroscopic evidence include: (a) about one-third of the aromatic rings contain free hydroxyl groups, as in A, H, and J; (b) about one-half of the aromatic rings are condensed and have only two aromatic hydrogens attached, as in B, E, H, I, and J; (c) about four protons are

(9) D. E. Bland and S. Sternhell, *Nature*, **196**, 985 (1962).

(10) I. A. Pearl and D. L. Beyer, *Tappi*, **39**, 171 (1956).

(11) K. Freudenberg, *Pure Appl. Chem.*, **5**, 9 (1962).

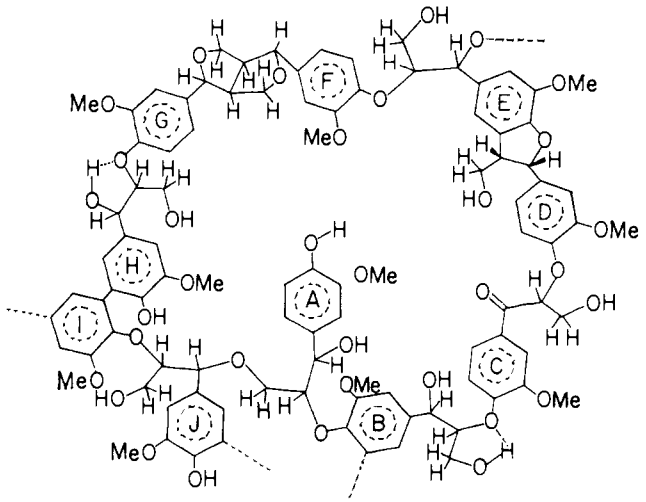

Fig. 12.—Speculative structure of segment of gymnosperm lignin molecule.

attached to side chain carbon atoms per phenylpropane unit; and (d) about eight aliphatic hydroxyl groups, of which about three are benzylic, are present per ten phenylpropane units. The presence of the large scale cross-linking or ring systems indicated in Fig. 12 as being present in lignin molecules is not proved, but the existence of a somewhat rigid structure is suggested by the broadness of the signals observed in the n.m.r. spectra. Evidence for hydrogen bondings of the types shown in BC and GH has been found with model compounds in chloroform solution, and such bonding may also occur in lignins in other solvents and in the dry state.

Experimental

The n.m.r. spectra were taken using the instrumentation and procedures previously described.[1] Solutions of 7 to 15% by weight of the acetylated lignins in $CDCl_3$ containing hexamethyldisiloxane internal reference were used in standard degassed and sealed 5-mm. tubes.

The integrations of n.m.r. spectra were done using an integrator described by Varian Associates.[12] Prior to integration of the n.m.r. spectrum of each lignin preparation a sample of ethanol was integrated to determine that the integrator was adjusted correctly. The integrator was considered to be properly adjusted only when the ratio of methylene protons to methyl protons in the ethanol was found to be 2.00 to 3.00 within confidence limits of 0.01. The stability of the integrator was further checked by allowing the sweep to cover several parts per million at the beginning and end of each spectrum to be sure there was no appreciable drift. Lignin solutions of 10% or greater concentration were found to give more reliable integration results than weaker solutions.

A correction for the small amount of signal from chloroform protons in the deuteriochloroform solvent was made in each case as follows. By integrating an n.m.r. spectrum of our solvent deuteriochloroform containing a known weight of hexamethyldisiloxane it was found to contain about 0.0042% protons by weight. Using this figure the percentage of the total integrated signal coming from chloroform protons, A, was found for each sample by applying the following equation in which x = the weight per cent of lignin preparation in the sample, $100 - x$ = the weight per cent of deuteriochloroform in the sample, and H = fraction of hydrogen in lignin. (The small amount of hexamethyldisiloxane was neglected.)

$$A = \frac{(0.0042)(100 - x)(100\%)}{(Hx) + (0.0042)(100 - x)}$$

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(12) The NMR-EPR Staff of Varian Associates, "NMR and EPR Spectroscopy," Pergamon Press, New York, N. Y., 1960, Chapter 15.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOTH, ISRAEL]

On the Synthesis of Cysteine Peptides[1]

BY MORDECHAI SOKOLOVSKY, MEIR WILCHEK, AND ABRAHAM PATCHORNIK

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The preparation of various peptides of cysteine is described. The basis of the method used is the selective removal of the carbobenzoxy group from either the amino or thiol group without causing racemization. The action of hydrogen bromide in acetic acid at room temperature removes the carbobenzoxy moiety from the amino group almost quantitatively, without affecting the S-carbobenzoxy group. On the other hand, the action of excess sodium methoxide in anhydrous solvent causes rapid alcoholysis of the S-carbobenzoxy group with a quantitative liberation of the free thiol group as determined iodometrically. The method described was successfully applied to the total synthesis of glutathione in 25% over-all yield.

In the course of recent studies on the development of a method for the nonenzymatic cleavage of peptide at cysteinyl residues[2] it was found necessary to prepare cysteine-containing peptides as model compounds.

Methods for the preparation of peptides containing S-protected cysteinyl residues have been known for a long time.[3] However, the methods used so far for the selective removal of the S-protecting group suffer from a number of disadvantages: *e.g.*, low yields, racemization, and side reactions such as splitting of peptide bonds.[4] The problems arising during the synthesis of such peptides have been reviewed recently by Young.[5]

In the present paper, the preparation of various S- and N-carbobenzoxy derivatives of cysteine and their application to the synthesis of cysteine peptides is described. The basis of this method is the selective removal of the carbobenzoxy group from either the amino or thiol group in high yields without causing racemization. The action of hydrogen bromide in acetic acid for 15 min. at room temperature removes the carbobenzoxy moiety from the amino group almost quantitatively without affecting the S-carbobenzoxy group. On the other hand, the action of excess sodium methoxide (5 equiv.) for 5–10 min. at room temperature, under nitrogen, causes rapid alcoholysis of the S-carbobenzoxy group with almost quantitative liberation of the free thiol group, as determined iodometrically.

Such selective removal of the protecting group may be conveniently used in the synthesis of long peptide

(1) A recent report by L. Zervas, I. Photaki, and N. Ghelis (*J. Am. Chem. Soc.*, **85**, 1337 (1963)), which reached us after this work had been completed, contains results similar to some of those described in this paper.

(2) A. Patchornik and M. Sokolovsky, "Vth European Peptide Symposium, Oxford, 1962," Pergamon Press, 1963, p. 253, and in the following papers.

(3) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961.

(4) S. Sarid and A. Patchornik, *Israel J. Chem.*, **1**, 63 (1963).

(5) G. T. Young, *Collection Czech. Chem. Commun.*, **24**, 114 (1959).