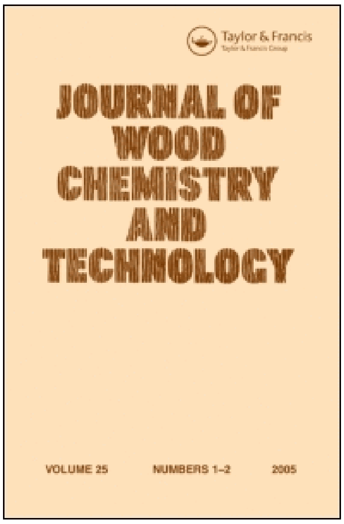

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Solid-Sample Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Lignin and Hydroxymethylated Lignin

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SOLID-SAMPLE CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF LIGNIN AND HYDROXYMETHYLATED LIGNIN

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ABSTRACT

Solid-sample carbon-13 NMR spectroscopy with cross polarization and magic angle spinning (CP/MAS) was employed to study the hydroxymethylation of lignin. Kraft pine and steam-exploded hardwood lignins were reacted with formaldehyde and the resultant structural changes in the lignins evaluated by comparing the spectra of the treated and untreated lignins. The results indicated that hydroxymethylation had occurred at the 5 position of phenolic guaiacyl units, in keeping with known reactions for phenol-formaldehyde resole formation. The degrees of formaldehyde substitution determined from CP/MAS NMR spectra were in excellent agreement with those determined by other methods. Intensities in the untreated kraft pine lignin spectrum implied the presence of unsaturated sidechains with some loss of γ carbons.

INTRODUCTION

The use of ^{13}C nuclear magnetic resonance (NMR) spectroscopy for studying lignin structure was first demonstrated by Lüdemann and Nimz[1]. Since then, ^{13}C NMR has found wide application in structural characterizations of different types of lignins, and the changes occurring in lignin structure through chemical reactions[2-5]. Compared with proton NMR spectroscopy[6], ^{13}C NMR offers convincing advantages for lignin studies, including a twenty-fold wider chemical shift range, greater chemical shift sensitivity to structural changes, and inherently higher structural detail.

While most ^{13}C NMR studies on lignin have been conducted using conventional solution spectroscopy, NMR on solid samples using cross polarization and magic angle spinning (CP/MAS NMR) is becoming common[7]. Although resolution is lower than in solution spectrometry, this technique circumvents the frequently low solubilities of lignins and yields adequate signal-to-noise ratios and quantitative spectra on polymers more rapidly than do solution techniques. For instance, CP/MAS NMR measurements of lignin contents in wood pulps generally agree with those determined by other methods[8] and require only a fraction of the time.

The structural chemistry of phenol-formaldehyde (PF) resin formation[9] and curing reactions[10] has been monitored with both solution- and solid-sample ^{13}C NMR. PF formation consists of base-catalyzed substitution by formaldehyde at positions ortho and para to the phenol hydroxyl group, yielding hydroxymethylphenol mixtures. Lignin reacts with alkaline formaldehyde similarly, substituting the 5-positions of phenolic (nonetherified) guaiacyl rings (Lederer-Manasse reaction) and sidechain carbonyl carbons by the Tollens reaction[11]. The potential of lignin as an alternative feedstock for PF resins has been well documented[12].

The purpose of this study was to utilize the CP/MAS NMR technique in demonstrating the structural changes in kraft and steam-exploded lignins resulting from their reactions with formaldehyde and in measuring formaldehyde uptake. The results could bring increased understanding of lignin-formaldedhye reaction chemistry and offer insight into the formulation of lignin-based PF resins.

EXPERIMENTAL

Lignin Preparations

Kraft lignin (Indulin AT, Lot 02261) was obtained from Westvaco and used as received. Steam-exploded fiber from mixed southern hardwood species was obtained from Masonite Corporation, Laurel, Mississippi. Steam explosion was carried out at 225°C for one minute. Lignin was extracted from the steam-exploded fibers with 2% NaOH solution, followed by acid precipitation of the extract with sulfuric acid. The precipitate, a mud-like dispersion, was washed with water and centrifuged several times to remove the major portion of soluble salt and carbohydrates. The lignin precipitate was dried in an oven at approximately 50°C and then ground and ball-milled. The chemical composition and elemental analyses of the lignins are shown in Table 1. Elemental and methoxyl content analyses were performed by Galbraith Laboratories, Knoxville, Tennessee. Both lignin preparations were analyzed for ash content after ignition at 700°C and for carbohydrate content by gas chromatography after hydrolysis and derivatization[13].

Hydroxymethylation and Formaldehyde Uptake

Kraft and steam-exploded lignins (10g) each were added to 50g 1N NaOH in a 250-mL Erlenemyer flask and sonicated two hours. Five and one-half grams of formaldehyde (39%) were added to the solution and allowed to react with agitation for 72 hours at ambient temperature. Formaldehyde uptake by lignin was determined by measuring the disappearance of formaldehyde from the reaction solution using the hydroxylamine procedure[14]. After the reaction, acid was added and the precipitate was washed by centrifugation with several changes of distilled water until the supernate appeared clear.

NMR Spectroscopy

^{13}C CP/MAS NMR spectra were obtained on a General Electric S-100 NMR spectrometer observing ^{13}C at 25.2 MHz, accumulating 12,000 to 19,000 transients for each sample with a 2-msec contact time, 500-msec recovery delay, 50.7-msec acquisition time, 10.1-kHz spectral width, and a spinning rate of 3360 to 3400 rps. The proton decoupling power was equivalent to 12 G. Data sets of 1024 points were treated with 10-Hz exponential line broadening and zero-filled to 4096 words for Fourier transformation. Frequency referencing was accomplished by setting the transmitter frequency daily so that the methyl carbon resonance of hexamethylbenzene appeared at 17.80 ± 0.02 ppm, which in turn was measured against the crystalline component of polyethylene at 33.63 ppm[22].

Integrated intensities were corrected for spinning sidebands from the aromatic carbons. The downfield sidebands appeared at 290 to 230 ppm, while the upfield sidebands appeared at about 30 to -30 ppm and partially overlapped the aliphatic carbon signals. The correction consisted of subtracting the downfield sideband intensity from the aliphatic intensity and adding twice this adjustment to the aromatic intensity; thus, the aromatic intensity included all the sideband intensity and the aliphatic intensity contained none. It is common in mixed aromatic and aliphatic samples for the aromatic carbons, having large chemical shift anisotropies, to produce spinning sidebands while the

TABLE 1

Analytical Data for Lignin Preparations

	Percent by Weight						
					Methoxyl and Elemental[b]		
Sample	Lignin[a]	Carbohydrate	Ash	Total	OCH_3[c]	C	H
Kraft pine lignin	94.0	0.4	2.5	96.9	13.98	61.51	5.96
Exploded hardwood lignin	89.0	2.0	7.0	98.0	19.46	54.98	5.82

[a] Klason and acid-soluble lignin.
[b] Ash-free sample basis.
[c] Lignin-only basis.

aliphatic carbons do not; in these spectra, spinning sidebands from the aliphatic carbons would appear at between -40 and -100 ppm, yet the spectra were flat in this region. The intensity corrections were not large: total sideband intensity was only 4% to 6% of the total integral.

RESULTS AND DISCUSSION

Analytical data for the isolated lignins are shown in Table 1. These preparations were particularly low in carbohydrates, so no significant NMR signals should arise from carbohydrate impurities; were they present, these would appear at 105, 85-90, 75 and 66 ppm. The methoxyl contents correspond to 0.87 and 1.29 methoxyl groups per C_9 unit in the kraft pine and exploded hardwood lignin, respectively, employing an idealized lignin unit formula of $C_9H_{10-n}O_3(OCH_3)_n$.

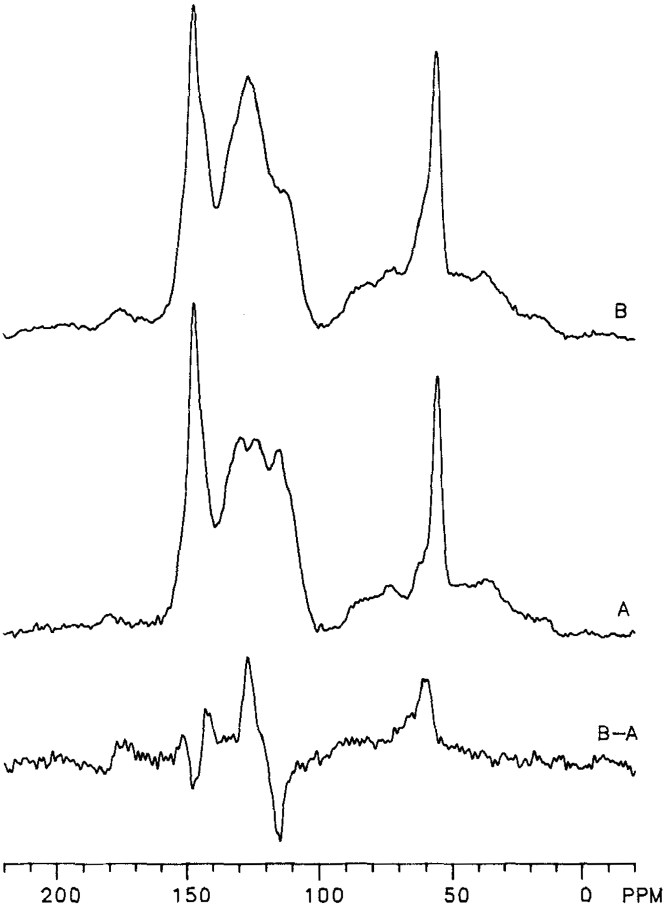


FIGURE 1. ^{13}C CP/MAS NMR spectra of untreated (middle) and formaldehyde-treated (top) kraft pine lignin and difference spectrum (bottom) of treated minus untreated lignin.

Hydroxymethylation Reactions

The top two traces in each of Figures 1 and 2 are the CP/MAS NMR spectra of the pine and hardwood lignins, respectively, before (A) and after (B) reaction with formaldehyde. Assignments for the major peaks are in Table 2. NMR peaks in CP/MAS spectra are wider

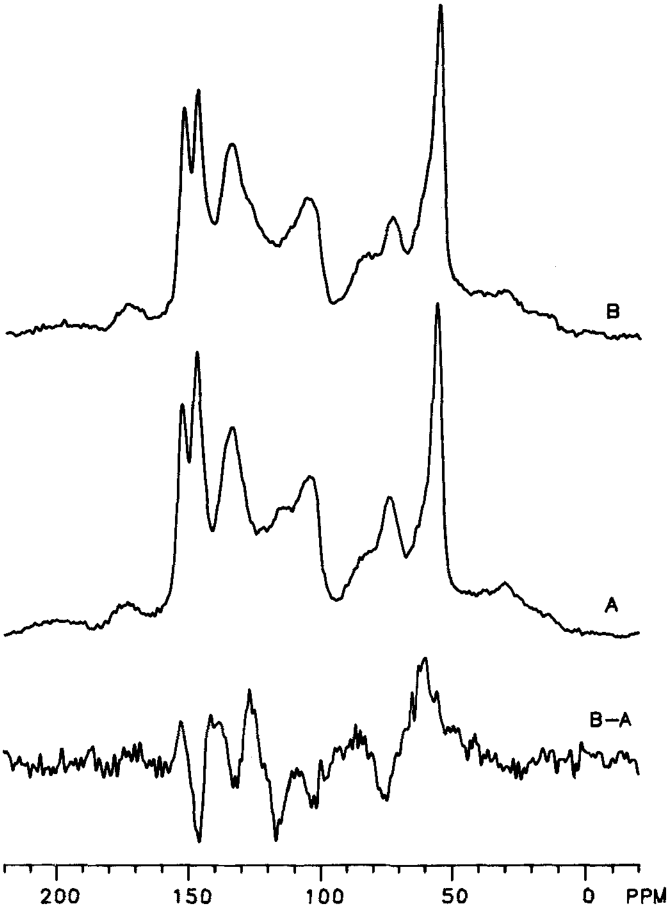


FIGURE 2. ^{13}C CP/MAS NMR spectra of untreated (middle) and formaldehyde-treated (top) exploded hardwood lignin and difference spectrum (bottom) of treated minus untreated lignin.

than in solution spectra of lignins, so considerable detail is lost by peak overlap; nonetheless, major changes in lignin structure are observed readily, as these spectra illustrate.

The kraft pine lignin spectrum exhibits major changes from formaldehyde treatment (Figure 1): some of the intensity at 115

TABLE 2

Assignments of Major ^{13}C CP/MAS NMR Signals from Untreated and Hydroxymethylated Lignins.

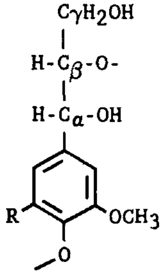
Frequency, ppm	Assignment[a]	Frequency, ppm	Assignment[a]
153	SOR' 3,5[b]	85-80	C_β-O-
148	SOH 3,5[b]; G 3,4	75	C_α-OH
142	G_{sub} 4[c]	65-60	G_{sub}-CH_2OH[d]
135-130	S 1,4; G 1	63	$C_\gamma H_2OH$
128	G_{sub} 5[c]	57	$-OCH_3$
124	G 6		
115	G 2,5		
105	S 2,6		

[a] For carbons indicated in syringyl (S) and guaiacyl (G) units.

[b] SOR' denotes syringyl with ether linkage at C4; SOH denotes phenolic syringyl.

[c] 5-Hydroxymethyl substituted.

[d] Assignment for hydroxymethyl carbon.



G: R = -H

G_{sub}: R = $-CH_2OH$

S: R = $-OCH_3$

ppm shifts approximately 13 ppm to lower field, appearing at 128 ppm; a shoulder on the G 3,4 peak appears at about 142 ppm; and intensity increases in the shoulder on the methoxyl peak, at 60 to 65 ppm. These changes are presented conveniently in the difference spectrum (Figure 1, bottom) of treated minus untreated pine lignin, scaled to null the methoxyl signal at 57 ppm. Positive peaks in the difference spectrum correspond to species created by formaldehyde treatment; negative peaks indicate carbons in the original lignin whose resonance frequencies were shifted by the treatment. These changes are fully consistent with hydroxymethyl substitution at C5 of phenolic guaiacyl units in the kraft pine lignin. The 115(-)/128(+) ppm pair represents C5 taking on a hydroxymethyl group; the 13-ppm shift is typical of the ortho carbon shift in PF resole formation[9]. The peak remaining at 114 ppm in the treated lignin spectrum is guaiacyl C2, which does not react with formaldehyde and should shift only a small amount on para substitution at C5[9,20]. Another negative/positive pair, those at 148 and 142 ppm, are assigned to phenolic guaiacyl C4, ortho to the substitution site, shifted to higher field, again in keeping with observations on PF resin spectra[9]. Finally, the 60 to 65(+) ppm peak is assigned easily to the hydroxymethyl carbons themselves. The tail out to 70 ppm could arise from methylene ether carbons in small amounts of dibenzyl ether condensation products or hemiformals of the C5 or $C\gamma$ hydroxymethyl groups. No evidence is seen for diphenylmethane $-CH_2-$ carbons at 30 to 35 ppm[9,21] arising from formaldehyde treatment.

The C5 shift and appearance of hydroxymethyl carbons were reported recently by Landucci[21] in solution ^{13}C NMR spectra of pine lignin reacted with $^{13}CH_2O$. He further reported the formation of condensed diphenylmethane products, based on intensity at 20 to 40 ppm for the methylene carbon, but these products appeared only at reaction temperatures much higher than the ambient conditions used in this work.

Similar hydroxymethylation effects are observed in the exploded hardwood lignin spectra in Figure 2: peaks at 116 and 146 ppm, negative in the difference spectrum, become positive peaks at 128 and 139 to 142 ppm, respectively. New peaks appear at 60 to 65 ppm. These changes match those in the pine lignin and are ascribed to hydroxymethyl subsitution in guaiacyl units. The lower guaiacyl content in hardwood relative to pine lignin causes the changes in the hardwood spectrum to be less obvious and the signal-to-noise ratio in the difference spectrum to drop. The small negative peaks at 103 and 75 ppm in the difference spectrum are difficult to interpret, if they are even significant, but may represent removal of part of the residual carbohydrate by the alkaline formaldehyde treatment.

Degree of Substitution

Essentially quantitative responses from carbon atoms in a CP/MAS NMR experiment employing reasonable parameters[15,16] make peak areas useful for determining compositions of complex samples. In the case of lignins, the spectra are split conveniently into two frequency ranges: a downfield range (165 to 95 ppm) containing aromatic and olefinic carbon signals, and an upfield part (95 to -30 ppm) containing sidechain, methoxyl and hydroxymethyl carbon resonances and the upfield aromatic spinning sidebands. After correcting them for spinning sideband contributions, the downfield intensity (I_{Ar}; 165 to 95 ppm) and aliphatic carbon intensity (I_{Al}; 95 to -30 ppm) were used to estimate the degree of hydroxymethyl substitution (DS). Two simplifying assumptions were made:

1. I_{Ar} corresponds to six aromatic carbons in each lignin C_9 unit; i.e., sidechain olefinic bonds were ignored.

2. Formaldehyde treatment causes no change in the number of carbon-carbon double bonds.

Since hydroxymethylation should increase the aliphatic carbon intensity, comparing the aliphatic/aromatic intensity, R_2, for a formaldehyde-treated lignin with that for the untreated lignin, R_1, gives DS directly:

$$DS = 6(R_2 - R_1) \qquad \text{Eqn (1)}$$

$$\text{where } R = I_{Al}/I_{Ar}$$

TABLE 3

CP/MAS NMR Intensity Ratios and Calculated DS Values for Hydroxymethylated Lignins.

	Kraft Pine Lignin	Exploded Hardwood Lignin
CP/MAS NMR intensity ratios:		
R_1	0.502	0.682
R_2	0.571	0.728
DS results (mol/lignin unit):		
CP/MAS NMR	0.41_4	0.27_6
HCHO uptake	0.39	0.25
^1H NMR[a]	0.38	0.18
^1H NMR[b]	0.35	0.15-0.20
Hydroxyl detn.[c]	0.39	--

[a] Values from reference 18.
[b] Values from reference 17.
[c] Values from reference 11.

The R values and DS results from the NMR analysis and some DS values obtained by other methods are listed in Table 3. The close agreement of CP/MAS NMR measurements with the others and the specific structural information revealed in Figures 1 and 2 show the utility of the NMR technique.

Kraft Pine Lignin Composition

The saturated/unsaturated carbon intensity ratio in the untreated kraft pine lignin CP/MAS NMR spectrum can reveal useful information on the overall composition of the lignin, within the quantitative accuracy limits of the experiment. A pure guaiacyl lignin should exhibit R = 4/6, with three saturated sidechain carbons and one methoxyl carbon for one phenyl ring. The R value of 0.50 found for the kraft lignin is in substantial disagreement with the classical concept of guaiacyl lignin. Lignin structures which can lower the R value include catechol and p-hydroxyphenyl systems (deficient in methoxyl content), and vinyl ether and stilbene structures resulting from elimination of the sidechain $C\gamma$ as formaldehyde and formation of an α,β-unsaturated bond. Vinyl ethers and stilbenes would be particularly effective in lowering R: The α and β carbons disappear from the upfield part of the spectrum, and contribute instead to the unsaturated carbon intensity. Some of the γ carbon formaldehyde recombines into lignin as hydroxymethyl groups or methylene linkages between aromatic rings during pulping and still contributes to I_{Al}; the rest presumably is consumed by Cannizzaro reactions and does not appear in the isolated lignin.

Various quantities, as moles per average lignin unit, can be denoted as N_{OMe}, number of methoxyl groups; N_{us}, number of unsaturated sidechain bonds formed; N_{fl}, number of γ carbons lost as formaldehyde and not recombined. Then the NMR intensity relationship can be written:

$$\frac{I_{Al}}{I_{Ar}} = R = \frac{3 + N_{OMe} - 2N_{us} - N_{fl}}{6 + 2N_{us}} \qquad \text{Eqn (2)}$$

Solving for N_{us},

$$N_{us} = \frac{3 + N_{OMe} - 6R - N_{fl}}{2R + 2} \qquad \text{Eqn (3)}$$

Since N_{fl} is not known, an exact solution is not possible. However, limits can be placed on N_{fl} by realizing that no more formaldehyde can be lost than was created by unsaturated bond formation; i.e., $0 \leq N_{fl} \leq N_{us}$. Inserting values of 0.87 and 0.502 for N_{OMe} and R, respectively, and then tabulating N_{us} as a function of N_{fl}, the upper limit of N_{fl} is found to be 0.21 for this kraft lignin (for $N_{fl} > 0.21$, $N_{us} < N_{fl}$); allowable ranges are $0 \leq N_{fl} \leq 0.21$ for $0.28 \geq N_{us} \geq 0.21$. Thus, consideration of only the methoxyl content and the NMR intensity ratio suggests that this lignin is composed of 13% p-hydroxyphenyl and 87% guaiacyl units, 21-28% of the units contain olefinic sidechain bonds, up to 20% of γ carbons have been lost, and the average repeating unit contains 9.7 to 9.9 carbons.

While this simplistic analysis cannot account for all aspects of lignin composition, it does support the common observation of vinyl ether and stilbene olefinic carbon resonances in solution ^{13}C NMR spectra of softwood lignin preparations[4,21]. Finally, for the analysis to be at all meaningful, the lignin must contain less than about 1% carbohydrates; these species cause a positive error in the R value which leads to a low estimate of the vinyl content. Also, the NMR parameters must be chosen to obtain a valid ratio: an error in R of 0.05 causes an uncertainty of -0.1 in N_{us} in this example.

Implications for DS Results

In all the DS results in Table 3, idealized lignin unit compositions were employed as calculation bases: vinyl bonds were neglected and all γ carbons assumed to be present. The above analysis of olefinic bond and γ carbon content indicates that the idealized composition deviates from reality and that the average unit formula weight of kraft pine lignin is lower than that used in converting analytical results to the per-unit basis. The calculations above, for instance, point to a pine lignin unit formula weight of 180 to 185 g/mol, down from 196 in idealized guaiacyl lignin. Thus, the DS values from HCHO uptake and hydroxyl determination could be raised by 5-8% of those listed for kraft pine lignin.

Correcting the CP/MAS NMR result for DS is simple: since I_{Ar} seems to represent about 6.5 carbons per unit (1 aromatic ring and 0.25 olefinic bond), the coefficient in Eqn (1) is changed from 6.0 to 6.5, raising the pine lignin DS result from 0.41 to 0.45, an 8% change.

CONCLUSIONS

CP/MAS NMR spectrometry of solid lignin preparations is a useful method for establishing some important compositional properties of lignin, identifying the structural changes in lignin on reaction with formaldehyde, and determining the degree of substitution by formaldehyde. The DS values from this technique agree well with those obtained by more time-consuming wet chemical or spectroscopic analyses; the results from this study show the utility of this convenient nondestructive technique in routine examinations of lignin preparations.

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