of the values reported in Tables II–V. Starting with trial $EM_1$ and $EM_2$ values rather different from those which generated the above yields, we succeeded in reproducing the correct $EM_1$ and $EM_2$ values.

## Concluding Remarks

As stressed in a previous paper in this series,[16] we believe that in a large number of available reports on the synthesis of many-membered rings, the physicochemical fundamentals underlying ring closure are either ignored or applied incorrectly and that many current interpretations in the field are based on concepts whose mechanistic feasibility still awaits substantiation. A major difficulty undoubtedly arises from the kinetic complexity of systems where extensive polymerization competes with macrocyclization. In order to fill the gap between the general physicoorganic foundation of intramolecular reaction and the synthetic strategies for ring closure, a sound kinetic background is needed. We have now shown that this problem can be satisfactorily solved by means of a computational procedure involving the computer aided numerical integration of the complex system of differential equations, coupled with meaningful sets of EM values. These are derived from the accumulation in recent years of extensive quantitative studies in the field,[6,10,11,17] which have put the dilution principle into proper perspective.

In the present work various cyclization reactions involving bifunctional reactants A–B (one-component ring closure) have been simulated under batchwise conditions. The results nicely show the way the various parameters determine the outcome of a given cyclization reaction in terms of distribution among cyclic oligomers. Hopefully, they can provide the synthetic chemist with useful guidelines to achieve maximum selectivity in a desired cyclooligomer.

For the sake of a close adherence of calculations to actual experimental procedures, current work is devoted to simulation of one-component ring closure under influxion conditions, i.e., under conditions where the bifunctional reactant is added slowly into the reaction medium[2] (Ziegler's high dilution conditions). Similarly, simulation of two-components ring closure,[18,19] i.e., A–A + B–B, is under current investigation under both batchwise and influxion conditions.

**Acknowledgment.** Financial support by the Ministero della Pubblica Istruzione, Rome, is greatly acknowledged.

## References and Notes

(1) Part 26 of the series "Ring-Closure Reactions". Part 25: Casadei, M. A.; Di Martino, A.; Galli, C.; Mandolini, L. *Gazz. Chim. Ital.* **1986**, *116*, 659.
(2) Rossa, L.; Vögtle, F. *Top. Curr. Chem.* **1983**, *113*, 1.
(3) Stoll, M.; Rouvê, A.; Stoll-Comte, G. *Helv. Chim. Acta.* **1934**, *17*, 1289.
(4) Morawetz, H.; Goodman, N. *Macromolecules* **1970**, *3*, 699.
(5) Galli, C.; Mandolini, L. *Gazz. Chim. Ital.* **1975**, *105*, 367.
(6) Mandolini, L. *Adv. Phys. Org. Chem.* **1986**, *22*, 1.
(7) For the numerical integration of rate equations by Euler method, see Ercolani and Mencarelli (Ercolani, G.; Mencarelli, P. *Educ. Chem.* **1986**, *23*, 176).
(8) Flory, P. J. *Chem. Rev.* **1949**, *39*, 137.
(9) Galli, C.; Mandolini, L. *J. Chem. Soc., Perkin Trans. 2* **1977**, 443.
(10) Kirby, A. *Adv. Phys. Org. Chem.* **1980**, *17*, 183.
(11) Illuminati, G.; Mandolini, L. *Acc. Chem. Res.* **1981**, *14*, 95.
(12) Morawetz, H. *Macromolecules in Solution*; Wiley: New York, 1975.
(13) Flory, P. J. *Statistical Mechanics of Chain Molecules*; Wiley: New York, 1969.
(14) Scheme III differs from that originally proposed by Morawetz and Goodman for the presence of the coefficient 2 in the crossed reaction $M_1 + P$ (cf. the discussion on the statistical coefficient 2 in the section "Kinetic Treatment").
(15) For the sake of convenience the solutions obtained by numerical integration can be interpolated in the range of $\alpha$ 0.01–1000 by the third-order polynomial $\% \, C_1 = -0.059 + 18.029a + 30.499a^2 - 7.289a^3$, where $a = (\ln (1 + 2\alpha))/\alpha$, with a standard deviation = 0.128.
(16) Galli, C.; Mandolini, L. *J. Chem. Soc., Chem. Commun.* **1982**, 251.
(17) Winnik, M. A. *Chem. Rev.* **1981**, *81*, 491.
(18) Hammerschmidt, E.; Schutter, H.; Vögtle, F. *J. Chem. Res., Synop.* **1980**, 86; *J. Chem. Res., Miniprint* **1980**, 1083.
(19) Fastrez, J. *Tetrahedron Lett.* **1987**, *28*, 419.

---

# Synthesis and Characterization of [$^{13}$C]Lignins

**Robert E. Botto**

*Chemistry Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439.* *Received July 29, 1987*

ABSTRACT: Synthetic lignins were prepared from coniferyl alcohols, 3-(4-hydroxy-3-methoxyphenyl)propenols, specifically enriched with the $^{13}$C isotope at the $\beta$-position in the propyl side chain or at the methoxyl carbon. A convenient synthetic route was developed to prepare reasonably large quantities (1–3 g) of [$^{13}$C]coniferyl alcohols in good yields using fairly inexpensive reagents. Chemical characterization of the synthetic polymers by spectroscopic methods (solid NMR, FTIR, MS) showed some of the similarities and differences in their structure to that of natural softwood lignins.

Synthetic lignins (termed DHP's, dehydrogenative polymerizates) were first prepared by Freudenberg and his associates[1-3] by enzyme-controlled oxidative coupling of coniferyl alcohol. These synthetic polymers have shown some resemblance to naturally occurring softwood lignins, containing largely the same structural features of the natural materials, but not necessarily in identical proportions.[4] The subsequent use of synthetic lignins having radioisotopic ($^{14}$C) labels incorporated at specific sites has proved to be an extremely valuable tool for elucidating lignin structure and biosynthetic pathways[5] and for quantifying lignin biodegradation processes.[6] The more recent application of $^{13}$C-labeling techniques combined with NMR spectroscopy[7-9] came as a natural extension of the earlier radioisotope methods.

Our interest in "polymer models" for natural lignins arises from their potential use in $^{13}$C-labeling experiments to examine chemical transformations of lignin during

0024-9297/88/2221-1246\$01.50/0

coalification. Earlier results obtained in this laboratory[10,11] had indicated that "lignin-like" polymers could be identified in low-rank coals. In a subsequent investigation, it was shown that conifer lignin could be readily transformed in the laboratory into an insoluble polymer resembling, to a remarkable degree, natural coal in terms of chemical composition, oxidative degradation products, and spectroscopic properties.[12]

In this paper, I describe the convenient and economical syntheses of coniferyl alcohols, 3-(4-hydroxy-3-methoxyphenyl)propenols, specifically labeled with the $^{13}$C isotope at the $\beta$-position in the propyl side chain and at the methoxyl carbon, and their subsequent conversion into [$^{13}$C]DHP's. Characterization of these synthetic polymers by solid $^{13}$C NMR, FTIR, and pyrolysis MS is presented, and their structural similarities and differences to natural softwood lignin are discussed. A preliminary account of their utilization in simulated coalification experiments has been reported.[13]

## Experimental Section

**General Procedure for the Preparation of Coniferyl Alcohols. 4-*O*-Acetylferulic Acid [3-(4-Acetoxy-3-methoxyphenyl)propenoic Acid] (1).** Vanillin (15.0 g) and anhydrous sodium acetate (15.0 g) ground in a mortar and pestle were added to a 250-mL round bottom flask fitted with a reflux condensor. Acetic anhydride (75.0 g) and 0.15 mL of pyridine were added, and the mixture was stirred for 12 h at 165 °C under an atmosphere of $N_2$. While still warm, the reaction mixture was poured into ca. 800 mL of $H_2O$, at which time a brown oil separated. This mixture was heated with stirring for 1–2 h and then filtered hot to give a lemon yellow solution. The solution was allowed to cool to room temperature and placed in a refrigerator for additional cooling overnight. The yellow solid that precipitated was filtered and subsequently recrystallized (with hot filtration) from ca. 500 mL of 3% aqueous acetic acid to give a pale yellow solid: mp 195–197 °C (lit.[14] mp 194–196 °C); typical yields ranged from 63 to 75% based on vanillin, NMR ($\delta$) 7.68 (d, $J$ = 16.0 Hz, 1 H, olefinic), 7.49 (d, $J$ = 1.3 Hz, 1 H, aromatic), 7.29 (d of d's, $J$ = 8.0, 1.3 Hz, 1 H, aromatic), 7.14 (d, $J$ = 8.0 Hz, 1 H, aromatic), 6.56 (d, $J$ = 16.0 Hz, 1 H, olefinic), 3.93 (s, 3 H, $OCH_3$), 2.28 (s, 3 H, OAc); MS, $m/z$ (relative intensity) 236 (6, M – 1), 194 (100, M – 43), 179 (13, $CH_3$) 151 (11, CO).

When bis(acetic-2-$^{13}$C)anhydride was employed as the reagent (18% or 45% isotopic enrichment), the reaction was carried out by using a 3:1 weight ratio of acetic anhydride/vanillin with only a slight reduction in yield (ranging from 58 to 65% based on vanillin). Excess reagent was distilled from the reaction flask under reduced pressure after the reaction was complete; a typical recovery was >80% of theoretical. Pertinent information on 45% $^{13}$C-labeled product 1: NMR ($\delta$) 6.56 ($J_{^{13}\text{C-H}}$ = 162.1 Hz), 2.28 ($J_{^{13}\text{C-H}}$ = 129.8 Hz); MS, $m/z$ (relative intensity) 237 (8, M – 1), 236 (9, M – 1), 195 (78, M – 43), 194 (100, M – 44).

Bis(acetic-2-$^{13}$*C*) anhydride was synthesized in quantitative yield from sodium acetate-2-$^{13}$*C* (Merck Isotopes, Inc.—90% $^{13}$C-enriched) by using the procedure of Radin and co-workers.[15] It was then diluted by the appropriate amount of acetic anhydride for use in the preceding reactions.

**Methyl 4-*O*-Acetylferulate [Methyl 3-(4-Acetoxy-3-methoxyphenyl)propenoate] (2).** In a typical preparation, desired acid 1 (0.22 g) was dissolved in 25 mL of acetone, and 0.24 g of $Ag_2O$ was added. Methyl iodide (ca. 0.5 g) was introduced into the reaction vessel which was then stoppered. The reaction mixture was stirred at room temperature for 8 h with the precipitation of a yellow solid (AgI). An additional 0.5 g of methyl iodide was added and the reaction stirred overnight. Filtration through a fine-porosity-sintered funnel and removal of solvent at reduced pressure gave a yellow solid residue. Recrystallization from a small volume of methanol at low temperature produced slightly yellow platelike crystals in 95% yield; mp 116–118 °C; NMR ($\delta$) 7.65 (d, $J$ = 16.0 Hz, 1 H, olefinic), 7.02–7.15 (m, 3 H, aromatic), 6.38 (d, $J$ = 16.0 Hz, 1 H, olefinic), 3.86 (s, 3 H, OMe), 3.81 (s, 3 H, $CO_2Me$), 2.32 (s, 3 H, OAc); MS, $m/z$ (relative intensity) 250 (5, $M^+$), 208 (100, M – 42), 177 (36, OMe).

**Coniferyl Alcohol [3-(4-Hydroxy-3-methoxyphenyl)propenol] (3).** The reduction of methyl ester 2 with "$AlH_3$" is based on the general procedure of Jorgenson.[16] For the reduction of 2.0 g of methyl ester 2, 0.61 g of lithium aluminum hydride was placed in a round-bottom flask (oven dried prior to use) fitted with a reflux condensor and a septum inlet and maintained under an atmosphere of dry $N_2$. About 80 mL of dry ethyl ether was added carefully. After the reaction flask was cooled in an ice bath, $AlCl_3$ (0.90 g) dissolved in 20 mL of ether was slowly added. The reaction mixture was allowed to warm to room temperature and was stirred for an additional $^1/_2$ h with the precipitation of LiCl. The solution was then filtered under a positive pressure of $N_2$ into a 500-mL three-neck flask (oven dried) by using a fine-porosity-sintered funnel to give a clear solution of "$AlH_3$". This flask, fitted with a condensor and 250-mL addition funnel, was transferred to an ice bath while a positive $N_2$ atmosphere was maintained. The desired methyl ester (2) dissolved in 175 mL of dry ether was added over a 1-h period, after which the reaction mixture was stirred at room temperature for an additional 6 h. A bright yellow precipitate was collected by filtration under nitrogen, washed with additional ether, and dried in vacuo. The aluminum salt could be stored dessicated under refrigeration for an indefinite period.

Hydrolysis of the aluminum salt[17] was carried out in the following manner: the salt was added in small portions to a rapidly stirred solution of 2.0 g of $(NH_4)_2CO_3$ in 100 mL of $H_2O$ and about 100 mL of ether. After addition was complete, the mixture was stirred an additional 2 h. The ether layer was then decanted and the aqueous phase extracted with two additional portions of ether. The combined ether extracts were dried over $Na_2SO_4$ and the solvent removed under reduced pressure to give a yellow oil. Recrystallization from 1,2-dichloroethane produced off-white needles: mp 72–75 °C (lit.[18] mp 74.6 C°); mixed mp with an authentic sample of coniferyl alcohol, 73–75 °C; yields from this reaction varied greatly, from 66 to 85%; NMR ($\delta$) 6.88–6.92 (m, 3 H aromatic), 6.54 (br d, $J$ = 15.9 Hz, 1 H, olefinic), 6.24 (d of t's, $J$ = 15.9, 5.8 Hz, 1 H, olefinic), 4.31 (d, $J$ = 5.8 Hz, 2 H, $CH_2O$), 3.91 (s, 3 H, $OCH_3$); MS, $m/z$ (relative intensity) 180 (85, $M^+$), 137 (100, M – 43), 124 (60), 91 (38), 77 (21).

**4-(Benzyloxy)-3-hydroxybenzaldehyde (4).** In a 50-mL round-bottom flask was placed 5.6 g of 3,4-dihydroxybenzaldehyde, 2.2 g of KOH, 5.6 g of benzyl chloride, 20 mL of ethanol, and 8 mL of water. The reaction mixture was refluxed for 5 h, poured onto crushed ice, and extracted with ether (3 × 50 mL). The ether extracts were combined, washed with aqueous sodium bicarbonate (a deep brownish red solution resulted), and dried over $MgSO_4$ and the solvent removed under vacuum (40 °C). A dark oily residue formed. Hexane was added to the residue, the mixture heated, and the hexane decanted to remove the oily substances. The remaining residue subsequently was dissolved in warm benzene to which was added to equal volume of hexane. The solution was left to cool overnight resulting in the formation of brown crystals. The crystals were then recrystallized twice from 1:5 (v/v) methanol/water to give long crystalline needles slightly yellow in color: mp 116–117 °C; typical yields obtained ~10–20%; NMR ($\delta$) 9.84 (s, 1 H, CHO), 7.32–7.44 (m, 7 H, aromatic), 6.99 (d, $J$ = 8.0 Hz), 1 H, aromatic), 5.25 (s, 2 H, $OCH_2$), 3.95 (s, 3 H, -$OCH_3$); MS, $m/z$ 228 (6, $M^+$), 91 (100).

**4-(Benzyloxy)-3-(methoxy-$^{13}$*C*)benzaldehyde (5).** In a 100-mL round-bottom flask was placed 4.6 g of aldehyde 4, 3.1 g of $K_2CO_3$, and 3.0 g of 90% methyl-$^{13}$*C* iodide (Merck Isotopes, Inc.). Acetone (50 mL) that had been stored over anhydrous $K_2CO_3$ was added, and the reaction mixture was refluxed at 60–70 °C for 6 h. After being refluxed, the reaction mixture was poured onto ice and extracted several times with ether. The ether combinations were washed with sodium hydroxide (the washes were yellowish in color), water, and saturated sodium chloride solution and dried over magnesium sulfate. Finally, the organic solvent was evaporated, resulting in the formation of a yellow solid. Recrystallization from hexane/benzene (4:1) afforded white crystals: mp 116–118 °C; typical yields ranged from 75 to 85% of the theoretical amount; NMR ($\delta$) 9.88 (1 H, CHO), 6.7–7.5 (m, 8 H, aromatic), 5.24 (d, 2 H, $OCH_2$), 3.88 (s, d, $J$ = 146.0 Hz, 3 H, $OCH_3$); MS, $m/z$ (relative intensity) 243 (8, $M^+$), 91 (100).

**[*methoxy*-$^{13}$C]Vanillin [4-hydroxy-3-(methoxy-$^{13}$*C*)benzaldehyde] (6).** To a mixture of 4 mL of acetic acid and 4 mL of concentrated hydrochloric acid in a 25-mL round-bottom flask fitted with a magnetic stirrer was added 0.40 g of benzyl ether **5**. The mixture was refluxed for 30 min, allowed to cool to room temperature, and poured into 50 mL of water. The aqueous solution was extracted several times with ether (total volume ~80 mL). The ether solution was washed several times with saturated $NaHCO_3$ solution and with water and dried over $MgSO_4$. Removal of solvent under vacuum gave a yellow solid material. Recrystallization from hexane/benzene (1:1) afforded 0.35 g (98% yield) of slightly yellow needles: mp 78–79 °C; the structure confirmed by a mixed melting point determination with an authentic vanillin sample, mixed mp 77–79 °C, NMR ($\delta$) 9.88 (s, 1 H, CHO), 7.49–6.46 (m, 3 H, aromatic), 6.18 (s, 1 H, OH), 3.97 (s, d, $J$ = 145.7 Hz, $OCH_3$); MS, $m/z$ (relative intensity) 153 (92.1, $M^+$), 152 (100, H), 137 (6.7, $^{13}CH_3$), 124 (11.6, CHO), 109 (25.2); 81 (44.6).

**General Procedure for the Preparation of Synthetic Lignins.** In a typical preparation, the desired coniferyl alcohol (0.63 g, 3.5 mmol) and 29.6 mg of peroxidase (EC 1.11.1.7, Type I, Sigma Chemical Co.) were dissolved in 148 mL of $N_2$-degassed 0.01 M sodium phosphate buffer adjusted to pH 7.5. This solution and 0.38 g of $H_2O_2$ (30% solution, 3.5 mmol) dissolved in 148 mL of buffer were added separately over a 16-h period by using a peristaltic pump to a vigorously stirred solution of 7.4 mg of peroxidase and 11.1 mg of vanillyl alcohol in 74 mL of buffer. All solutions were kept under $N_2$ and in the dark. After the final addition of reactants, the mixture was stirred an additional 24 h; total reaction time was 40 h. The insoluble polymer was isolated by centrifugation and then filtered as a suspension in water and washed with additional water and methanol. Drying overnight at 50 °C in a vacuum oven gave synthetic lignins, which varied in color from tan to reddish brown, in essentially quantitative yields. The synthetic lignins could be stored indefinitely in a desiccator below 0 °C.

**Characterization and Identification Procedures.** All solid-probe mass spectra were obtained on a Kratos MS25 mass spectrometer with a DS55 data system operating in the precise mass-measurement mode. Solids were evaporated and pyrolyzed in the source by using a direct heating platinum-mesh filament probe designed in this laboratory. For GCMS analysis, separations were made with no column injection by using a 60 m × 0.25 mm bonded OV-1701 fused silica column, temperature programmed 50–280 °C at 8°/min.

Infrared spectra were obtained by the KBr disk method on an IBM FTIR spectrometer, Model 98/4A vacuum instrument, with a 4 $cm^{-1}$ resolution and 200 scans.

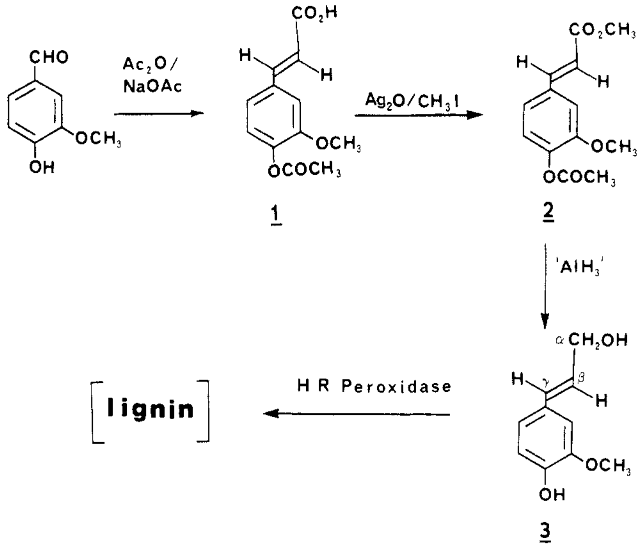
Proton NMR spectra were obtained at 200.07 MHz on a Nicolet NTC-200 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) employing chloroform-*d* as the lock solvent. Coupling constants are reported in hertz by using the following designations for peak multiplicities: (s) singlet, (d) doublet, (t) triplet, (m) multiplet.

Solid-state CP/MAS spectra were obtained at 25.18 MHz on a Bruker CXP-100 spectrometer with a doubly-tuned single coil probe and a dual air-bearing spinning apparatus. The ceramic spinners with an internal volume of 300 $\mu$L were spun at approximately 4 kHz. Contact time experiments were carried out on a few samples in order to select a contact time which gave representative aromatic/aliphatic intensities for the entire suite of samples. The experimental conditions used for the measurements were a contact time of 1.5 ms, a 2-s pulse repetition time, a 30-ms acquisition time, and a 67-kHz proton decoupling field (3.75-$\mu$s 90° pulse width).

## Results and Discussion

The present investigation was concerned with the synthesis of [$^{13}$C]lignins from coniferyl alcohols specifically enriched with the $^{13}$C isotope at the $\beta$-position in the propyl side chain and at the methoxyl carbon. The general synthetic scheme (Scheme I) developed for the preparation of [$^{13}$C]coniferyl alcohols employs vanillin as the starting material. Extension of the carbon side chain of vanillin was carried out with acetic anhydride in the presence of sodium acetate and pyridine by the Perkin reaction[19] to produce 4-*O*-acetylferulic acid (**1**). For the preparation of the [$\beta$-$^{13}$C]analogue of **1**, bis(acetic-*2*-$^{13}$*C*) anhydride was used. In this case, a limited amount of the labeled reagent was employed with only a slight reduction in yield; unreacted reagent was recovered in >80% of the theoretical amount by distillation under vacuum after the reaction was complete. Protection of the labile 4-hydroxyl group in compound **1** as the acetate during this reaction provides a synthetic intermediate that is stable toward autocatalytic polymerization during subsequent manipulations. Mild esterification of **1** with $CH_3I/Ag_2O$ gave methyl 4-*O*-acetylferulate (**2**) in 95% yield. Selective reduction of the two ester groups with "$AlH_3$" produced [$^{13}$C]coniferyl alcohols in overall yields of 40–63% from vanillin. Synthesis of [*methoxy*-$^{13}$C]vanillin was achieved by selective benzylation of the 4-hydroxyl group of 3,4-dihydroxybenzaldehyde. Methylation of the HO-3 group with methyl-$^{13}$*C* iodide/$K_2CO_3$ and subsequent removal of the protecting group via mild hydrolysis with dilute HCl produced labeled vanillin in an overall yield of 85% based on labeled methyl iodide. A controlled polymerization of the labeled coniferyl alcohols with horseradish peroxidase and $H_2O_2$ using the general procedure of Kirk and co-workers[20] gave synthetic [$^{13}$C]lignins in essentially quantitative yields.

Scheme I



The synthetic lignins were characterized by elemental analysis, FTIR, and solid-probe MS. Elemental analysis gave values for synthetic lignin that are similar to those for softwood conifer lignin. Synthetic (unlabeled); C, 61.7; H, 5.4; N, 0.3; O, 32.6 (diff) [$C_{100}H_{104}O_{40}$]. Softwood lignin; C, 65.6; H, 5.9; N, 0.5; O, 28.5 (diff) [$C_{100}H_{108}O_{34}$]. The IR spectra of synthetic and natural lignins, shown in Figure 1, were also very similar; both had distinct bands at approximately 3440, 2930, 1600, 1510, 1460, 1270, 1210, 1140, 1090, 1030, 860 and 820 $cm^{-1}$. However, bands in the synthetic lignin spectrum at 1420 and 970 $cm^{-1}$, which were absent from the spectrum of the natural sample, indicate a higher proportion of coniferyl alcohol end groups in the synthetic polymer. Furthermore, greater intensity of the band near 1700 $cm^{-1}$ suggests a higher degree of oxidation of the synthetic lignin sample and is consistent with the microanalytical results.

Solid-probe MS analysis of the natural and synthetic lignin samples documents the similarity in their essential structural subunits. Similar findings have been observed previously by others.[21,22] Pyrolysis mass spectra of the lignins are characterized by the presence of thermal degradation fragments derived from the main structural building blocks. Total-ion and selected-ion mass pyro-

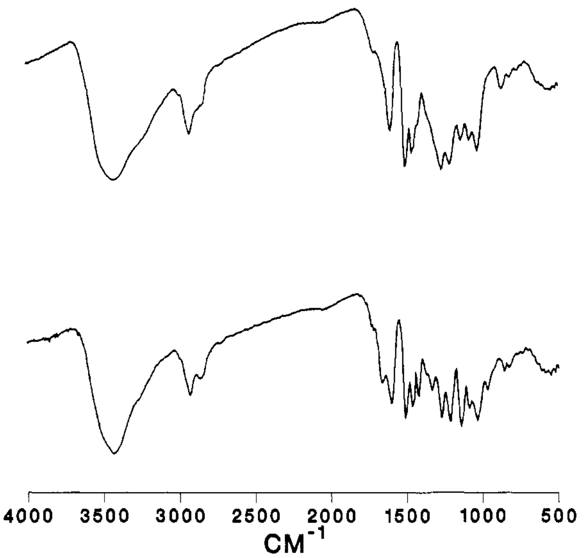


**Figure 1.** FTIR spectra of softwood conifer lignin (top) and synthetic lignin (bottom).

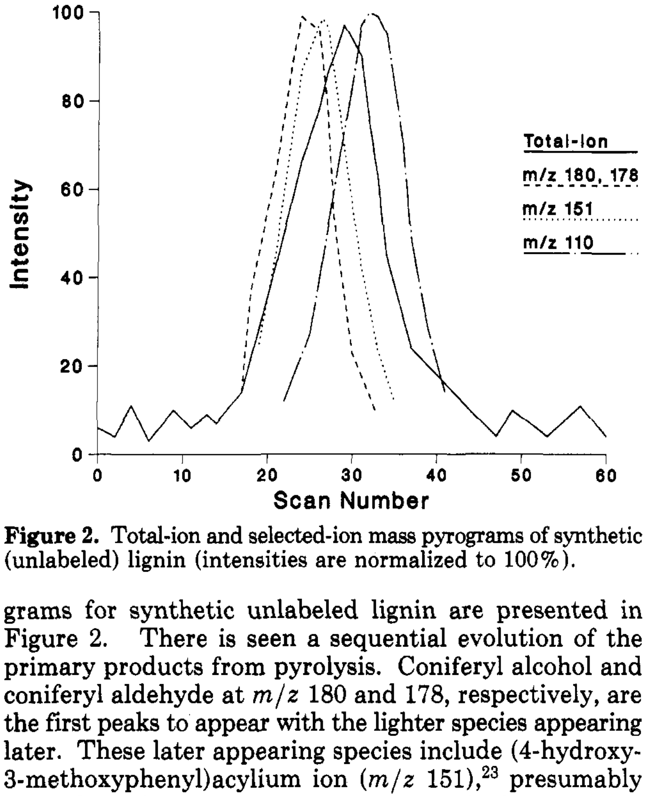


**Figure 2.** Total-ion and selected-ion mass pyrograms of synthetic (unlabeled) lignin (intensities are normalized to 100%).

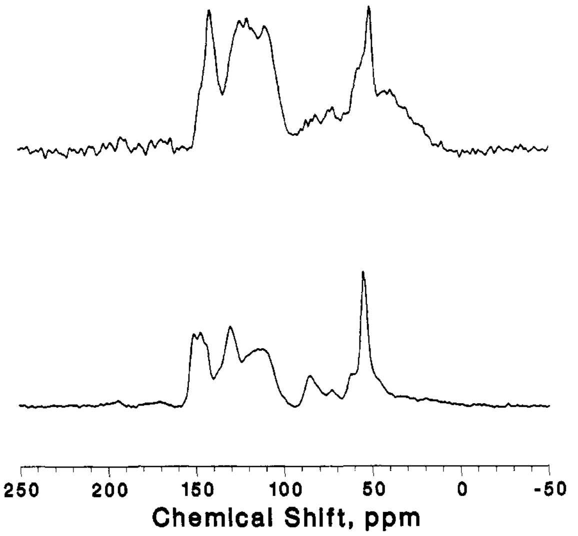


**Figure 3.** CP/MAS $^{13}$C-NMR spectra of softwood conifer lignin (top) and synthetic lignin (bottom).

grams for synthetic unlabeled lignin are presented in Figure 2. There is seen a sequential evolution of the primary products from pyrolysis. Coniferyl alcohol and coniferyl aldehyde at $m/z$ 180 and 178, respectively, are the first peaks to appear with the lighter species appearing later. These later appearing species include (4-hydroxy-3-methoxyphenyl)acylium ion ($m/z$ 151),[23] presumably formed in the fragmentation of $\beta,\beta$-resinol structures, followed by dihydroxybenzenes and methoxyphenols, including methylguaiacol ($m/z$ 110, 124, and 138) and lesser amounts of phenol ($m/z$ 94) and methylphenols ($m/z$ 108). The evolution of alkylbenzene fragments is observed only at higher temperatures (>400 °C) and is primarily due to secondary pyrolysis reactions. Mass pyrograms for the $^{13}$C-labeled lignins show a similar distribution and comparable rates of evolution of the major pyrolysis products having the expected isotope composition.

The cross polarization/magic-angle spinning (CP/MAS) $^{13}$C NMR spectrum of unlabeled synthetic lignin shows some similarity to that obtained for Klason conifer lignin (Figure 3). The spectrum of the Klason lignin (top) is characterized by an absorption centered at 55 ppm for the methoxyl carbons, a reasonably broad resonance at 120–140 ppm for aromatic carbons, and an intense, narrow band at 155 ppm typical of phenolic carbons. A shoulder appearing to higher field of the aromatic carbon absorption (~115 ppm) is indicative of aromatic carbons ortho to phenolic groups. In addition, there are less intense resonances which appear in the aliphatic region (0–45 ppm) and absorptions of low intensity centered at 72 and 85 ppm, which are characteristic of $\alpha$- and $\beta$-carbons in the side chain that are bonded to aryl ether groups.[24] The spectrum of the synthetic lignin sample (bottom) shows a similar distribution of the various absorption bands with some notable differences. Among these include a sharper, more intense methoxyl absorption at 55 ppm and the absence of appreciable intensity in the aliphatic region. These spectral features are more in line with those for dioxane spruce lignin and milled spruce wood lignin found previously by Hatcher and co-workers.[25] Furthermore, the differences point to the structural alteration of the Klason lignin sample that may have occurred during its isolation with mineral acid.[4] The observed splitting of the aromatic C–O absorption into two distinct bands at 148 and 153 ppm is consistent with the $^{13}$C resonances expected for coniferyl alcohol subunits. Moreover, greater intensity of the resonance centered at 128 ppm in the spectrum of the synthetic lignin corroborates the preceding IR evidence for the existence of a proportionately higher number of coniferyl alcohol end groups in the synthetic sample.

CP/MAS $^{13}$C NMR of 45% enriched [*methoxy*-$^{13}$C]lignin produces a spectrum, shown in Figure 4 (top), in which the signal from the $^{13}$C-labeled methoxyl carbons is considerably intensified compared to those with the natural $^{13}$C abundance. Thus the spectrum consists of a single, relatively sharp resonance centered at 55 ppm. The CP/MAS spectrum of 45% enriched [$\beta$-$^{13}$C]lignin is also shown in Figure 1 (bottom). Because its NMR spectrum is considerably less complex than the unlabeled sample, it is possible to assign the major structural subunits which characterize the synthetic lignins on the basis of the $^{13}$C chemical shifts of lignin model compounds recorded in solution.[24] An inspection of the [$\beta$-$^{13}$C]lignin spectrum reveals that $\beta$-*O*-4 aryl alkyl ether structures centered at 85 ppm and $\beta,\beta$-resinol structures at 54 ppm are predominant, whereas dihydrofuran (phenylcoumaran) structures at 45 ppm are present in smaller amounts. Moreover, the presence of coniferyl alcohol end groups is indicated by the intense resonance centered at 128 ppm.

Previous studies on the structure characterization of natural lignins suggest that almost all $C_\beta$ atoms are involved in inter-subunit bonds.[27] A comparison of the

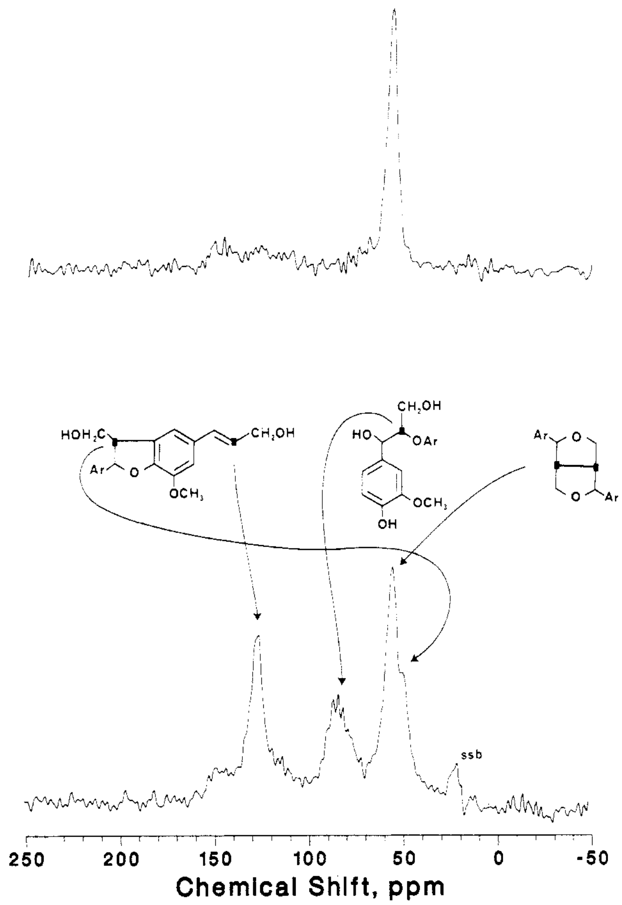


**Figure 4.** CP/MAS $^{13}$C NMR spectra of 45% enriched [*methoxy*-$^{13}$C]lignin (top) and [$\beta$-$^{13}$C]lignin (bottom). Structures designated from left to right are dihydrobenzofuran (phenylcoumaran), $\beta$-aryl ether, and $\beta,\beta$-resinol subunits, where Ar represents a 4-hydroxy-3-methoxyphenyl group.

**Table I**
**Frequency of Common $\beta$-Carbon Linkages Found in Softwood and Synthetic Lignins**[a]

| linkage | softwood lignin[b] | synthetic lignin[c] |
|---|---|---|
| $\beta$-*O*-4-aryl alkyl ether | 5.0–5.6 | 3.0 |
| $\beta,\beta$-resinol | 1.9–2.5 | 2.5 |
| phenylcoumaran | 1.0–2.2 | 1.6 |
| coniferyl alcohol end groups | 1.0 | 3.5 |

[a] Expressed as percentage of total number of carbon bonds. [b] Range of values converted from data compiled in ref 4, 5, and 26. [c] This work.

distribution in common $\beta$-carbon linkages found in softwood lignins, from data obtained in the literature,[4,5,26] with the distribution determined by NMR analysis of the synthetic lignins is presented in Table I. The relative intensities of resonances in the spectrum of [$\beta$-$^{13}$C]lignin have been measured to determine the distribution of $\beta$-carbon linkages in the synthetic lignins. The ratios for $\beta$-*O*-4 aryl alkyl ether and $\beta,\beta$-resinol structures were then used in conjunction with the integrated intensity of the 85 ppm region in the spectrum of unlabeled synthetic lignin to provide the results shown in the table.[28] It is seen that substantially fewer $\beta$-*O*-4 aryl alkyl groups and approximately 3.5 times the number of coniferyl alcohol end groups are found in the synthetic polymers.

While the experimental evidence presented here supports a structure for synthetic lignins containing largely the same common inter-subunit linkages as natural softwood lignins, the distribution of the common linkages in synthetic lignins is found to be substantially different. It is felt that knowledge of the structural dissimilarities between synthetic and natural lignins may be helpful in understanding differences in their reactivity with various substrates.

**Acknowledgment.** I am grateful to Robert Scott, who performed the mass spectral analyses, and to Debbie Willoughby (ANL Summer Undergraduate Research Participant), who carried out some of the organic preparations. This work was performed under the auspices of the Office of Basic Energy Sciences, Division of Chemical Sciences, U.S. Department of Energy, under Contract Number W-31-109-ENG-38.

**Registry No.** 1, 2596-47-6; 2, 2309-08-2; 3, 458-35-5; 3 (methoxy-$^{13}$C, homopolymer), 113568-90-4; 3 ($\beta$-$^{13}$C, homopolymer), 108562-16-9; 4, 4049-39-2; 5, 113568-89-1; 6, 86884-84-6; 3,4-$(HO)_2C_6H_3CHO$, 139-85-5; $C_6H_5CH_2Cl$, 100-44-7; lignin, 9005-53-2.

## References and Notes

(1) Freudenberg, K.; Richtzenhain, H. *Chem. Ber.* **1943**, *76*, 997. Freudenberg, K.; Richtzenhain, H. *Holzforschung* **1947**, *1*, 90. Freudenberg, K. *Angew. Chem.* **1949**, *61*, 228.
(2) Freudenberg, K.; Heimberger, W. *Chem. Ber.* **1950**, *83*, 519. Freudenberg, K.; Siebert W.; Heimberger, W.; Kraft, R. *Chem. Ber.* **1950**, *83*, 533.
(3) Freudenberg, K.; Neish, A. C. *Constitution and Biosynthesis of Lignin*; Springer-Verlag: Berlin-Heidelberg, 1968; p 84.
(4) Sarkanen, K. U. in *Lignins: Occurence, Formation, Structure and Reactions*; Sarkanen, K. U., Ludwig, C. H., Eds.; Wiley-Interscience: New York, 1971; Chapter 4.
(5) Freudenberg, K.; Neish, A. C. *Constitution and Biosynthesis of Lignin*; Springer-Verlag: Berlin-Heidelberg, 1968.
(6) Crawford, R. L.; Robinson, L. E.; Cheh, A. M. In *Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications*; Kirk, T. K., Chang, T. H., Eds.; CRC Press: Boca Raton, FL, 1980; Chapter 3.
(7) Ellwardt, P.-C.; Haider, K.; Ernst L. *Holzforschung* **1981**, *35*, 103.
(8) Gagnaire, D.; Robert, D. *Makromol.* Chem. **1977**, *178*, 1477.
(9) Lapierre, C. Monties, B. *Holzforschung* **1984**, *38*, 333.
(10) Hayatsu, R.; Winans, R. E.; McBeth, R. L.; Scott, R. G.; Moore, L. P.; Studier, M. H. *Nature (London)* **1979**, *278*, 41.
(11) Hayatsu, R.; Winans, R. E.; McBeth, R. L., Scott, R. G.; Moore, L. P.; Studier, M. H. In *Coal Structure*; Gorbaty, M. L., Ouchi, K., Eds.; Advances in Chemistry 192; American Chemical Society: Washington, DC; pp 133–149.
(12) Hayatsu, R.; McBeth, R. L.; Scott, R. G.; Botto, R. E.; Winans, R. E. *Org. Geochem.* **1984**, *6*, 463.
(13) Botto, R. E. *Energy Fuels* **1987**, *1*, 228.
(14) Fosdick, L. S.; Starke, A. C., Jr. *J. Am. Chem. Soc.* **1940**, *62*, 3352.
(15) Radin, N. S.; Rittenberg, D.; Shemin, D. *J. Biol. Chem.* **1950**, *184*, 745.
(16) Jorgenson, M. J. *Tetrahedron Lett.* **1962**, *13*, 559.
(17) Allen, C. F. H.; Byers, J. R., Jr. *J. Am. Chem. Soc.* **1949**, *71*, 2683.
(18) Freudenberg, K.; Hübner, H. *Chem. Ber.* **1952**, *85*, 1181.
(19) Perkin, W. H. *J. Chem. Soc.* **1877**, *31*, 388.
(20) Kirk, T. K.; Connors, W. J.; Blearn, R. D.; Hackett, W. F.; Zeikus, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *72*, 2515.
(21) Saiz-Jimenez, C.; de Leeuw, J. W. *Org. Geochem.* **1984**, *6*, 417.
(22) Saiz-Jimenez, C.; de Leeuw, J. W. *Org. Geochem.* **1986**, *10*, 869.
(23) Duffield, A. M. *J. Heterocycl. Chem.* **1967**, *4*, 16.
(24) Kringstad, K. P.; Mörck, R. *Holzforschung* **1983**, *37*, 237.
(25) Hatcher, P. G.; Maciel, G. E.; Dennis, L. W. *Org. Geochem.* **1981**, *3*, 43.
(26) Harkin, J. M. In *Oxidative Coupling of Phenols*; Taylor, W. I., Battersby, A. R., Eds.; Marcel Dekker: New York, 1967; pp 256–263.
(27) Reference 5, p 86.
(28) The chemical shifts of $C_\beta$ in $\beta$-*O*-4 aryl alkyl ethers and $C_\alpha$ in $\beta,\beta$-resinol are found at 80–85 ppm.