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Registry No. P(2VPy), 25014-15-7; (L-ALA NCA)(SARNCA) (copolymers), 108561-50-8; L-ALANCA, 2224-52-4; SARNCA, 5840-76-6.

References and Notes

(1) Szwarc, M. *J. Polym. Sci.* **1954**, *13*, 317.
(2) Ballard, D. G. H.; Bamford, C. H. *Proc. R. Soc. London, A* **1956**, *A236*, 384.
(3) (a) Suzuoki, K.; Imanishi, Y.; Higashimura, T.; Okamura, S. *Biopolymers* **1969**, *7*, 917. (b) Kabanov, V. A.; Aliev, K. V.; Kargina, O. V.; Patrikeeva, T. I.; Kargin, V. A. *J. Polym. Sci., Polym. Symp.* **1967**, *No. 16*, 1079. (c) Ferguson, J.; Shah, S. A. O. *Eur. Polym. J.* **1968**, *4*, 611. (d) Bartels, T.; Tan, Y. Y.; Challa, G. *J. Polym. Sci., Polym. Chem. Ed.* **1977**, *15*, 341.
(4) Suzuoki, K.; Imanishi, Y.; Hagashimura, T.; Okamura, S. *Biopolymers* **1969**, *7*, 925.
(5) Szwarc, M. *Adv. Polym. Sci.* **1965**, *4*, 1.
(6) Leuchs, J. L. *Ber.* **1906**, *39*, 857.
(7) Leuchs, J. L.; Manare, W. *Ber.* **1907**, *40*, 3235.
(8) Idelson, M.; Blout, E. R. *J. Am. Chem. Soc.* **1957**, *79*, 3948.
(9) Natta, G.; Mazzanti, G.; Pong, A.; Dall'asta, G.; Bernardini, F. J. *J. Polym. Sci.* **1961**, *51*, 487.
(10) Kricheldorf, H. R.; Schilling, G. *Makromol. Chem.* **1977**, *178*, 3115.
(11) Kricheldorf, H. R.; Schilling, G. *Makromol. Chem.* **1978**, *179*, 1175.
(12) Flory, P. J. *Statistical Mechanics of Chain Molecules*; *Interscience*: New York, **1969**.
(13) Yau, W. W.; Kirkland, J. J.; Bly, D. D. *Modern Size-Exclusion Liquid Chromotography*; Wiley: New York, 1979.

Determination of Bonding Patterns of ^{13}C Specifically Enriched Dehydrogenatively Polymerized Lignin in Solution and Solid State

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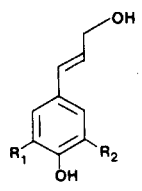
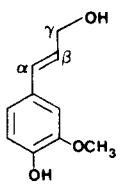
ABSTRACT: Synthetic dehydrogenatively polymerized (DHP) lignins were prepared by the oxidative polymerization of various coniferyl alcohols, enriched with ^{13}C at C_γ, C_β, $C_{\beta\gamma}$, and C_α. The solution and solid-state ^{13}C NMR spectrum of each was then recorded. The solution spectra further confirmed assignments for unlabeled DHP lignin. Solid-state spectra, while subject to extensive line broadening, nevertheless gave valuable information. It was established that the major bonding patterns, namely, substructures containing coniferyl alcohol (A), β-*O*-(4-aryl)glycerol (C), and pinoresinol (D) moieties could readily be distinguished. It was also shown that coniferaldehyde (B) substructures were artifacts. With the labeling methods used it was not possible to distinguish between phenylcoumaran (E) and β-1 (F) moieties. The bonding patterns of a DHP lignin (enriched at C_γ to 4% with ^{13}C) in a cellulose matrix (ratio DHP:cellulose, 24% w/w) could be identified. It therefore seems feasible to examine the bonding patterns of lignin in situ, if specifically labeled lignified tissue enriched to the level of >4% can be obtained.

Introduction

Lignin, a widely distributed vascular plant polymer, is nature's second most abundant naturally occurring organic material. It is generally considered to be formed *exclusively* via the random, dehydrogenative polymerization of the three trans (*E*) monolignols, *p*-coumaryl (**1**), coniferyl (**2**), and sinapyl (**3**) alcohols.[1] This polymerization reaction is thought to be catalyzed by cell-wall-bound peroxidases, whose action on the monomers **1–3**, in the presence of H_2O_2, results in the formation of highly reactive free radical intermediates which combine randomly to give lignin.[2] The ratio of each monomer in the lignin polymer has been shown to be both species[3] and morphological origin[4,5] dependent.



1, $R_1, R_2 = H$
2, $R_1 = H$, $R_2 = OCH_3$
3, $R_1, R_2 = OCH_3$



4a, $C_\alpha = {}^{13}C$
b, $C_\beta = {}^{13}C$
c, $C_\gamma = {}^{13}C$
d, $C_{\beta,\gamma} = {}^{13}C$

Lignin is relatively intractable and isolation procedures, whether chemical or biochemical, are drastic. Irreversible changes to the original lignin macromolecule are therefore *unavoidable*. Lignins are thus classified according to their source, e.g., hardwoods, or softwoods,[6] and method of isolation, e.g., kraft, sulfite, and milled wood lignins.[6] Consequently, the bonding pattern of lignin in situ has never been established.

Much of our current understanding of lignin structure comes from the comparison of isolated lignins with an artificial preparation, known as dehydrogenatively polymerized (DHP) lignin. This material is produced by the in vitro dehydrogenative polymerization of monolignols 1–3; a reaction catalyzed by peroxidase/H_2O_2. DHP lignin is considered to more or less represent native lignin, although differences between it and isolated lignin derivatives have been noted.[7,8] In the absence of a better model, DHP lignin

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has found fairly broad application as a substrate with which to study lignin biodegradation.[9,10]

In terms of their ^{13}C nuclear magnetic resonance (NMR) spectra, DHP lignin and lignin derivatives are fairly complex. Many of the resonances have, however, been assigned by means of comparison with a rather large number of model compounds, which were either monomeric or dimeric in character.[11]

Specifically enriched (^{13}C) DHP lignins have also been prepared by the in vitro polymerization of either [α-^{13}C]-(**4a**)[12] or [β-^{13}C] coniferyl (**4b**) alcohol,[9] and the resulting enhanced resonances in solution have been assigned to various bonding environments.

More recently, solid-state (^{13}C) CP/MAS NMR of plant tissue, e.g., wood,[13-17] and its isolated components, e.g., lignin[13,15-18] and cellulose,[16,17,19-22] has received much attention. This technique is currently one of the most promising methods for probing the bonding environment of *insoluble* lignin preparations. Its usefulness in examining natural-abundance lignified tissue, however, is limited because of the severe masking of resonances by carbohydrate signals.

In this study, we wish to describe the optimized syntheses of γ-^{13}C **4c**, β,γ-$^{13}C_2$ **4d**, β-^{13}C **4b**, and α-^{13}C **4a** DHP lignins. As discussed below solution and solid-state ^{13}C NMR of these substances were recorded and compared and the results interpreted.

Materials and Methods

^{13}C NMR spectra were recorded on a Varian XL-300 spectrometer in the Fourier-transform mode at 75.4 MHz. The pulse width was 9.0 μs, giving a flip angle of 37°. Acquisition time and pulse repetition time were 0.91 s. The spectral width was 16 500 Hz, using 30 000 data points in the double-precision mode. Carbon–proton coupling was eliminated by broad-band decoupling.

Me_4Si was used as internal standard. DHP lignin samples (180 mg) were dissolved in deuteriated Me_2SO to make a 10% solution, and 35 000–80 000 transients were collected.

Free induction decays (FIDs) were weighted with an exponential function to give a line broadening of 6 Hz, before performing the Fourier transform. Difference spectra were obtained by subtracting FIDs on the spectrometer's software, and then weighting and transforming the resulting FID as for a regular spectrum.

Solid-state ^{13}C NMR spectra were recorded on a Bruker CXP 180 spectrometer at 45.27 MHz. The pulse repetition time was 2 s with a cross-polarization contact time of 1 ms. On alternate scans the ^{13}C spin temperatures were reversed and the FID alternately added and subtracted, to cancel out magnetization from sources other than cross polarization.

Samples of 250–300 mg were packed in bullet-type Delrin rotors and spun at 3 KHz. For each spectrum 2400 scans were recorded except for the spectra of mixed DHP lignin and cellulose where 8000 scans were collected.

Preparation of DHP Lignin. To a 2-L round-bottom flask, equipped with two polyethylene capillary inlet tubes, a magnetic stirring bar, and a nitrogen inlet, were added buffer solution (150 mL, 0.025 M phosphate, pH 6.5, degassed) and horseradish peroxidase (1100 units) (Boehringer Mannheim).

Peroxidase activity was determined spectrophotometrically as previously described.[23] The suspension was stirred continuously during the preparation.

Coniferyl alcohol (2) (400 mg, 2.22 mmol) was dissolved in buffer solution (350 mL, 0.025 M phosphate, pH 6.5, degassed) to give solution A. Solution B was prepared by dissolving hydrogen peroxide (2.67 mmol, 1.2 equiv) in buffer (350 mL, 0.025 M phosphate, pH 6.5, degassed).

Solutions A and B were added simultaneously to the peroxidase solution via a three-channel Pharmacia P-3 peristaltic pump at the rate of 8 mL/h each.

After 24 h, another 500 units of peroxidase was added to the reaction mixture. (The enzyme retained its activity for 24 h in the presence of 2 alone but gradually lost activity when H_2O_2 was present in the solution). When all of the reagents had been delivered (48 h), the yellow-orange suspension was immediately centrifuged for 4 h at 65000*g*.

The clear supernatant was decanted and the sediment resuspended in distilled water. The DHP suspension was centrifuged a second time. The resulting sediment was then suspended in 5 mL of distilled water and freeze-dried to give an amorphous light-beige product (340 mg, 85% yield).

Synthesis of ^{13}C Labeled DHP Polymers. ^{13}C labeled coniferyl alcohols **4a–d** were synthesized as previously described.[24] The resulting DHP polymers were synthesized as described above.

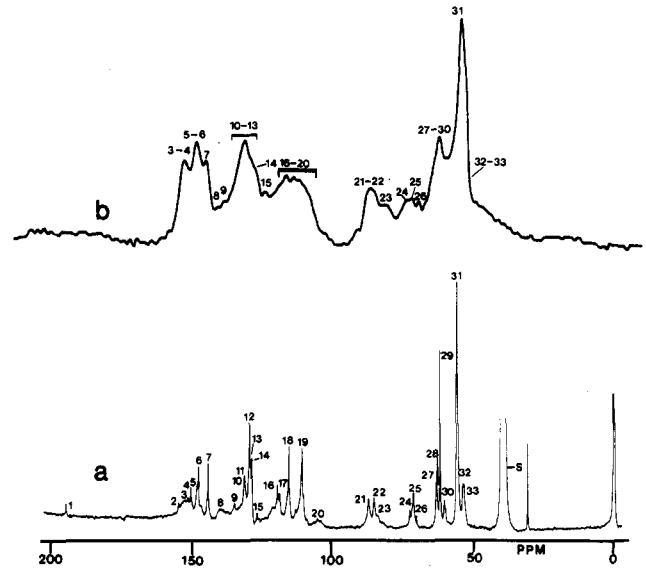


Figure 1. ^{13}C NMR of natural-abundance DHP lignin: (a) solution; (b) solid state; S = solvent (deuteriated Me_2SO).

Results and Discussion

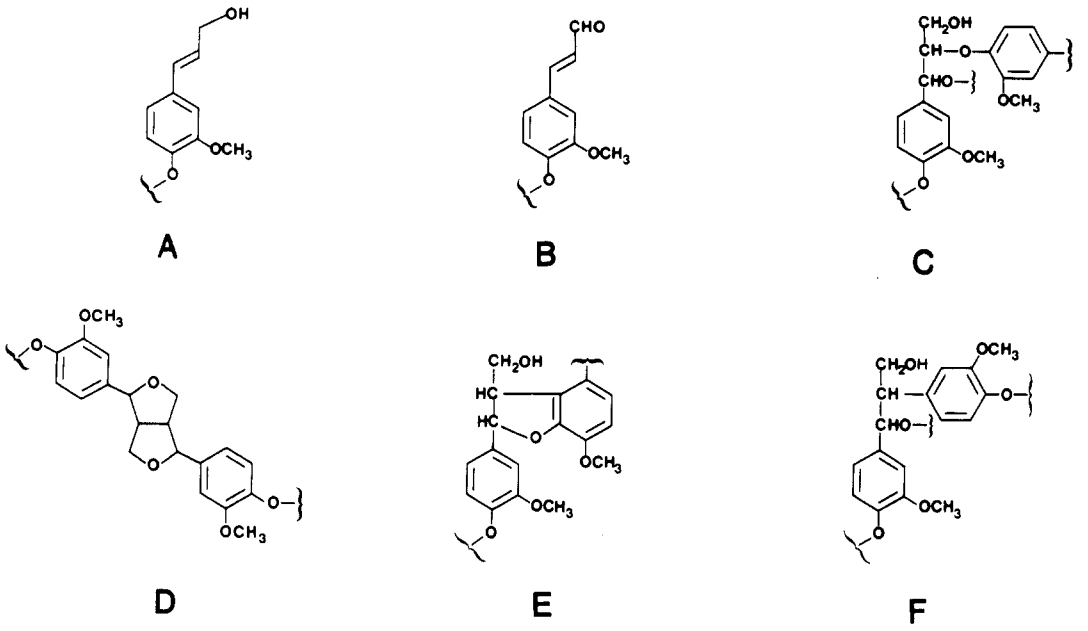
Synthesis of DHP Polymers. [γ-^{13}C]- (**4c**), [β,γ-^{13}C]-(**4d**), and [α-^{13}C]coniferyl (**4a**) alcohols were prepared as described recently.[24] [β-^{13}C]Coniferyl alcohol (**4b**) was synthesized by conventional means.[9] These carbons were labeled since they are involved in the vast majority of lignin interunit linkages.

Two procedures were reported by Freudenberg for the preparation of DHP lignin.[1] In the first (Zulaufverfahren) method, oxygen was bubbled through a solution of coniferyl alcohol (**2**) in the presence of the dehydrogenating enzyme and H_2O_2. This approach, though, only resulted in the formation of low molecular weight dimers and oligomers.

The second method (Zutropfverfahren) was carried out by independently adding solutions of coniferyl alcohol (**2**) and hydrogen peroxide, simultaneously and slowly, into a buffered solution of horseradish peroxidase. This gave a polymeric product more closely resembling lignin derivatives[11] and was the method employed in our studies.

Numerous researchers have synthesized DHP lignins at pH's ranging from 5.5 to 7.5.[25-27] In our investigations, several DHP polymers were prepared at pH values of 5.5, 6.5, and 7.5. These gave average yields of 17%, 85%, and 65%, respectively. Indeed, best results were achieved with a pH of 6.5, a 20% excess of H_2O_2, and addition of an additional 500 units of peroxidase to the reaction mixture after 24 h. This was because, although the enzyme retained its activity for 24 h in the presence of coniferyl alcohol (**2**), it gradually lost its activity when H_2O_2 was present in the medium. These optimized conditions were used in the synthesis of specifically ^{13}C-enriched DHP polymers.

^{13}C NMR Analysis of DHP Lignins in Solution and Solid State. Previous studies on DHP lignin preparations have showed that the oxidative polymerization of coniferyl



alcohol (2) gives a Me_2SO-soluble polymer whose main substructures are A–F.[11,12]

Figure 1a shows the ^{13}C spectrum of natural-abundance DHP lignin. Several of the individual resonances have been assigned to substructures A–F.[11,12] These will be discussed, as appropriate, in the text.

Figure 1b shows the solid-state ^{13}C NMR spectrum of unlabeled DHP lignin. The most obvious feature, on comparison to Figure 1a, is the much greater line width due to the lack of averaging in the solid state; anisotropicities which are eliminated by molecular tumbling in solution are not completely removed by magic angle spinning and the very strong proton–carbon dipole interactions remain in spite of high power decoupling.

As can be seen, many individual resonances in the solid-state spectrum overlap so greatly that individual bonding arrangements can no longer be recognized. Therefore, the synthesis of specifcally labeled DHP lignins was carried out. We envisaged that this would permit the determination of (1) the actual bonding environments for a particular carbon atom and (2) whether a particular substructure (i.e., A–F) could be detected in a solid-state DHP (or lignin) sample.

DHP Lignin from [γ-^{13}C]Coniferyl Alcohol. γ-^{13}C DHP lignin was produced from [γ-^{13}C]coniferyl alcohol (**4c**), enriched with ^{13}C at C_γ to the extent of 11 atom %. [99 atom % ^{13}C alcohol **4c** was mixed with unlabeled coniferyl alcohol **2** to give this level of enrichment].

Figure 2a,b shows the DHP resonances corresponding only to ^{13}C enrichment at C_γ for both solution and solid state, respectively. These are difference spectra and were obtained by subtracting natural-abundance resonances from those of the enriched spectra.

In the solution-state spectrum (Figure 2a), enrichment at C_γ produced six enhanced peaks. These are assigned to substructures A–F (Table I) and are in agreement with previous tentative designations.[11,12]

In the solid-state spectrum (Figure 2b), resonances for substructures A, C, E, and F all appear as a single peak centered at 62 ppm because of the greater line broadening. However, substructure D can be identified as a shoulder at 71 ppm and is the only resonance that can be individually assigned. It is also noteworthy that there is no evidence for the aldehyde (substructure B) which is clearly visible in the solution spectrum (peak 1). It therefore

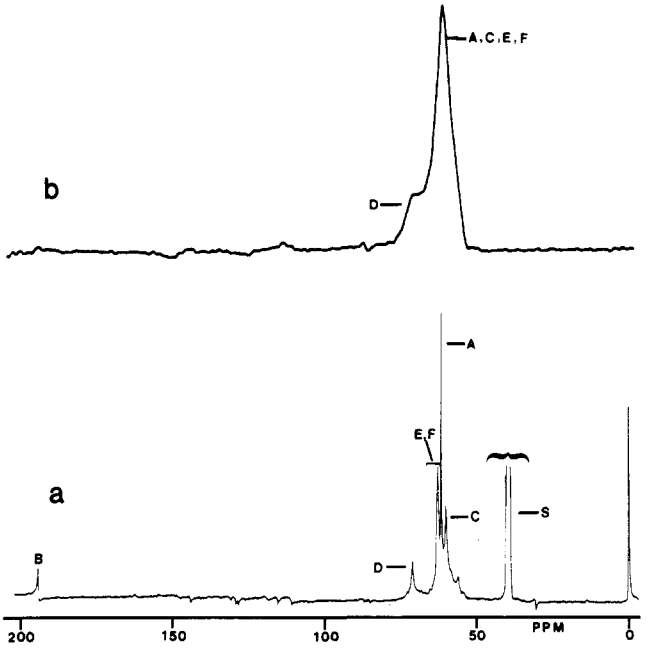


Figure 2. ^{13}C NMR difference spectra of γ-^{13}C DHP lignin showing only enhanced resonances in (a) solution and (b) solid state. (Natural-abundance resonances were subtracted from the spectra.) S = solvent (Me_2SO); ppm values for substructures are given in Table I.

Table I
^{13}C NMR Assignments of γ-^{13}C DHP Lignin

solution spectrum			solid-state spectrum		
peak[a]	shift, ppm	substructure	peak[a]	shift, ppm	substructure
1	193.93	B			
25	70.90	D	25	71	D
27	62.85	E or F			
28	62.58	E or F	27–30	62	A, C, E, F
29	61.56	A			
30	60.00	C			

[a] Refers to numbering system used in Figure 1.

follows that allylic oxidation has occurred in the Me_2SO solvent. This is not an unexpected result since Me_2SO is a known oxidizing reagent.[28]

DHP Lignin from [β,γ-^{13}C]- (4d) and [β-^{13}C]Coniferyl (4b) Alcohols. Figure 3a shows the solution dif-

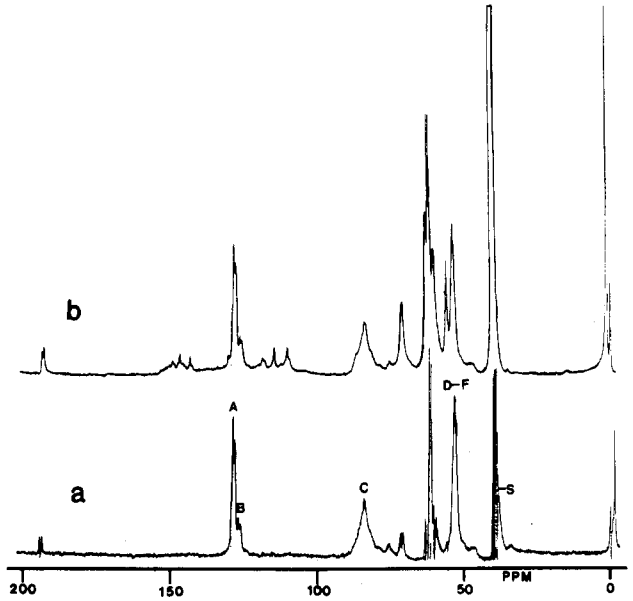


Figure 3. ^{13}C NMR difference solution spectra of (a) β,γ-^{13}C and (b) β-^{13}C DHP lignin showing only enhanced resonances. The latter was obtained by subtraction of γ-^{13}C DHP resonances from those of [β,γ-^{13}C] DHP. S = solvent; ppm values for substructures are given in Table II.

Table II
^{13}C NMR Assignments of β-^{13}C DHP Lignin

solution spectrum			solid-state spectrum		
peak[a]	shift, ppm	substructure	peak[a]	shift, ppm	substructure
14a	128.33	A	14	127.6	A
14b	127.75	A			
15a	126.47	B			
15b	125.74	B			
23	83–84	C	23	83–84	C
32, 33	53	D–F	32, 33	53–54	D–F

[a] Refers to numbering system used in Figure 1.

ference spectrum of β,γ-^{13}C-labeled DHP obtained from coniferyl alcohol **4d** enriched at C_β and C_γ to 11 atom % ^{13}C. Splitting of 30–60 Hz for each enriched resonance is noted because of ^{13}C–^{13}C coupling. We also synthesized β-^{13}C DHP lignin using 11 atom % β-^{13}C-enriched coniferyl alcohol (**4b**) and then recorded its solid-state ^{13}C NMR (Figure 3b). As can be seen, enrichments were observed at 128 (substructure A), 126 (substructure B), 83 (substructure C), and 53 ppm (substructures D–F). These assignments are given in Table II and are in agreement with those of Haider.[9]

Figure 4a shows the solid-state ^{13}C difference spectrum of DHP lignin enriched to 5% with ^{13}C at carbons β and γ. Unfortunately, the large dipolar couplings between the adjacent ^{13}C-enriched carbons are not suppressed in the solid state. The resulting spectrum is therefore of limited usefulness and shows the current limitation of double-labeled experiments for solid-state purposes.

Figure 4b shows the solid-state ^{13}C NMR difference spectrum of β-^{13}C-labeled coniferyl alcohol (**4b**) in the solid state. Resonances due to substructures A (127.6 ppm), C (83–84 ppm), and D–F (53–54 ppm) can clearly be distinguished, as previously noted for the solution (Figure 3b).

α-^{13}C-Labeled DHP. Figure 5a,b shows the ^{13}C difference spectra of α-^{13}C DHP in both solution and solid state, respectively. This was prepared from [α-^{13}C]coniferyl alcohol (**4a**) enriched to 11 atom % with ^{13}C at C_α.

Several enhanced resonances due to C_α in different bonding environments can be seen from inspection of the

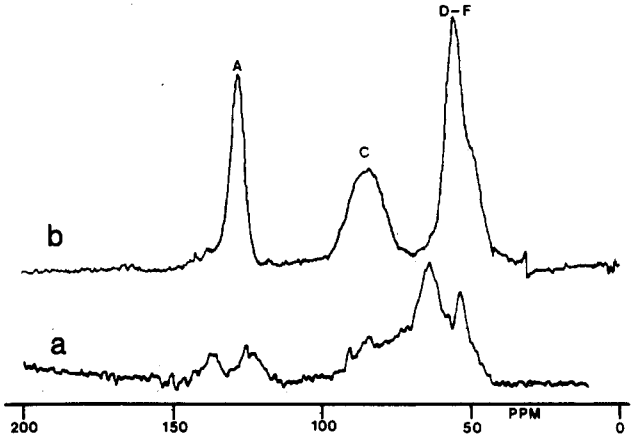


Figure 4. ^{13}C NMR difference solid-state spectra of (a) β,γ-^{13}C and (b) β-^{13}C DHP lignin samples. (Natural-abundance resonances were subtracted from the spectra.) Ppm values for substructures are given in Table II.

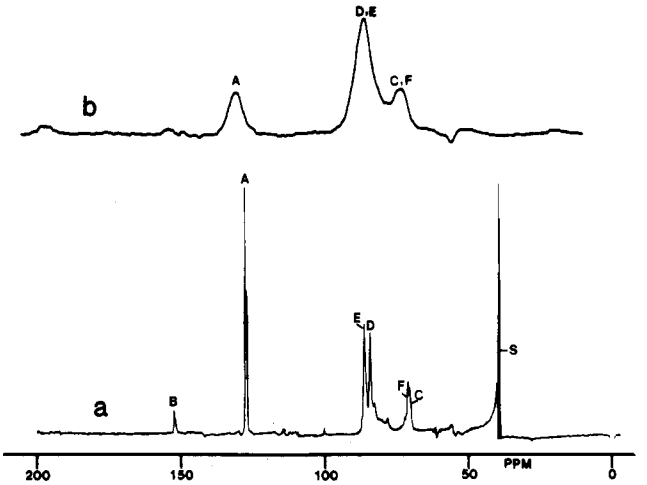


Figure 5. ^{13}C NMR difference spectra of α-^{13}C DHP lignin showing only enhanced resonances in (a) solution and (b) solid state. (Natural-abundance resonances were subtracted from the spectra.) S = solvent; ppm values for substructures are given in Table III.

Table III
^{13}C NMR Assignments of α-^{13}C DHP Lignin

solution spectrum			solid-state spectrum		
peak[a]	shift, ppm	substructure	peak[a]	shift, ppm	substructure
2	153.97	B			
2a	153.60	B			
12	128.90	A	12, 13	132	A
13	128.47	A			A
21	86.91	E	21, 22	86	D/E
22	84.91	D			
24	71.47	F	24, 26	72	C/F
26	70.83	C			

[a] Refers to numbering system used in Figure 1.

solution spectrum. Assignments are given in Table III and correspond to those of Gagnaire and Robert.[12]

From the solid-state spectrum, only substructure A (~132 ppm) can clearly be distinguished. Substructures D/E (~86 ppm) and C/F (~72 ppm) overlap preventing their unambiguous differentiation.

DHP Lignin/Cellulose Mixtures. The lignin content of woody plants is normally ca. 20–30%. The remainder is made up largely of cellulose and hemicellulose. As a *crude* approximation to the environment of lignin in intact plant tissue, we blended a natural-abundance DHP lignin sample (24% w/w) in cellulose. The spectrum is shown

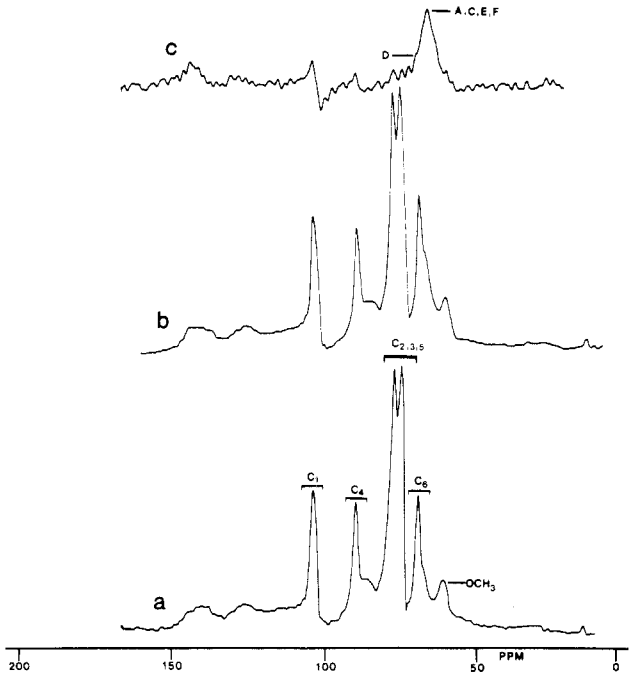


Figure 6. ^{13}C NMR spectrum of (a) natural-abundance DHP lignin (24% w/w) in cellulose, (b) 4% enriched γ-^{13}C DHP lignin (24% w/w) in cellulose, and (c) difference spectrum due to γ-^{13}C DHP lignin enrichment [(b) − (a)].

in Figue 6a. Only the methoxy resonance (~56 ppm) and the aromatic and olefinic resonances (120–160 ppm) of the DHP lignin can celarly be seen. The major signals correspond to cellulose: namely, the anomeric carbon (C_1) at 105 ppm, C_4 at 83–89 ppm, $C_{2,3,5}$ at 74 and 72 ppm, and C_6 at ~64 ppm.[16,17,19-22]

We then blended DHP lignin and cellulose in the same proportions, but now with C_γ enriched to the extent of 4% with carbon-13. The purpose of this experiment was to establish the minimum detectable levels of enrichment in simulated plant tissue. The spectrum is shown in Figure 6b. The difference spectrum is shown in Figure 6c and was obtained by subtracting the natural-abundance DHP spectrum (Figure 6a) from that of the enriched sample (Figure 6b). In the difference spectrum (Figure 6c), the resonances corresponding to substructures A and C–F (62 ppm) can clearly be seen.

Concluding Remarks

Since before 1900, there has been considerable interest in the structure of lignin and its reactions, both chemical and biochemical. Because of the lack of suitable scientific methodology, the structure (i.e., bonding patterns) of lignin in situ have not been elucidated. Consequently, organic solvent soluble synthetic DHP lignin preparations have evolved as being "representative" materials and are being widely used to study lignin chemistry and biodegradation.

In this study, we have synthesized DHP lignins labeled at $\alpha,\beta,\beta\gamma$, and γ and recorded their solid-state spectra. Although line broadening is a severe problem in all of the spectra obtained we were able to unambiguously determine that substructure B was an artifact and that substructures A, D, and C could be definitely identified. Resonances for substructures E and F in the solid state overlapped irrespective of which carbon atom was labeled with ^{13}C. Thus to unambiguously differentiate these bonding environments in the solid state, labeling of the aromatic ring at carbons 1 and 5 may be necessary or new pulse sequences need to be developed. This is currently under investigation.

Most importantly, changes to specifically carbon-13-enriched DHP substrates can now be monitored in the solid state and this will provide important information about chemical and biodegradative reactions.

It is also very important to note that the bonding environments of a 4 atom % ^{13}C DHP lignin in a cellulose matrix can be detected. It therefore seems possible that, with the employment of suitable specifically labeled ^{13}C precursors, the bonding patterns of lignin in situ in intact plants can now be pursued. This is currently under investigation.

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Registry No. 2 (homopolymer), 26745-55-1; **4a**, 63057-69-2; **4a** (homopolymer), 108562-15-8; **4b**, 108562-12-5; **4b** (homopolymer), 108562-16-9; **4c**, 108562-13-6; **4c** (homopolymer), 108562-17-0; **4d**, 108562-14-7; **4d** (homopolymer), 108562-18-1; lignin, 9005-53-2; cellulose, 9004-34-6.

References and Notes

(1) Freudenberg, K.; Neish, A. C. In *Constitution and Biosynthesis of Lignin*; Springer-Verlag: Berlin, 1968.
(2) Grisebach, H. In *Biochemistry of Plants*; Conn, E. E., Ed.; Academic: New York, 1981; Vol. 7, p 457.
(3) Gross, G. G. *Recent Adv. Phytochem.* **1979**, *12*, 177–220.
(4) Musha, Y.; Goring, D. A. I. *Wood Sci. Technol.* **1979**, *9*, 45–58.
(5) Whiting, P.; Goring, D. A. I. *Wood Sci. Technol.* **1982**, *16*, 261–267.
(6) Fengel, G.; Wegener, D. *Wood: Chemistry, Ultrastructure, Reactions*; W. de Gruyter: Berlin, 1984; p 132.
(7) Brunow, G.; Wallis, H. *The Ekman Days 1981, Chemistry and Biochemistry of Wood-Based Processes and Products*; International Symposium on Wood and Pulping Chemistry, Stockholm, June 9–12, 1981; Vol. 4, p 125.
(8) Glasser, W. G.; Barnett, C. A.; Sano, Y. *J. Appl. Polym. Sci.: Appl. Polym. Symp.* **1983**, *No. 37*, 441–460.
(9) Haider, K.; Ellwardt, P.-C.; Ernst, L. *Holzforschung* **1981**, *35* (3), 103–109.
(10) Haider, K.; Trojanowski, J. In *Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications*; Kirk, T. K., Higuchi, T., Chang, H. M., Eds.; CRC Press: Boca Raton, FL, 1980; Vol. I, p 111–134.
(11) Nimz, H.; Mogharab, I.; Ludemann, H. D. *Makromol. Chem.* **1974**, *175*, 2563–2576.
(12) Gagnaire, D.; Robert, D. *Makromol. Chem.* **1977**, *178*, 1477–1495.
(13) Bartuska, V. J.; Maciel, G. E.; Bolker, H. I.; Fleming, B. I. *Holzforschung* **1980**, *34*, 214–217.
(14) Hatcher, P. G.; Breger, I. A.; Earl, W. L. *Org. Geochem.* **1980**, *2*, 49–55.
(15) Hatcher, P. G.; Breger, I. A.; Suererenyi, N.; Maciel, G. E. *Org. Geochem.* **1982**, *4*, 9–18.
(16) Kolodziejski, W.; Frye, J. S.; Maciel, G. E. *Anal. Chem.* **1982**, *54*, 1419–1424.
(17) Barron, P. F.; Frost, R. L.; Doimo, L.; Kennedy, M. J. *J. Macromol. Sci. Chem.* **1985**, *A22* (3), 303–322.
(18) Maciel, G. E.; O'Donnell, D. J.; Ackermann, J. J. H.; Hawkins, G. H.; Bartuska, V. J. *Makromol. Chem.* **1981**, *182*, 2297–2304.
(19) Atalla, R. H.; Gast, J. C.; Sindorf, D. W.; Bartuska, V. J.; Maciel, G. E. *J. Am. Chem. Soc.* **1980**, *102*, 3249–3251.
(20) Maciel, G. E.; Kolodziejski, W. L.; Bertran, M. S.; Dale, B. E. *Macromolecules* **1982**, *15*, 686–687.
(21) Earl, W. L.; VanderHart, D. C. *J. Am. Chem. Soc.* **1980**, *102*, 3251–3252.
(22) Earl, W. L.; VanderHart, D. L. *Macromolecules* **1981**, *14*, 570–574.
(23) Putter, J. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim; Academic Press: London, New York, 1974; p 685.
(24) Newman, J.; Rej, R. N.; Just, G.; Lewis, N. G. *Holzforschung* **1986**, *40*, 369–373.
(25) Freudenberg, K. *Chem. Ber.* **1963**, *96*, 1844–1848.
(26) Kirk, T. K.; Connors, W. J.; Bleam, R. D.; Hackett, W. F.; Zeikus, J. G. *Proc. Nat. Acad. Sci. U.S.A.* **1975**, *72*, 2515–2519.
(27) Tanahashi, M.; Higuchi, T. *J. Jpn. Wood Res. Soc.* **1981**, *67*, 29–42.
(28) Traynelis, V. J.; Hergenrother, W. L. *J. Am. Chem. Soc.* **1964**, *86*, 298–299.