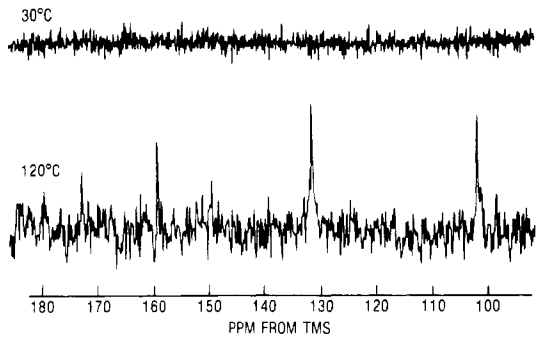

Figure 4. Partial ^{13}C NMR spectra of the carbonyl and the backbone carbons of poly3BCMU in CD_3NO_3 in yellow solution (120 °C) and blue gel (30 °C) (also see Tables I and III).

the effect of lowering temperature is more pronounced in the spectrum of poly3BCMU than in the spectrum of poly4BCMU.

Discussion

Though =C= and —C≡ carbons have the same sp hybridization, they have different chemical shifts; =C= absorbs around 220 ppm while —C≡ absorbs around 100 ppm. The ^{13}C NMR spectra were carefully searched for =C= of the butatriene structure of the backbone, beyond 200 ppm, but to no avail. The peaks for —C= and —C≡ at 130 ± 1 and 100 ± 1 ppm, respectively, suggest that the poly(diacetylene) backbone has the acetylenic structure (=C—C≡C—C=) in the yellow solutions. The peaks for —C= and —C≡ disappear when the yellow solutions form blue or red gels at lower temperatures because the backbone acquires a rigid planar conformation[5,6] (Figure 1). It is noteworthy that with either polymer the carbonyl peaks are severely broadened in the low-temperature spectra, an observation which suggests the formation of hydrogen bonds between >C=O and >N—H functionalities of the adjacent side groups, which, while not conclusive from ^{13}C NMR alone, is compatible with vibrational spectroscopic results[6] which list the urethane carbonyl at 1725 cm^{-1} in yellow solution and 1689 cm^{-1} in the red and blue gels.[6] The rigidity is reinforced by the two hydrogen bond chains: one on each side of the backbone (see Figure 1) decreases the mobility of methylene groups between the two hydrogen bond chains. That is why the signals of the methylene groups directly attached to the backbone are missing and those for the others are broadened in the low-temperature spectra. The low-temperature ^{13}C NMR spectra (Tables II and III) indicate that poly3BCMU has a more rigid conformation than poly4BCMU. The mobility of CH_2 of the *n*-butyl groups is not seriously affected by the formation of the hydrogen bonds and hence their peaks remain almost unchanged. The spectra of the polymers in chloroform are not affected by variation in temperature because the conformation of the molecules remains unchanged.[5,6] Future work utilizing solid-state ^{13}C NMR should prove beneficial for determination of the structure of the backbone in the solid gels and bulk poly(diacetylenes) through the thermochromic transitions.

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References and Notes

(1) Wegner, G. *Z. Naturforsch., B* **1969**, *24*, 824. *Makromol. Chem.* **1970**, *134*, 219. *Ibid.* **1972**, *154*, 35. *Ibid.* **1971**, *145*, 85. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 355.
(2) Wegner, G. In "Molecular Metals"; Hatfield, W. E., Ed.; Plenum Press: New York, 1979; pp 209–42.
(3) Baughman, R. H. In "Contemporary Topics in Polymer Science"; Pearce, E. M., Schaefgen, I. R., Eds.; Plenum Press: New York, 1977; Vol. 2, p 205.
(4) Baughman, R. H.; Witt, J. D.; Yee, K. C. *J. Chem. Phys.* **1974**, *60*, 4755.
(5) Patel, G. N.; Chance, R. R.; Witt, J. D. *J. Chem. Phys.* **1979**, *70*, 4387.
(6) Patel, G. N.; Witt, J. D.; Khanna, Y. P. *J. Polym. Sci., Polym. Phys. Ed.* **1980**, *18*, 1383.
(7) "Sadtler Standard ^{13}C NMR Spectra"; Sadtler Research Laboratories: Philadelphia, Pa.
(8) Patel, G. N. *Polym. Prepr., Am. Chem. Soc., Div. Polym. Chem.* **1978**, *19* (2), 154.
(9) van Dongen, J. P. C. M.; de Bie, M. J. A.; Steur, R. *Tetrahedron Lett.* **1973**, 1371.
(10) Bohlmann, F.; Brehm, M. *Chem. Ber.* **1979**, *112*, 1071.

Characterization of the Catabolic Transformation of Lignin in Culture Using Magic-Angle Carbon-13 Nuclear Magnetic Resonance

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ABSTRACT: Catabolic transformation of softwood kraft lignin in culture with the white-rot fungus *Coriolus versicolor* increases lignin functionality, thereby improving its potential for incorporation into useful end products. Cross-polarization, magic-angle spinning ^{13}C NMR spectra of the total solid products from 1-, 4-, and 8-week lignin cultures show lignin is not massively transformed by *C. versicolor* in these experiments, although the spectra do reveal significant differences. Analysis of the spectrum of the solids from the 8-week fermentation shows that about 7% of the carbons have been oxygenated. Of these, almost half appear to be vinyl aldehydes and this represents a fourfold increase compared to the starting lignin.

As a major constituent of all vascular plants, lignin represents a major renewable source of reduced carbon. Over half of the photosynthetic activity in these plants is devoted to the conversion of atmospheric carbon dioxide to lignocellulosic materials. The lignin component of the lignocellulosic cell wall material presents the greatest technological barrier to utilization of this biomass resource. Progess in lignin research is hampered by the chemical and

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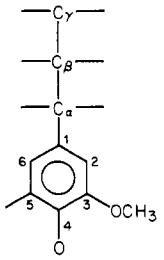
Table I
Simplified Representation of the Chemical Structure of Lignin[a]



interunit linkages[b]		functional groups[b]	
β-O-4	45	aliphatic OH	100
α-O-4	7	phenolic OH	25
5-5	17	methoxyl	93
4-O-5	8	carbonyl	18
β-5	14	end groups	9
β-1	10	uncondensed guaiacyl groups	45
β-β	3		
misc	10		
total	114		

[a] Glasser, W. G. In "Pulp and Paper"; Wiley: New York, 1980; Vol. I, p 58. [b] Approximate number prominent per 100 softwood phenylpropane units.

biological stability of the material and by its complex and varied structure which defies quantitative analytical investigation. We report here the use of cross-polarization and magic-angle spinning (CPMAS) carbon-13 NMR to assay chemical changes brought about in lignin by fungal transformation.

Lignin is comprised of a class of structurally related macromolecules formed by an essentially random free-radical polymerization of phenylpropane monomer units related to *p*-hydroxycinnamyl alcohol.[1,2] Although the polymerization is initiated by the plant peroxidases, the subsequent oxidative coupling reactions are controlled not by enzymes but by ordinary kinetic and thermodynamic parameters; this accounts for the heterogeneous and random nature of lignin (Table I). In spite of this complexity the biogenesis[1,2] and chemical structure of lignin are reasonably clear.[3] The principal uses for lignin (produced in large quantities as a byproduct of paper mills) are as fuel and as a source of low and high molecular weight chemicals. Of these, direct utilization of the high molecular weight lignin is attractive but difficult in practice due to its chemical stability. Chemical modification of lignin functionality has improved incorporation of lignin into polyurethanes[4] and phenol–formaldehydes.

Hall, Drew, and Glasser[5] have suggested the microbial transformation of kraft lignin as an alternative to chemical modification. In this scheme microorganisms are allowed to catabolize partially the lignin, possibly leaving chemically reactive functionalities which can be exploited in the lignin end use.

In the present study the white-rot fungus *Coriolus versicolor* was cultured in the presence of a reprecipitated softwood kraft lignin, Westvaco Indulin ATR-Cl, as the major carbon source, after which the extent of lignin transformation was evaluated by solid-state ^{13}C NMR. *Coriolus versicolor* is well-known among the strains of white-rot fungi for its superior ability to metabolize kraft lignin.[5] Typically, 1-L flasks containing a basal salt fermentation medium were charged with 1 g of phosphoric acid swollen cellulose and 10 g of Indulin ATR-Cl lignin. The flasks were then inoculated from a seed culture of *C. versicolor* (~20-mL wet cells). Details of the culture basal media and conditions of growth are similar to those of fermentation CE-12 in ref 5. Cellulose was added to provide a carbohydrate source, which has been found to be a necessary cofactor for the *C. versicolor* catabolism of lignin.[5] The fermentation flasks were harvested after 1, 4, and 8 weeks in a rotary shaker/incubator. The solids were washed, centrifuged, lyophilized, and examined intact, thereby avoiding possible anomalies due to extraction procedures. As a control, a 1-week fermentation was performed as above but in the absence of lignin. The 15.1-MHz ^{13}C NMR spectra were obtained from approximately 0.5-g samples of the lyophilized solids in a 700-μL Kel-F hollow Beam-Andrews rotor spinning at 2.2 kHz. Single 2-ms matched spin-lock contacts and 60-kHz H_1's were used.

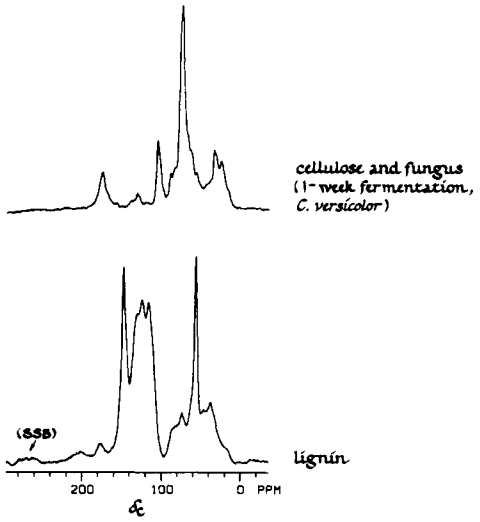


Figure 1. Magic-angle cross-polarization 15.1-MHz ^{13}C NMR spectra of the total solids of a 1-week submerged culture of a white-rot wood fungus with cellulose as the sole carbon source (top) and of a reprecipitated softwood kraft lignin (bottom).

The top trace of Figure 1 shows the CPMAS spectrum of the lyophilized materials from the control fermentation. This spectrum consists primarily of resonances from cellulose (anomeric carbons at ~105 ppm and nonanomeric carbons at ~75 ppm) and from the fungus (protein amide carbons at ~170 ppm, vinyl and aromatic carbons at ~130 ppm, carbohydrate carbons at ~65 ppm, and aliphatic carbons at ~20–30 ppm, the last-mentioned due to both protein and structural lipids). The spectrum of the reprecipitated lignin is shown at the bottom of Figure 1. The chemical heterogeneity of the lignin results in a broad distribution of isotropic chemical shifts. The propanoid carbon lines appear as a broad band in the region 0–100 ppm and the phenyl-carbon lines lie between 100 and 160 ppm. The low-field bands centered near 175 and 200 ppm are carbonyl-carbon resonances of ketones and vinyl aldehydes, respectively. The narrow intense resonance at 56 ppm is due to the phenylmethoxy carbons of the coniferyl and sinapyl moieties of the lignin structure. The sharp resonance near 147 ppm is due to the corresponding methoxy-substituted phenyl carbons. Chemical shift assignments for many of the structural components of lignin may be found in the publications of Nimz and Ludemann.[7]

Spectra of the total solids from the submerged culture after 1 and 8 weeks are shown in Figure 2. The spectra have the same general appearance as that of the uncatabolized lignin (Figure 1, bottom), evidence that the lignin was not massively transformed in this experiment. Lignin is catabolized with difficulty and so provides a poor growth

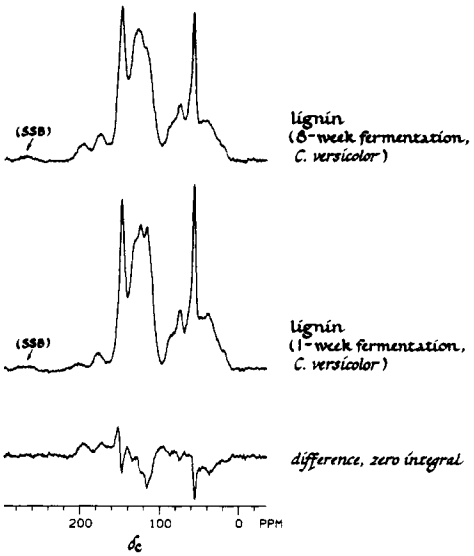


Figure 2. Magic-angle cross-polarization 15.1-MHz ^{13}C NMR spectra of the total solids of a submerged culture of a white-rot wood fungus with lignin as the major carbon source after 8 weeks (top) and 1 week (middle). The bottom spectrum is the difference between the upper two spectra (middle subtracted from top), with intensities scaled to produce an integral equal to zero.

medium even for the *C. versicolor* culture.[5]

Due to the complexity and similarity of the spectra of the solids from 1- and 8-week cultures, we used the technique of computer subtraction[8] to enhance the differences due to the fungal catabolism. The difference spectrum is shown at the bottom of Figure 2. The subtraction parameters were adjusted so that the integral across the difference spectrum was zero. In this way, lignin which was catabolized to soluble products does not contribute to the difference spectrum. Since the spectra in Figure 2 were obtained from samples of the total solids in the culture after 1 and 8 weeks, some of the NMR signal intensity is due to the fungus and residual cellulose. However, the absence in the *difference* spectrum of characteristic features found in the spectrum of the control culture (Figure 1, top) confirms that rapid growth of the fungus did not occur (cf. above).

Distinctive features of the difference spectrum (bottom, Figure 2) are the general loss (negative-going signal) of lignin-carbon resonances (as in Figure 1, bottom) and a gain of low-field carbonyl-carbon lines. One interpretation of this result is that the *C. versicolor* catabolic transformation involves a random oxidation of the phenylpropane moieties of lignin. Oxidation of specific lignin functionalities or selective side-chain cleavage during the catabolic transformation would result in a more specific loss of carbon resonances in the difference spectrum, and this is not observed. An alternative interpretation is that catabolic cleavage occurs only at the wide variety of phenylpropanes which are not highly oxidized. Both interpretations are consistent with the elemental and functional analysis of the transformed lignin, which shows an increase in the ratio of oxygen per phenylpropane C_9 unit but no change in the methoxyl/C_9 ratio.[5]

Comparison of the positive areas of the difference spectrum with the spectrum of the 8-week fermentation shows that about 7% of the carbons have been oxygenated through the catabolic action of the *C. versicolor*. Of these, almost half appear to be vinyl aldehydes, based on their chemical shift (195 ppm)[7] and represent about a fourfold increase compared to the starting lignin. Further experimentation is needed to answer the question of whether the increased functionality can be exploited to improve the incorporation of lignin into polymers and resins.

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References and Notes

(1) Freudenberg, K.; Neish, A. C. "Constitution and Biosynthesis of Lignin"; Springer-Verlag: New York, 1968.
(2) Higuchi, T. *Adv. Enzymol.* **1971**, *34*, 207.
(3) Sarkanen, K. V.; Ludwig, C. H., Eds.; "Lignin: Occurrence Formation, Structure and Reactions"; Wiley-Interscience; New York, 1971.
(4) Glasser, W. G. Abstracts, 178th National Meeting of the American Chemical Society, Washington, D.C., Sept 1979; American Chemical Society: Washington, D.C.; Abstr. CARB 26.
(5) Hall, P. L.; Drew, S. W.; Glasser, W. G. In "Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications"; CRC Press: West Palm Beach, Fla., 1979.
(6) Marton, J. In ref 3.
(7) Nimz, H. H.; Ludemann, H.-D. *Holzforschung* **1976**, *30*, 33 and references therein.
(8) Sefcik, M. D.; Stejskal, E. O.; McKay, R. A.; Schaefer, Jacob *Macromolecules* **1979**, *12*, 423.