Using ¹³C nuclear magnetic resonance spectroscopy for the study of northern hardwood tissues

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Abstract: Nuclear magnetic resonance (NMR) spectroscopy is a useful tool for examining the structural chemistry of natural organic matter. The use of cross-polarization and magic-angle spinning to study ¹³C functionality (CPMAS ¹³C NMR) is convenient, but not always quantitative. We used various ¹³C NMR techniques to examine the structural chemistry of bark and wood of sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.). Spin counting experiments showed that 87%–97% of the ¹³C in the samples was observable by CPMAS ¹³C NMR. A comparison of CPMAS and Bloch decay experiments revealed few differences in spectral properties. Together, these results suggest that CPMAS ¹³C NMR is quantitative for these tissues. We observed little variation in the structural chemistry of wood, either among samples of the same species or among species. Within-species variations in bark chemistry were greater than in wood, probably because of variations in environmental conditions. However, we observed no significant differences in bark chemistry among the species. Bark and wood chemistry differed significantly, with the bark spectra displaying greater contributions from lignin, suberin, waxes, and resins. Hardwood spectra differ from softwood spectra in the aromatic C regions because of the contribution of syringyl units to hardwood lignin. Hardwood bark appears to contain less tannins than softwood bark. Together, the quantitative and qualitative features of CPMAS ¹³C NMR spectra are useful for studying the ecology of living and detrital wood and bark.

Résumé : La spectroscopie par résonance magnétique nucléaire (RMN) est un outil utile pour étudier la structure chimique de la matière organique naturelle. L'utilisation de la polarisation croisée et de la rotation à l'angle magique pour étudier la fonctionnalité de ¹³C (CPMAS ¹³C RMN) est pratique mais cette technique n'est pas toujours quantitative. Les auteurs ont utilisé diverses techniques de ¹³C RMN pour étudier la structure chimique de l'écorce et du bois d'érable à sucre (*Acer saccharum* Marsh.), du hêtre à grandes feuilles (*Fagus grandifolia* Ehrh.) et du bouleau jaune (*Betula alleghaniensis* Britt.). Les expériences de comptage de spin ont montré que 87 % – 97 % du ¹³C dans les échantillons pouvait être observé par la technique CPMAS ¹³C RMN. Une comparaison entre des expériences de CPMAS et de relaxation a révélé qu'il y avait peu de différence dans les propriétés spectrales. Globalement, ces résultats indiquent que la CPMAS ¹³C RMN est une technique quantitative pour ces tissus. Ils ont observé peu de variation dans la structure chimique du bois, ni entre les échantillons de la même espèce, ni entre les espèces. Au sein d'une même espèce, les variations dans les caractéristiques chimiques étaient plus importantes dans l'écorce que dans le bois, probablement à cause des variations dans les conditions environnementales. Cependant, ils n'ont observé aucune différence significative entre les caractéristiques chimiques de l'écorce des différentes espèces. Les caractéristiques chimiques de l'écorce et du bois étaient significativement différentes; les spectres de l'écorce montraient une plus forte contribution de la lignine, de la subérine, des cires et des résines. Les spectres des feuillus différaient de ceux des conifères dans les régions correspondant au C aromatique à cause de la présence du groupe syringyl dans la lignine des feuillus. L'écorce des feuillus semble contenir moins de tannins que l'écorce des conifères. Ensemble, les caractéristiques quantitatives et qualitatives des spectres CPMAS ¹³C RMN sont utiles pour étudier l'écologie du bois et de l'écorce, qu'ils soient vivants ou non.

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Introduction

Solid-state nuclear magnetic resonance (NMR) spectroscopy is an effective tool for examining the nature of chemical bonding in natural materials. Using ^{13}C as the target nucleus provides insight into the nature and abundance of bonds involving carbon (C). Analyses of plant tissues, soils, humic substances, and dissolved organic matter have offered useful insight into processes such as decomposition (e.g., Preston et al. 1990; Baldock et al. 1997; Irbe et al. 2001), soil organic matter dynamics (e.g., Golchin et al. 1997; Dai et al. 2001; Mathers and Xu 2003), and the sorption of synthetic organic compounds to soils (e.g., Witte et al. 1998; Roy et al. 1999; Benoit and Preston 2000).

The cross-polarization with magic-angle spinning (CPMAS) technique is widely used in the study of natural organic matter because spectra with acceptable signal to noise ratios can often be obtained in 1–2 h. The CPMAS spectrum can be integrated to estimate the fraction of C in various resonance regions (e.g., alkyl C, *O*-alkyl C, aromatic C, carbonyl C). However, the CPMAS technique is not always quantitative (e.g., Fründ and Lüdemann 1989; Skjemstad et al. 1994; Kinchesh et al. 1995). C atoms that are remote from ^1H nuclei and those that are in structures with high molecular mobility tend to be underestimated in CPMAS ^{13}C NMR (e.g., Jurkiewicz and Maciel 1995; Mao et al. 2000; Preston 2001).

The problem of CPMAS quantitation can be avoided by using the more robust Bloch decay (BD) technique, otherwise known as direct polarization (DP). The drawback of the BD technique is that it is much less sensitive than CPMAS. A typical BD spectrum requires 24 h or more of instrument time to achieve a reasonable signal to noise ratio, although there have been some recent attempts to improve the sensitivity of BD (Mao et al. 2000, 2002; Keeler and Maciel 2003). Quantitation in CPMAS spectra can also be addressed by analyzing spectra acquired with a range of cross-polarization contact times (Davis et al. 1994a; Kinchesh et al. 1995; Preston et al. 1998), rather than just a single contact time (usually 1 ms).

Spin counting (Smernik and Oades 2000a, 2000b) provides a way to identify potential problems with quantitation in a CPMAS spectrum, with virtually no additional cost in terms of spectrometer time. Spin counting simply involves calibrating the total signal intensity (across the whole spectrum) of each sample against that of a reference material known to produce a quantitative NMR signal. If the sample produces an equivalent amount of signal per unit C to that of the reference material, then the sample spectrum must be quantitative. If the sample produces less signal than the reference material, then the analysis may not be quantitative.

A number of NMR investigations have focused on the analysis of tree tissues. These studies have used NMR to study decomposition processes (e.g., Preston et al. 1990, 1998; Baldock and Preston 1995; McColl and Powers 1998; Ganjegunte et al. 2003), forest harvesting effects (Mathers et al. 2003), and wood fiber properties (e.g., Newman and Hemmingson 1990; Baldock and Smernik 2002; Maunu 2002; Preston and Forrester 2004). Wood and bark should be good candidates for quantitative CPMAS ^{13}C NMR because they contain small quantities of paramagnetic elements (e.g., Fe, Mn), they have high C content, and few C atoms are distant from H.

The objective of this study was to determine the conditions under which CPMAS ^{13}C NMR might be quantitative in the analysis of wood and bark. We used tissue samples from the dominant trees of the northern hardwood forest, which covers much of the northeastern United States and southeastern Canada. Our specific objectives were (i) to assess quantitation through spin counting experiments; (ii) to compare CPMAS and BD spectra to determine the nature of any CPMAS bias; (iii) to study the effect of spinning speed on spectral properties of bark and wood samples; (iv) to compare standard CPMAS analyses to ramped-amplitude analyses (Metz et al. 1994); and (v) to examine the variations in tissue chemistry within and among tissue types (i.e., wood vs. bark) and tree species.

Materials and methods

Sample collection and preparation

The wood and bark samples used in this study were collected at the Hubbard Brook Experimental Forest (HBEF) in central New Hampshire, USA (43°56'N, 71°45'W). Most of the HBEF is northern hardwood forest, dominated by sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.). In July 1990 and May 1991, we collected samples of tissues from six freshly felled trees of each of these three dominant species. The trees were approximately 70 years old. A segment of each bole, approximately 1 m long, was isolated with a chain saw.

After measuring the dimensions of the bole-segments, disks about 8–10 cm thick were cut from one end of each segment. The bark was separated from the wood of each disk, and both were dried at 80 °C to constant mass. Subsamples were collected from the wood disks by drilling from the side to the center with a 2.5 cm diameter drill bit, to ensure that a representative sample was collected from the entire diameter of the disk. The dried bark samples and the wood shavings were ground in a Wiley mill. Samples from the same log were composited for analysis. Total C was measured on oven-dried samples using an Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyzer (Eurovector, Milan, Italy).

NMR spectroscopy

Most of the CPMAS ^{13}C NMR analyses were performed on a Bruker AVANCE 300 spectrometer, operating at 75.47 MHz for ^{13}C . In these analyses, 2000 scans were collected with a 1-ms contact time, acquisition time of 17.5 ms, and a recycle delay of 3 s. The samples were spun in 7-mm zirconia rotors with Kel-F caps. Half of the samples were spun at 5 kHz, and the other half at 7 kHz to examine the effects of spinning speed. The CPMAS experiments were set up using glycine and the spinning speed at which the acquisitions were to be made (5 or 7 kHz). The CPMAS spectra were zero-filled to 8192 data points and processed with 50-Hz Lorentzian line broadening. Chemical shift values were internally referenced to the anomeric C peak at 105 ppm.

After baseline correction, we integrated the spectra between 280 and –50 ppm. Assignments for various spectral regions are given in Table 1 and were based on those used by Baldock and Smernik (2002). The 45–60 ppm region in-

Table 1. Chemical shift assignments for carbon structures and spinning sidebands (SSB); assignments are based on those of Baldock and Smernik (2002).

Chemical shift region (ppm)	Assignment	Integrated intensity calculation (ppm)
5-kHz spinning speed		
0–45	Alkyl C	(0–45)
45–60	Methoxyl C	(45–60)
60–94	<i>O</i> -alkyl C	(60–94) – (215–230)
94–110	Di- <i>O</i> -alkyl C	(94–110) – (230–250)
110–144	Aryl and unsaturated C	(110–144)
144–165	<i>O</i> -aryl C	(144–165) + 2(215–230)
165–190	Carbonyl and amide C	(165–190) + 2(230–250)
190–215	Ketone C	(190–215)
215–230	<i>O</i> -aryl SSB	
230–250	Carbonyl SSB	
250–280	No assignment	
7-kHz spinning speed		
0–45	Alkyl C	(0–45) – (215–235)
45–60	Methoxyl C	(45–60) – (235–260)
60–94	<i>O</i> -alkyl C	(60–94) – (260–280)
94–110	Di- <i>O</i> -alkyl C	(94–110)
110–144	Aryl and unsaturated C	(110–144) + 2(215–235)
144–165	<i>O</i> -aryl C	(144–165) + 2(235–260)
165–190	Carbonyl and amide C	(165–190) + 2(260–280)
190–215	Ketone C	(190–215)
215–235	Aryl SSB	
235–260	<i>O</i> -aryl SSB	
260–280	Carbonyl SSB	

cludes signals from methoxyl and *N*-alkyl C. However, since the N content of wood and bark is low, we attributed the intensity in this region solely to methoxyl C. We accounted for the effect of spinning sidebands as described in Table 1. The integrated area between 0 and –50 ppm was never more than 1.8% of total spectral area. Thus, we estimated the fraction of C associated with various structures by dividing the integrated intensities shown in Table 1 by the spectral area between 0 and 280 ppm.

The analyses required for the comparison of CPMAS and BD spectra, the spin counting experiments, and the acquisition of dipolar dephasing (DD) spectra were carried out on a Varian Unity200 spectrometer (Varian Inc., Palo Alto, California, USA) at the University of Adelaide. One sample of beech wood and one sample of sugar maple bark were selected for these analyses. The samples were analyzed at a ^{13}C frequency of 50.3 MHz, with an acquisition time of 15 ms. They were spun at 5 kHz in 7-mm zirconia rotors with Kel-F caps. After zero-filling to 8192 data points, the spectra were processed with a 50-Hz Lorentzian line broadening and 0.005-s Gaussian broadening. The chemical shifts were externally referenced using the methyl resonance of hexamethylbenzene at 17.36 ppm. For the CPMAS analyses, a 1-ms contact time was used, with a 4-s recycle delay; the Hartmann–Hahn match was set using hexamethylbenzene at a MAS rate of 5 kHz. For the BD analyses, a 6.2- μs (90°) ^{13}C pulse was applied, followed by a 90-s recycle delay. Approximately 1000 scans were collected in both the CPMAS and BD experiments. The BD spectra were corrected for back-

ground signal as described in Smernik and Oades (2001). DD spectra were acquired with cross-polarization and a dephasing delay of 45 μs , using the pulse sequence of Harbison et al. (1985). The recycle delay was 1 s, and the contact time was 1 ms.

Spin counting experiments

Spin counting experiments were performed using the method of Smernik and Oades (2000a, 2000b). Glycine (AR grade, Ajax Chemicals, Seven Hills, NSW, Australia) was used as an external intensity standard (i.e., the glycine spectrum was acquired separately from those of the tissue samples). A known mass (367 mg) of glycine was analyzed by CPMAS and BD, as described previously, to determine the amount of signal generated per mass of C. The beech wood and sugar maple bark samples were then analyzed under identical conditions. The amount of signal generated in these analyses was used to estimate the mass of “NMR-observable” C in the sample (C_{obs}). The actual amount of C in the rotor (C_{act}) was the product of the mass of sample and the total C concentration. If C_{obs} is approximately 100% of the C_{act} , then the spectrum is considered quantitative.

For CPMAS spin counting experiments, differences in spin dynamics between the sample and the glycine standard were accounted for using the method of Smernik and Oades (2000a), except that a variable spin lock rather than a variable contact time experiment was used to determine $T_{1\rho}\text{H}$ (Smernik et al. 2002). Errors in C NMR observabilities (C_{obs} values) are estimated to be $\pm 10\%$ in the CPMAS and $\pm 15\%$ in BD experiments (Smernik and Oades 2000a). Also, it is worth noting that some NMR operating systems do not provide intensity data in a form suitable for spin counting experiments.

Results and discussion

Spin counting

We performed spin counting experiments on samples of beech wood and sugar maple bark using both BD and CPMAS techniques. The BD results indicated that the NMR observability of C (C_{obs}) in these materials was 104% and 103%, with respect to the standard, glycine (Table 2). These results indicate that the BD technique is quantitative for these tissue samples, as expected. Smernik et al. (2000b) reported that C_{obs} ranged from 84% to 106% in BD analyses of HF-treated soils, and Baldock and Smernik (2002) reported a C_{obs} value of 90% for BD analysis of red pine (*Pinus resinosa*) wood, suggesting that the BD technique is quantitative for a wide range of environmental samples.

In the CPMAS analyses, C_{obs} was 97% for the C in beech wood (Table 2). We did not perform spin counting experiments on other species of wood. However, there were minimal differences in the NMR properties of wood from the three species we examined (discussed in the next section). Baldock and Smernik (2002) reported a C_{obs} value of 91% for CPMAS analysis of red pine wood. Thus, it appears that CPMAS ^{13}C NMR spectroscopy of wood under these analytical conditions is quantitative. In contrast, C_{obs} for sugar maple bark was 87% using the CPMAS method, suggesting a lesser degree of quantitation for the analysis of bark (Table 2).

The C_{obs} values that we observed in our CPMAS spin counting experiments were high compared with reported values

Table 2. Results of spin counting experiments on American beech wood and sugar maple bark.

Sample	Carbon mass, C_{act} (mg)	Spectral area (dimensionless)	Signal/mass (mg^{-1})	NMR-observable C, C_{obs} (mg)	C_{obs}/C_{act} (%)
Bloch decay					
Glycine (standard)	117.5	38.00	0.323	117.5	100
Beech wood	90.78	30.61		94.62	104
Sugar maple bark	93.25	30.93		95.62	103
CPMAS					
Glycine (standard)	117.5	127.99	1.090	117.5	100
Beech wood	90.78	95.83		87.95	97
Sugar maple bark	93.25	88.55		81.26	87

for soils (54%–83%; Smernik et al. 2000b), humic substances (30%–62%; Wilson 1987), and soil fractions (14%–30%; Preston et al. 1994). Smernik et al. (2000a) found that cellulose is detected quantitatively by CPMAS ^{13}C NMR and lignin is very nearly so. Thus, the high C_{obs} values that we observed for wood were no surprise. Our bark samples exhibited up to 24% alkyl C, which may help explain the lower degree of quantitation relative to the wood tissue. Model compounds rich in alkyl C (collagen, chitin) also exhibited relatively low C_{obs} values in the spin counting experiments conducted by Smernik et al. (2000a). Many of these C forms exhibit a high degree of molecular mobility, which limits the efficiency of the cross-polarization process.

Comparison of CPMAS and BD spectra

Figure 1 shows CPMAS and BD spectra for the same beech wood and sugar maple bark samples used for the spin counting experiments. The CPMAS and BD spectra for beech wood are nearly identical, although the BD spectrum includes more noise on the baseline. The distribution of signal intensity among spectral regions was also similar (Fig. 2). Slightly greater percentages of aryl and alkyl C in the BD spectrum may have been partly due to the uncertainty in the BD baseline.

The CPMAS and BD spectra for the bark sample, while similar to one another, showed greater differences than the wood spectra (Fig. 1). The alkyl C (0–45 ppm) and carbonyl C (165–190 ppm) regions are less prominent in the CPMAS spectrum, whereas the anomeric C (94–110 ppm) region is somewhat more prominent. These differences are also evident in the integration results (Fig. 2). As with the wood sample, some of the difference in the integrated values may be due to the uncertainty in the BD baseline. In the BD spectrum there is greater noise and the “valleys” between peaks lie higher above the baseline than in the CPMAS spectrum. Upon integration, these factors will influence the smaller peaks disproportionately.

Researchers studying soils (e.g., Knicker and Skjemstad 2000; Smernik et al. 2000b) and humic substances (e.g., Keeler and Maciel 2003) have observed substantial differences between CPMAS ^{13}C and BD spectra. In contrast, the differences we observed were very small. In a study of the effects of thermal alteration, Baldock and Smernik (2002) also found few differences between CPMAS and BD spectra of red pine at their lowest temperature treatment (70 °C). Our results suggest that the fraction of signal intensity in various resonance regions differs by up to 2.5% of the total integrated intensity.

Fig. 1. Bloch decay (BD), CPMAS ^{13}C (CP), and dipolar dephasing (DD) spectra for American beech wood and sugar maple bark. Each spectrum was collected with the same number of scans at a ^{13}C frequency of 50.3 MHz.

Standard versus RAMP CPMAS

The standard CPMAS analysis involves the magnetization of 1H in the sample using a single pulse. This magnetization is then passed to nearby ^{13}C atoms, which gives rise to the term “cross-polarization”. At high magnetic field strengths and fast spinning speeds, the efficiency of cross polarization can be affected and can vary among C structures. The ramped-amplitude (RAMP) CPMAS method addresses this problem by linearly increasing the 1H field during cross-polarization (Metz et al. 1994). This technique has proved especially valuable in the study of soils and humic substances (Cook et al. 1996; Cook and Langford 1998; Mao et al. 2002).

We compared RAMP and standard CPMAS analyses of beech wood and sugar maple bark samples. The beech wood sample was the same sample that was used for the spin counting experiments. The RAMP spectra are virtually iden-

Fig. 2. Distribution of spectral intensity among the major regions of ^{13}C NMR resonance. Data are shown for the CPMAS (CP) and Bloch decay (BD) analyses of American beech wood and sugar maple bark shown in Fig. 1. The labels, read down in the legend, correspond to the bars viewed left to right.

Fig. 3. CPMAS ^{13}C NMR spectra for American beech wood and sugar maple bark analyzed by standard (single-pulse) cross-polarization and ramped amplitude cross-polarization. Each spectrum was collected with the same number of scans at a ^{13}C frequency of 75.47 MHz.

tical to the CPMAS spectra, including shoulders and sub-peaks (Fig. 3). The differences in the integration results were also minimal (Fig. 4). Thus, it appears that the RAMP method does not significantly improve the analysis of these tissues. This is not surprising given the high C_{obs} values for the CPMAS spectra.

Effects of spinning speed

Spinning sidebands (SSBs) are artifacts that appear as peaks in the NMR spectrum, displaced from the correspond-

ing main peaks (the "center bands") by integral multiples of the spinning speed (Conte et al. 2004). C atoms that are sp^2 -hybridized, such as carbonyl-C and aryl-C, tend to produce SSBs, whereas carbons that are sp^3 -hybridized, such as alkyl-C and O-alkyl-C, do not (Smernik 2005). The size of SSBs decreases with increased spinning speed and increases with increasing magnetic field strength (Preston 2001). In our experiments, carried out at a field strength of 75.47 MHz for ^{13}C , SSBs were located $5000/75.47$, or ± 66.2 ppm, from the center band at 5-kHz spinning speed, and $7000/75.47$, or

Fig. 4. Distribution of spectral intensity for the standard (single-pulse) CPMAS (denoted "Std") and ramped amplitude CPMAS (RAMP) analyses of American beech wood and sugar maple bark shown in Fig. 3. The labels, read down in the legend, correspond to the bars viewed left to right.

± 92.7 ppm at 7-kHz spinning speed. Since the compounds that most commonly produce SSBs are associated with high chemical shifts, SSBs are most easily recognized at the high-ppm end of the spectrum (>220 ppm).

Spectra collected at 5 kHz displayed small, but clear, SSBs at about 220 ppm (*O*-aryl C) and 240 ppm (carbonyl C) (Fig. 5). When integrated, these peaks always accounted for less than 1% of the total intensity between 280 and -50 ppm. However, since the corresponding main peaks typically accounted for only 2%–6% of the total intensity, the areas of the SSBs were a significant fraction of the total signal generated by *O*-aryl and carbonyl structures. For example, in the wood samples (all tree species), the carbonyl SSB at 239 ppm contributed $0.47\% \pm 0.12\%$ of total spectral intensity (mean $\pm$ standard deviation, $n = 9$), while the center band contributed $3.03\% \pm 0.35\%$. The carbonyl SSB in bark accounted for $0.38\% \pm 0.13\%$ of total spectral intensity, compared with $4.11\% \pm 0.49\%$ for the center band. The *O*-aryl SSB at 219 ppm contributed $0.61\% \pm 0.17\%$ and $0.56\% \pm 0.21\%$ of total spectral intensity in wood and bark, respectively, representing about 15% of the area in the center band (wood: $4.11\% \pm 0.49\%$; bark: $4.63\% \pm 0.40\%$).

When samples were analyzed at a spinning speed of 7 kHz, no SSBs were apparent (Fig. 5). The integrated intensity in the entire 215–280 ppm region was less than 1% of the total spectral intensity. Furthermore, when the same samples were analyzed at both spinning speeds, the spectra were nearly identical (Fig. 5), including the presence of shoulders and subpeaks. The integration results also indicated a high degree of similarity (Table 3). The estimated fractions of carbonyl C and *O*-aryl C were somewhat lower in the 7-kHz

analyses, while aryl C and di-*O*-alkyl C tended to be somewhat greater. It is worth noting, however, that the analyses reported in Fig. 5 and Table 3 were performed on different aliquots of the same samples. Therefore, some of the differences in the integrated intensities may be due to sampling variation. Baldock and Smernik (2002) found variations of similar magnitude among three aliquots of thermally treated wood analyzed by CPMAS ^{13}C NMR under similar experimental conditions.

Our data suggest that spinning the samples at a higher speed improves spectral quality by eliminating SSBs, without substantially altering the features of the spectrum. Faster spinning speeds often require the use of smaller sample rotors. The use of smaller samples decreases the total signal generated, thereby increasing the analysis time required to obtain the same signal to noise ratio (Preston 2001). However, since more of the signal generated at higher spinning speeds is focused on the center bands, this effect is partially offset.

Sample to sample variation

The CPMAS ^{13}C NMR spectra of beech bark collected from six different trees are shown in Fig. 6. Although all the spectra contain the same major features, there are some differences. In particular, the magnitude of the alkyl C resonance (0–45 ppm) and the relative importance of the peaks at 21 and 30 ppm vary considerably among the samples. Although not obvious in Fig. 6, there is also substantial variation in the carbonyl C region (165–190 ppm).

The variations in the integrated intensities of the alkyl, *O*-alkyl (60–94 ppm), aryl (110–144 ppm), and carbonyl regions are shown for the wood and bark of all species in

Fig. 5. CPMAS ^{13}C NMR spectra for wood and bark of yellow birch, analyzed at spinning speeds of 5 and 7 kHz. Each spectrum was collected with the same number of scans at a ^{13}C frequency of 75.47 MHz.

Table 3. Spectral integration results from CPMAS ^{13}C NMR analyses of yellow birch wood and bark at 5- and 7-kHz spinning speeds, where values are percentages of the spectral intensity in each region.

Resonance region	Wood		Bark	
	7 kHz	5 kHz	7 kHz	5 kHz
Ketone C	1.0	1.5	1.1	2.1
Carbonyl C	3.4	4.1	3.9	5.5
<i>O</i> -aryl C	4.1	5.3	4.9	6.5
Aryl C	7.1	6.3	10.3	8.9
Di- <i>O</i> -alkyl C	13.5	12.4	10.1	9.3
<i>O</i> -alkyl C	47.8	46.1	37.4	36.4
Methoxyl C	19.1	18.9	14.7	14.1
Alkyl C	4.0	5.4	17.5	17.4

Note: The 5 and 7 kHz samples were different aliquots of the same material. Spectral assignments and corrections for spinning sidebands are shown in Table 1.

Fig. 7. Data from analyses at both 5- and 7-kHz spinning speeds are combined in the figure and indicate that there are no consistent differences in spectra collected at the two speeds. This is consistent with our replicate analyses of yellow birch tissues (Fig. 5, Table 3).

The wood tissues exhibited much less sample to sample variation than bark (Fig. 7, Table 4). Since bark is the exposed part of the tree, factors such as weather, pests, fungal infection, and animal activity are likely to cause greater variation in bark chemistry than in wood.

The variations we observed in tissue chemistry among replicate samples, especially bark, indicate that a single sam-

Fig. 6. CPMAS ^{13}C NMR spectra for replicate samples of American beech bark. Each spectrum was collected with the same number of scans at a ^{13}C frequency of 75.47 MHz. The spinning speed for each spectrum is shown for reference, along with the identification number of the log.

ple is not likely to provide a representative characterization of trees in a stand. Researchers examining differences in tissue chemistry between different species or different stand conditions should examine as many samples as possible. Because NMR access is generally limited and expensive, the CPMAS technique may be the only viable approach in such studies. Fortunately, our data suggest that, applied to tree tissues, CPMAS techniques can be highly quantitative. For studies looking at treatment effects on tissue chemistry — decomposition studies, for example — experimental designs based on paired sampling would substantially reduce the effect of high sample to sample variation.

Chemistry of wood and bark

For all resonance regions except methoxyl C, we found statistically significant differences in the tissue composition of wood and bark (one-way ANOVA, F values ranging from 7.1 to 236, $P = 0.001$ for all resonance regions except methoxyl C). Wood has a greater proportion of *O*-alkyl C and di-*O*-alkyl C, while bark is richer in the other components, especially alkyl C (Table 4). The greater fraction of *O*-alkyl and di-*O*-alkyl C in wood, and the more prominent peak at

Fig. 7. Variation in the fraction of spectral intensity in four NMR resonance regions. Chemical shift assignments are given in Table 1. The spinning speed at which each sample was analyzed is indicated by the symbol.

Table 4. Means and standard deviations for replicate CPMAS ^{13}C NMR analyses of northern hardwood tissues, where values for the various carbon (C) forms are the percentages of C in the structures indicated, estimated by integrating the spectra using the assignments in Table 1.

Tissue	Total C (g kg ⁻¹)	Ketone C	Carbonyl C	O-aryl C	Aryl C	Di-O-alkyl C	O-alkyl C	Methoxyl C	Alkyl C
Wood									
Beech	402±25	1.1±0.3	4.2±0.5	4.9±0.9	6.0±0.6	13.1±0.3	59.4±1.5	7.6±0.4	3.9±0.4
Sugar maple	394±30	1.1±0.3	3.9±0.9	5.0±0.8	6.0±0.6	13.0±0.7	58.9±1.8	8.0±0.8	4.1±0.7
Yellow birch	413±21	1.1±0.3	3.5±0.6	4.3±1.0	5.9±0.4	13.3±0.7	60.0±2.3	7.4±0.5	4.5±0.7
Bark									
Beech	413±20	1.2±0.3	5.3±0.9	6.0±0.8	9.1±0.2	10.5±0.7	46.9±2.1	8.0±0.7	13.1±2.3
Sugar maple	408±13	1.3±0.2	5.0±0.6	6.2±0.7	8.9±1.4	9.9±1.1	45.0±4.2	7.5±0.9	16.1±4.4
Yellow birch	431±14	1.5±0.5	4.8±0.8	5.9±1.0	9.7±1.5	9.4±0.7	45.3±3.6	6.3±1.3	17.0±4.0

Note: In all cases, $n = 6$. Means for a row may not sum up to 100 because of rounding.

64 ppm, reflect greater cellulose content (e.g., VanderHart and Attala 1984; Newman and Hemmingson 1990). The single alkyl C peak at 21 ppm (see Figs. 1, 3, 5) is indicative of acetyl methyl C in hemicellulose (VanderHart and Attala 1984; Preston et al. 1990), consistent with the high cellulose concentration of the wood samples. The signal at 56 ppm, the broad peak centered at about 135 ppm, and the peak at 153 ppm reflect the methoxyl, aromatic, and phenolic structures of lignin, respectively (Hatfield et al. 1987; Preston et al. 1990). The location of the *O*-aryl peak at 153 ppm is characteristic of hardwood lignin, which contains both syringyl and guaiacyl units. The aromatic C–O bond in syringyl resonates at

153 ppm, whereas the peak for guaiacyl is located at 148 ppm (Martínez et al. 1991). Softwood lignin contains only guaiacyl units and therefore does not show a peak at 153 ppm.

The wood samples showed a remarkable consistency in composition, within and among species (Table 4). We found no significant species effects for any of the resonance regions (one-way ANOVA, $P > 0.05$ for all resonance regions), and the variations within species tended to be small (Fig. 7, Table 4). Therefore, the structural chemistry of the wood in these three northern hardwood species is very similar.

Our wood spectra are also similar to other CPMAS ^{13}C NMR analyses of hardwoods (Manders 1987; Martínez et al.

1991; Davis et al. 1994b). Martínez et al. (1991) estimated that European beech (*Fagus sylvatica* L.) wood contained 3.2% alkyl C (0–46 ppm), 13.4% aromatic C (110–160 ppm), and 80.8% in their “carbohydrate” fraction (46–110 ppm). Using slightly different integration limits, we found an average of 3.9% alkyl C (0–45 ppm), 10.8% aromatic C (110–165 ppm), and 80.1% in the 45–110 ppm region for American beech (Table 4). Davis et al. (1994b) studied the NMR properties of paper birch (*Betula papyrifera* Marsh.). They did not report integration results, but their spectra were very similar to our yellow birch wood spectra (Fig. 5), including the split peaks at 62–65 and 73–75 ppm.

In contrast to wood, the bark samples showed considerable variation within species (Fig. 6, Table 4). Only methoxyl C exhibited a significant difference among the three species (one-way ANOVA, $P = 0.029$; yellow birch > beech; $P > 0.05$ for all other resonance regions). Because of the high within-species variation, only large differences among species were statistically detectable. Nevertheless, the absence of significant differences in bark chemistry among the three species is surprising because they have very different physical characteristics. The bark of yellow birch is sheet-like, thin, and resistant to decomposition. In contrast, sugar maple has thick, rough, friable bark and American beech has a smooth, tight bark.

As in the wood samples, the large percentages of di-*O*-alkyl and *O*-alkyl C in bark, along with the acetyl methyl C peak at 21 ppm, indicate the important contribution of cellulose and hemicellulose (Figs. 1, 3, 5, 6). In the alkyl C region, the bark samples also contain a large split peak at 30 and 33 ppm. In the yellow birch bark and beech bark spectra (Figs. 5, 6), the 30-ppm peak is larger, whereas in the sugar maple bark spectra (Figs. 1, 3), the 33-ppm peak dominates. These peaks, which are absent in the wood spectra, indicate the presence of long-chain polymethylene structures $(CH_2)_n$, often attributed to fats, waxes, and resins (e.g., Baldock and Preston 1995). The peak at 30 ppm is associated with more mobile components of long-chain aliphatic C, whereas the 33-ppm peak is due to more rigid alkyl C structures. Thus, while we observed no significant differences in the content of alkyl C in bark among the three species, there are differences in the distribution of alkyl C among more mobile and rigid structures.

The bark spectra also indicated a greater abundance of lignin units. The bark samples had higher percentages of aryl and *O*-aryl C than the wood (Table 4). The greater aromaticity of the bark samples could also indicate the presence of tannin structures. While it can be difficult to distinguish tannins from lignin, tannins lack a methoxyl C peak at 56 ppm. This peak was prominent in our bark samples (Figs. 3, 5). Also, condensed tannins have a characteristic split peak in the *O*-aryl region at 144–145 and 154–155 ppm, whereas lignin typically shows signals at 153 and 147–148 ppm (Preston et al. 1997; Lorenz et al. 2000). In our bark spectra, the secondary *O*-aryl peak was often a broad peak in the 144–150 ppm range. However, the largest *O*-aryl peak was always found at 153 ppm, with no evidence of a shoulder at higher chemical shifts (Figs. 1, 3, 5, 6), suggesting that lignin, rather than tannins, was the principal source of these signals.

The dipolar dephasing (DD) results support the conclusion that lignin units, rather than tannins, are primarily re-

sponsible for the aromatic character of the bark samples we analyzed. The DD spectra of tannins are characterized by a prominent peak at 105 ppm and no significant peaks in the 0–100 ppm range (Preston et al. 1997). In contrast, DD spectra of lignin have a prominent peak at 56 ppm and little or no signal at 105 ppm (Preston et al. 1997). Our bark DD spectrum exhibited a major peak at 56 ppm and a clear, but much smaller, peak at 105 ppm (Fig. 1), suggesting that lignin is more abundant than tannins in these samples. Indeed, the peak at 105 ppm may be partly due to ketose sugars rather than tannins. C atoms that experience strong dipolar interactions with hydrogen do not contribute to the DD signal. Most anomeric C, which is responsible for the peak at 105 ppm, is bonded to hydrogen and loses signal during the dephasing period. Thus, the anomeric C peak that is so prominent in the BD and CPMAS spectra of both wood and bark nearly disappears in the DD spectra (Fig. 1). The anomeric C in ketose sugars is not bonded to hydrogen. Thus, it can retain signal during the dephasing period and contribute to the DD spectrum at 105 ppm.

Suberin is an important component of bark that occurs in phellem (cork) and epidermal cells of woody species (Bernards 2002). It is a complex substance that contains both aliphatic and aromatic structures. Perra et al. (1995) studied suberin extracted from the bark of European beech and attributed peaks occurring in the alkyl C region to fatty acids present in suberin. The aromatic character of suberin is broadly similar to that of lignin (Perra et al. 1995). Thus, suberin in bark tissues may help explain why both the aromatic-C and alkyl-C content was greater in our bark samples than in our wood samples.

The CPMAS ^{13}C NMR properties of bark have rarely been reported in the literature, especially for hardwoods. Bark of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) from Vancouver Island, British Columbia, contained less alkyl and carbonyl C and more *O*-aryl C than any of the hardwoods we studied (Preston and Forrester 2004). The methoxyl peak for Douglas-fir bark was much smaller than in our hardwood spectra, and DD analyses revealed a prominent peak at 105 ppm, much larger than the peak at 56 ppm, suggesting an abundance of tannins. McColl and Powers (1998) analyzed the bark of red fir (*Abies magnifica* A. Murr.) from northern California. Their samples contained more aryl and *O*-aryl C than our hardwood barks and less *O*-alkyl and di-*O*-alkyl C. Although they did not perform DD experiments, they also concluded that tannins were an important component of the bark samples they analyzed. Thus, tannins appear to be a much more important component in softwood barks than in hardwood barks.

Conclusions

Because tree tissues have low concentrations of paramagnetic materials and few C atoms remote from 1H , CPMAS ^{13}C NMR should be an effective means of characterizing tree tissue chemistry. Our work indicates that tissues from the principal northern hardwood trees (American beech, sugar maple, yellow birch) can be quantitatively analyzed by the CPMAS method. Spin counting and BD experiments on a typical wood sample showed that quantitation in the CPMAS spectra of all of the wood samples is likely to be very good.

The degree of quantitation in the CPMAS spectra of a typical bark sample was also very high, though not quite as high as for the wood.

Analyzing at a higher spinning speed (7 vs. 5 kHz) eliminated spinning sidebands while preserving spectral properties. The RAMP CPMAS method did not substantially improve the analyses.

We observed a high degree of sample to sample variation in bark properties, whereas the spectra from wood samples were much more consistent. The percentages of C in the various spectral regions differed significantly between wood and bark. However, there were no significant species effects on either wood or bark spectral properties. The wood samples show signatures of cellulose and lignin. The bark spectra showed stronger lignin-derived signals, along with a substantial contribution of long-chain polymethylene structures. These are consistent with the presence of suberin in the bark.

The CPMAS ^{13}C method combines quantitative information, such as the distribution of C among key structural groups, with qualitative indicators of the presence of specific chemical structures. This powerful combination makes CPMAS ^{13}C NMR a useful approach for examining the differences or changes in tissue chemistry caused by experimental treatments, decomposition, environmental gradients, and other ecologically relevant factors.

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