Quantitative Analysis of Cellulose in Tobacco by ^{13}C CPMAS NMR

Randal A. Hall[†] and Jan B. Wooten*[,‡]

2500 North Lakeview Avenue, Suite 1902, Chicago, Illinois 60614, and Philip Morris Research Center, P.O. Box 26583, Richmond, Virginia 23261-6583

A method utilizing ^{13}C CPMAS NMR was developed for the quantitative determination of cellulose in tobacco. The method was employed to measure cellulose in hot water extracted laminae and stems from bright and burley tobacco. A reference spectrum of tobacco cellulose was subtracted from the spectrum of each sample to obtain a sub-spectrum of the noncellulose components. This background spectrum was subtracted in turn from the original spectrum to give an appropriately scaled sub-spectrum of the cellulose alone. A calibration curve was constructed from the integrals of the C-1 resonance of a set of standard cellulose samples. The integrals were measured relative to an intensity standard contained in a ceramic cell inside the MAS sample rotor. The weight percentage of cellulose in each tobacco residue was then determined by comparison of the integral of the cellulose C-1 resonance in its cellulose sub-spectrum to the calibration curve.

Keywords: *Tobacco; ^{13}C CPMAS NMR; plants; cellulose*

INTRODUCTION

In the cigarette industry, cellulose content is a useful quantity for characterizing tobacco blends. In agriculture, cellulose content has been used to determine the nutritional value of forages (Van Soest, 1982). The chemical methods employed to measure the amount of cellulose in herbaceous plants involve tedious fractionation or extraction procedures which utilize harsh reagents to degrade the plant cell walls (Van Soest and Robertson, 1985). The potential of ^{13}C CPMAS NMR for the nondestructive analysis of plants, on the other hand, was recognized at its inception (Schaefer and Stejskal, 1976). In particular, NMR offers the prospect of the analysis of complex plant biopolymers in intact plant tissue including polysaccharides, hemicellulose, lignin, and protein (Himmelsbach, 1983; Maciel et al., 1985; Wooten, 1995). Broad resonance lines and the complexity of the ^{13}C NMR spectra of plants, however, have limited the number of useful qualitative and quantitative applications. Moreover, the need to consider differences in the rates of cross-polarization of individual resonances can make quantitative measurements by NMR time-consuming as well (Irwin, 1989). Nevertheless, various approaches have been successfully applied to remove spectral interference due to overlapping resonances (Davis et al., 1994; Bates and Hatcher, 1992), and the quantitative validity of ^{13}C CPMAS NMR is well established (Irwin, 1989).

The quantitative determination of cellulose in plants by chemical means can be especially troublesome since the nonglucan substances associated with cellulose in the plant cell wall (i.e., hemicellulose, lignin, and minerals) are difficult to remove without the destructive degradation of the cellulose (Van Soest, 1982). A spectroscopic method for measuring cellulose is consequently very desirable. We have developed a method for the quantitative determination of cellulose in cured tobacco utilizing ^{13}C CPMAS NMR. The only sample treatment utilized is hot water extraction. Several innovations minimize some of the experimental problems associated with previous NMR methods involving variability in cross-polarization dynamics, manipulation and handling of intensity standards, effects of radio frequency field mismatch on cross-polarization, and spectral deconvolution to remove interfering resonance lines. A reliable analysis can be accomplished on as little as 250 mg of sample with an absolute precision of ca. 0.5 wt %.

MATERIALS AND METHODS

Experimental Samples. Aged, cured, bright and burley tobacco laminae and stems were employed for the analyses. The weighed, oven-dried samples were extracted with boiling water for 1 h, rinsed with hot deionized water, and then dried again in an oven. The percentage of hot water-soluble material in each sample was determined by weight difference. The dry residue from the hot water extraction was used for the NMR analysis.

Reference Materials. 4-(*N*-Methylpyrrolidino)bicyclo-[3.2.1]octan-8-one trifilate ("d321"), 96.9% ^{13}C enriched in the carbonyl group, was employed both as an intensity and as a chemical shift reference; its synthesis and application to quantitative measurements have been described previously (Hall et al., 1993). None of the natural-abundance ^{13}C resonances of d321 appear inside the critical region 85−110 ppm used for the cellulose analysis and therefore were not a consideration in our measurements. The encapsulation of the d321 into a permanent cell provides a convenient source of constant resonance intensity against which other resonances can be measured, and allows the same reference sample to be employed repetitively. A very similar approach was used by Zhang and Maciel (1989), who used the background resonance of a MAS rotor machined from Delrin. In rotors larger than 7 mm, a glass capillary containing the intensity reference embedded within the sample seems to work quite satisfactorily (Jurkiewicz and Maciel, 1994).

The cellulose employed to establish a set of standard ^{13}C CPMAS NMR reference spectra was isolated by Bokelman et

* Address correspondence to this author at Philip Morris Research Center, P.O. Box 26583, Richmond, VA 23261-6583. Telephone: 804 274-3465. Fax: 804 274-2160. E-mail: wooten@talos.pm.com.

[†] 2500 N. Lakeview Ave., Chicago, IL 60614.

[‡] Philip Morris Research Center.

S0021-8561(97)00813-3 CCC: $15.00
Published on Web 03/18/1998

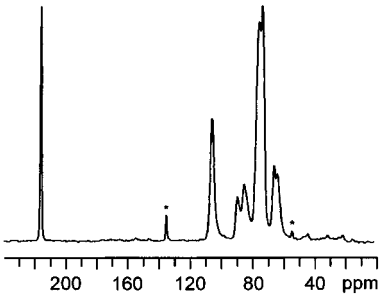

Figure 1. ^{13}C CPMAS NMR of the insoluble residue from the acid detergent extraction of tobacco. This material was employed as the reference sample for the quantitative determination of cellulose in tobacco. The peak at 216.5 ppm is the carbonyl resonance of the [^{13}C]-d321 intensity standard. Spinning sidebands of this resonance are marked by asterisks.

al. (1983) by acid detergent extraction of bright tobacco. This residue is predominantly cellulose, but contains some inorganic minerals and minor amounts of other polysaccharides and lignin. The actual cellulose content was determined to be 69.7 wt %. The fractionation procedure used to isolate the cellulose reference and the analysis procedures have been described in detail (Bokelman et al., 1983). Since the identical material was employed in the current study, any experimental errors associated with the previous measurements are consequently propagated into the NMR results. However, using the same material allows us to compare our NMR results directly with those obtained by chemical fractionation, and its resonance line shapes appear to closely match those of indigenous tobacco cellulose.

The ^{13}C CPMAS NMR spectrum of the cellulose reference material is free of resonances that might interfere significantly with the analysis. Although the resonances of the nonprotonated aromatic carbons of guaiacyl units (108 ppm) in lignin partially overlap the C-1 carbon resonance (105 ppm) of cellulose (Maciel et al., 1985; Davis et al., 1994), the integral of the *entire* aromatic region of the reference spectrum is only ca. 5% of the intensity of the C-1 resonance. Consequently, the aromatic contribution to the cellulose C-1 resonance is at the base line noise level. Hemicellulose polymers contribute ca. 9% of the weight of the reference material, but less than half of the hemicellulose resonance overlaps the cellulose C-1 resonance (Davis et al., 1994). Nevertheless, hemicellulose may contribute to the small discrepancy between the results of the NMR and fractionation methods (see Results and Discussion).

NMR Spectroscopy. The ^{13}C CPMAS NMR spectra were obtained on a Varian Unity 200 spectrometer at a carbon resonance frequency of 50.3 MHz. The NMR probe was a Doty Scientific (Columbia, SC) high-speed magic angle spinning probe. The carbonyl resonance of the d321 reference was assigned a chemical shift of 216.5 ppm, and the observe transmitter frequency was set near this peak. For the cross-polarization measurements, the initial proton $\pi/2$ pulse was 4.5 μs, corresponding to a radio frequency field of 56 kHz. The Hartmann−Hahn match was made at this field strength, whereas the proton decoupling field during the acquisition was ca. 60 kHz. The acquisition time was 50 ms, and the spectral width was 40 kHz. The pulse repetition time was 5 s. The time domain signal of all the spectra was weighted with a Gaussian apodization function prior to Fourier transformation to improve the signal-to-noise in the frequency domain. The sample cells were 7 mm cylindrical zirconia rotors with kel-F end caps (Doty Scientific). The long version of the end caps was used in order to confine the samples to the central region of the radio frequency coil. For all the measurements, the magic angle spinning speed was 4094 kHz. The spinning rate was regulated with a Doty Scientific MAS speed controller to within ca. 5 Hz of the target spinning speed. Base line flattening was employed to improve the accuracy of the integrals. The best results were obtained when fewer integral set points were used for the base line correction than were employed for making the resonance area measurements.

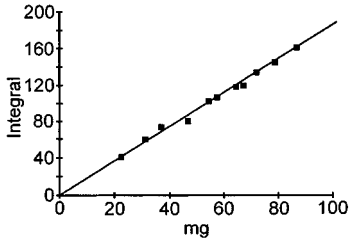

Figure 2. Calibration curve for the determination of cellulose in tobacco by ^{13}C CPMAS NMR. The integrals of the cellulose anomeric carbon were measured relative to the d321 carbonyl resonance integral set to 100.

The Hartmann−Hahn match condition was set using a conventional single-contact, cross-polarization pulse sequence. The proton radio frequency power was varied while holding the ^{13}C radio frequency power constant to obtain the maximum intensity for the d321 carbonyl resonance. All quantitative measurements, however, were made employing a variable-amplitude cross-polarization pulse sequence to minimize possible radio frequency mismatch between samples (Peerson et al., 1993). Signal acquisition was made over a period of 8−24 h to obtain suitable signal-to-noise for accurate measurements. The ^1H T_1 values for the cellulose anomeric resonance at 105.5 ppm and the d321 carbonyl resonances were made by inversion recovery measurements via cross-polarization to the ^{13}C nuclei. The values for T_{CH} and ^1H $T_{1\rho}$ were determined from multiple contact time experiments employing 18 contact times ranging from 15 to 40 000 μs. Regression analysis was performed on the peak intensities utilizing Varian software.

Sample Referencing. Approximately 1.1 mg of the [^{13}C]-d321 reference material was encapsulated in a cylindrical cell machined from aluminum nitride ceramic rod (Goodfellow, Malvern, PA) to precisely fit inside the 7 mm zirconia NMR rotor. To minimize variations in the cross-polarization intensities of both the analyte and d321 resonances due to radio frequency field inhomogeneity within the NMR coil, the reference cell was always placed in the same end of the NMR rotor. The sample (typically 55−65 mg) was packed into the opposite end of the rotor. The same rotor end caps were used for all measurements, and the zirconia rotor itself was always oriented in the same direction inside the NMR probe stator.

In solid-state NMR, finding appropriate materials for use as internal references has been problematic. The broad ^{13}C line widths that are typical of noncrystalline materials greatly increase the likelihood of spectral overlap among the resonances of any potential reference and the analyte, particularly when there are significant spinning sidebands. Moreover, most low molecular weight compounds commonly exhibit very slow proton relaxation rates, thus precluding their convenient application. The intensity reference [^{13}C]-d321 was introduced specifically to overcome these limitations. Its proton relaxation time is an acceptable 0.77 s, and it has favorable cross-polarization dynamics. The carbonyl reference appears at the extreme high-frequency end of the spectrum where it does not interfere with analyte resonances. The MAS rate was adjusted to place the spinning sidebands of the carbonyl resonance out of the spectral region of interest.

A calibration curve for the cellulose analysis was established from the ^{13}C CPMAS NMR spectra of a set of 12 cellulose samples with weights ranging from 10 to 65 mg. The same MAS rotor containing the d321 intensity reference cell was used for all the measurements. A representative spectrum is shown in Figure 1. The ratios of the integral of the region from 99 to 112 ppm, corresponding to the cellulose anomeric carbon resonance, to the integral of the d321 carbonyl resonance between 210 and 222 ppm were plotted versus the sample weights, as shown in Figure 2. Linear regression analysis was performed with the intercept constrained to pass through zero, yielding a straight line with a correlation coefficient $r^2 = 0.991$.

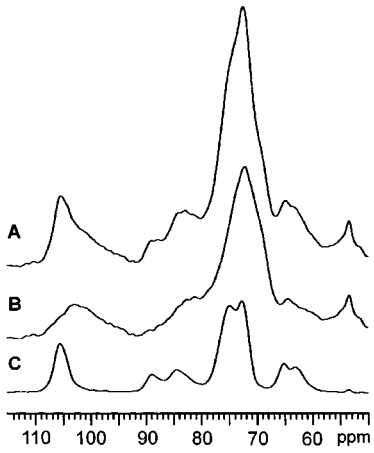

Figure 3. Deconvolution of the tobacco spectrum by spectral subtraction. (A) Carbohydrate region of the ^{13}C CPMAS spectrum of bright lamina. (B) Noncellulose background spectrum obtained by subtraction of the reference cellulose spectrum (Figure 1). (C) Cellulose-only sub-spectrum of tobacco obtained by subtraction of spectrum (B) from (A).

Cellulose Analysis. For each tobacco sample, a desiccated portion of the residue from the hot water extraction was loaded into the NMR rotor with the d321 reference cell and weighed to within ±0.1 mg on an analytical balance. Its ^{13}C CPMAS NMR spectrum was obtained with instrument settings identical to those used to obtain the cellulose reference spectra. Typically, the signal-to-noise ratio for the C-1 resonance at 105.5 ppm in our tobacco spectra was in the range of (50−100):1. A sub-spectrum of only the cellulose component of the tobacco residue was generated from this spectrum by the method shown in Figure 3. A spectrum of the cellulose reference material with a very high signal-to-noise ratio was subtracted from the tobacco residue spectrum to yield a sub-spectrum of the noncellulose components (mostly pectin and hemicellulose).

Two spectral peaks were chosen to aid the subtraction process, 105 and 89 ppm, corresponding to the C-1 and C-4 (crystalline) resonances, respectively, of cellulose I (Vanderhart and Attala, 1984). At the point where the scaling between the tobacco residue spectrum and the cellulose reference spectrum is close to correct, the exact scaling becomes somewhat subjective. The scaling of the reference cellulose spectrum was chosen such that the resulting difference spectrum had the minimum intensity possible without any dips or negative peaks at 105 or 89 ppm. If necessary, a zero-order phase correction was applied to the reference spectrum in order to maintain a level base line from 60 to 110 ppm and to afford a symmetric resonance for the noncellulose polysaccharides between 95 and 105 ppm in the difference spectrum. It should be noted that the resonance background in the vicinity of 89 ppm is small but nonzero for some tobacco types, and the line shape of the background resonances is slightly different for each type of tobacco. The first-order phase correction was kept constant for *all* the spectra.

Keeping the vertical scaling constant, the background spectrum from the deconvolution step was next subtracted from the original tobacco residue spectrum. The difference spectrum obtained from this operation consists of a sub-spectrum of only cellulose in which the resonance intensities are preserved from the original tobacco residue spectrum. Finally, the ratio of the cellulose anomeric carbon resonance in this difference spectrum to the reference d321 carbonyl resonance in the tobacco residue spectrum was compared to the standard calibration curve to determine the weight percentage of cellulose in the hot water residue. The result was multiplied by a factor of 0.697 to correct for the weight of the noncellulose components in the reference material (Bokelman et al., 1983). The percentage of cellulose in the dry tobacco was then computed on the basis of the percentage of hot water solubles in the original nonextracted sample.

Table 1. Weight Percentage of Cellulose in Bright and Burley Tobaccos Determined by ^{13}C CPMAS NMR

		% cellulose		
sample	repetitions	hot water residue	dry tobacco[a]	literature[b]
bright lamina	16	14.4 ± 0.7[c]	5.8 ± 0.1[d]	5.9
bright stems	7	27.2 ± 0.7	12.6 ± 0.3	11.7
burley lamina	5	13.0 ± 0.6	7.1 ± 0.3	6.4
burley stems	9	29.7 ± 0.8	17.5 ± 0.5	15.4

[a] Estimated from the percentage of cellulose in the residue of the hot water extracted tobacco and the percentage weight loss due to the extraction. [b] Bokelman and Ryan, 1985. [c] 95% confidence interval based on a sample *t*-distribution. [d] Since the analysis for each sample repetition was performed on the same batch of hot water extracted tobacco, the statistical uncertainties arise from the NMR measurements alone.

RESULTS AND DISCUSSION

Quantitative analyses of cellulose in bright and burley lamina and stems were performed by ^{13}C CPMAS NMR utilizing the procedure described under Materials and Methods. Measurements were made on each of at least five separately weighed samples for each tobacco type. The average weight percentages of cellulose in the hot water residue and in the nonextracted, dry samples are given in Table 1. The values obtained compare favorably with the cellulose percentages obtained by Bokelman and Ryan (1985) by chemical fractionation. The NMR results for the burley lamina and the bright and burley stems were slightly higher (8−14%) than the results obtained by chemical fractionation, suggesting a possible systematic discrepancy between the two methods. The NMR results reproduce the trend found in the Bokelman study which showed that burley lamina and stems generally contain slightly more cellulose than the corresponding bright tobacco samples.

For the most part, applications of quantitative ^{13}C CPMAS NMR have not attempted to make absolute weight measurements. Rather, the common approach has been to make comparisons between resonances in the same spectrum whereupon relative molar quantities can be determined (Himmelsbach et al., 1983; Davis et al., 1994). By including a standard such as d321 into the sample rotor with the sample, absolute molar quantities can be determined. For polymeric materials having indefinite chain lengths, however, molar quantities may not always be useful. In our application, absolute cellulose weights were estimated by correlating the resonance intensities of a set of standards (measured relative to the permanent d321 reference) with known sample weights. This approach obviates the need to make corrections for differences in cross-polarization dynamics, provided that the cross-polarization rates of the calibration set and the analyte samples are the same, and that they remain constant throughout the analysis. *It should be noted that, with our sampling arrangement, the latter consideration can potentially produce significant errors (see discussion below).* Because the sample spinning rate is kept constant with a spin controller, there is no need to consider the resonance intensity contained in the spinning sidebands of either the d321 or the analyte since regulation ensures that the sideband intensities remain constant throughout the analysis.

To make a quantitative estimate of the amount of cellulose in tobacco, it is necessary to obtain an undistorted sub-spectrum of the cellulose, free of interfering resonances from other plant components. The spectral

region containing the polysaccharide anomeric carbon (C-1) resonances between 85 and 110 ppm contains the fewest background resonances and also affords good spectral intensity and well-defined line shapes. In the analysis of wood, both graphical (Bates and Hatcher, 1992) and curve-fitting (Davis et al., 1994) methods have been employed for spectral deconvolution with reasonable success. In our method, we found that the line shapes of the reference cellulose match the cellulose resonances of tobacco very well. Thus, it is a simple matter to subtract out the cellulose spectrum to obtain the spectral background of all the noncellulose resonances including lignin, pectin, and hemicellulose resonances. Nonetheless, we chose to extract the tobacco with hot water solely to remove some of the resonance background due to water-soluble sugars in the carbohydrate region of the spectrum. It is crucial to the final results to make an accurate discrimination between the C-1 resonance and the background resonances. Having a tobacco spectrum with well-defined cellulose resonances and a high signal-to-noise facilitates the spectral subtraction process.

Throughout the analysis it is essential that the cross-polarization intensities of the analyte samples maintain the same proportionality to the reference d321 resonance peak as the standard cellulose samples. This requirement is another incentive for employing a spin rate controller, since the cross-polarization transfer time, T_{CH}, is affected by the magic angle spinning rate for many types of samples (Stejskal et al., 1977). A small change in the spinning rate can cause a significant change in cross-polarization rates, and individual resonances may be affected differently. The efficiency of cross-polarization is also influenced by the relative radio frequency power levels between the proton and carbon channels of the probe (the Hartmann−Hahn match condition). To ensure constant cross-polarization rates for all the samples, a variable-amplitude cross-polarization pulse sequence was employed. Variable cross-polarization helps to minimize errors due to mis-setting of the Hartmann−Hahn match, which can easily occur from sample to sample (Peerson et al., 1993).

The calibration chart shown in Figure 2 demonstrates that cross-polarization resonance intensities can be measured with a high degree of precision. However, we observed significant deviations from linearity whenever there were small changes in the sample and reference geometry. Changing the sample rotor end caps, for example, led to serious errors. The problem apparently arises from the radio frequency inhomogeneity inside the transmitter coil of the probe, which is exacerbated by our sample referencing method. Although variable cross-polarization preserves the match condition between proton and carbon radio frequency levels, the absolute rate of cross-polarization will vary if the radio frequency field strength changes (Stejskal et al., 1977). Because we employed only 1.1 mg of the ^{13}C-enriched d321 in the reference cell, the intensity standard is confined to a very small region of the transmitter coil. Thus, even small changes in sample position can make the relative intensities of the d321 and cellulose resonances sensitive to small variations in the radio frequency field. Potential users should be aware of this pitfall. Similar difficulties have not been reported for larger sample rotors where the sample reference is spread more uniformly over the radio frequency coil volume (Jurkiewicz and Maciel, 1994).

Table 2. Cross-Polarization Rate Constants for the Cellulose C-1 Resonance (105.5 ppm) in the Standard Cellulose Sample and Hot Water Extracted Tobacco Lamina and Stems

sample	T_{CH} (ms)	$T_{1\rho}$ (ms)	M/M_0[a]
standard	0.070	8.43	0.92
bright lamina	0.063	6.93	0.91
burley stems	0.070	7.36	0.91

[a] Computed from the relation $M/M_0 = [1/(1 - T_{CH}/T_{1\rho})](e^{-\tau/T_{1\rho}}) \times (1 - e^{-\tau/T_{CH}})$ for a contact time of $\tau = 750$ μs and the experimental values of T_{CH} and $T_{1\rho}$.

Our method also requires that the cross-polarization rates for the cellulose standard and the analyte samples be the same. To affirm this condition, the cross-polarization time constants T_{CH} and $T_{1\rho}$ for both the standard cellulose and representative samples of hot water extracted tobacco lamina and stems were measured via multiple contact time experiments (Davis et al., 1994). From the experimental values of T_{CH} and $T_{1\rho}$, the expected magnetization of the cellulose C-1 resonance at a contact time of 750 μs relative to full magnetization (M/M_0) can be calculated. The results are given in Table 2. Although small differences for T_{CH} and $T_{1\rho}$ were observed between the cellulose standard, lamina, and stems, the values for M/M_0 were found to be the same within ca. 1%. This means that the same proportionality between the resonances of the cellulose standard and the d321 intensity reference and between the analyte samples and the intensity reference is maintained.

The analysis of cellulose in other types of plants by our method should be straightforward. An important consideration may be the extent of the background interference from noncellulose resonances in the vicinity of 105.5 ppm. If the noncellulose resonances have comparable intensity to the cellulose resonances, the accuracy of the spectral deconvolution step may be adversely affected. In the case of cured tobacco, the background is due predominant to hemicellulose and pectin, which together comprise only ca. 20% of the resonance intensity at 105.5 ppm (Wooten, 1995). The interfering resonances due to simple sugars can easily be removed by hot water extraction. The analysis of green tobacco, however, can be expected to be complicated by the presence of large amounts of starch (Ryan et al., 1985). Presumably, pretreatment to remove the starch would facilitate the analysis. In general, herbaceous plants that are low both in starch and in lignin can probably be analyzed with minimal sample preparation, perhaps only with hot water extraction, as in the case of tobacco.

The extension of our method to woody plants may be more difficult. There is significant overlap among the cellulose C-1 carbon resonance and some of the aromatic resonances of guaiacyl lignin (Bates and Hatcher, 1992; Davis et al., 1994). The lignin components cannot be removed simply by extraction. Nonetheless, judging from the well-defined cellulose features in the spectra of some types of wood (Davis et al., 1994; Sosanwo et al., 1995), spectral deconvolution with an appropriate reference cellulose sample may be effective for some lignified plants as well as for herbaceous plants.

ACKNOWLEDGMENT

We gratefully acknowledge Bill Ryan, Karen Torrence, Gordon Bokelman, and Antoni Jurkiewicz for technical discussions and providing samples.

LITERATURE CITED

Bates, A. L.; Hatcher, P. G. Quantitative solid-state ^{13}C nuclear magnetic resonance spectrometric analyses of wood xylem: Effect of increasing carbohydrate content. *Org. Geochem*. **1992**, *18*, 407.

Bokelman, G. H.; Ryan, W. S., Jr.; Oakley, E. T. Fractionation of bright tobacco. *J. Agric. Food Chem*. **1983**, *31*, 897.

Bokelman, G. H.; Ryan, W. S., Jr. Analyses of bright and burley tobacco laminae and stems. *Beitr. Tabak. Forsch. Int.* **1985**, *13*, 29.

Davis, M. F.; Schroeder, H. R.; Maciel, G. E. Solid-state ^{13}C nuclear magnetic resonance studies of wood decay: I. White rot decay of Colorado blue spruce. *Holzforschung* **1994**, *48*, 99.

Hall, R. A.; Jurkiewicz, A.; Maciel, G. E. A bicyclic ketone as a solid-state ^{13}C NMR intensity reference. *Anal. Chem*. **1993**, *65*, 534.

Himmelsbach, D. S.; Barton, F. E., II; Windham, W. R. Comparison of carbohydrate, lignin, and protein ratios between grass species by cross-polarization-magic angle spinning carbon-13 nuclear magnetic resonance. *J. Agric. Food Chem*. **1983**, *31*, 401.

Irwin, P. L. The structure and degradation of intact plant cell wall matrixes by ^{13}C CPMAS NMR and related techniques. In *Nuclear Magnetic Resonance in Agriculture*; Pfeffer, P. E., Gerasimowicz, W. V., Eds.; CRC Press: Boca Raton, FL, 1989; Chapter 11.

Jurkiewicz, A.; Maciel, G. E. Spin dynamics and spin counting in the ^{13}C CP/MAS analysis of Argonne premium coals. *Fuel* **1994**, *73*, 828.

Maciel, G. E.; Haw, J. F.; Smith, D. H.; Gabrielsen, B. C.; Hatfield, G. R. Carbon-13 nuclear magnetic resonance of herbaceous plants and their components, using cross-polarization and magic-angle spinning. *J. Agric. Food Chem*. **1985**, *33*, 185.

Peerson, O. B.; Wu, X.; Kustanovich, I.; Smith, S. O. Variable-amplitude cross-polarization MAS NMR. *J. Magn. Reson. Ser. A* **1993**, *104*, 334.

Ryan, W. S., Jr.; Bokelman, G. H.; Sun, H. H.; Terrill, T. R. Influence of genetic and cultural factors on chemical and physical properties of tobacco. II. Cell wall biopolymers. *Beitr. Z. Tabak. Int.* **1985,** *13*, 88.

Schaefer, J.; Stejskal, E. O. Carbon-13 nuclear magnetic resonance of polymers spinning at the magic angle. *J. Am. Chem. Soc.* **1976**, *98*, 1031.

Sosanwo, O. A.; Fawcett, A. H.; Apperly, D. C. ^{13}C CPMAS NMR spectra of tropical hardwoods. *Polym. Int.* **1995**, *36*, 247.

Stejskal, E. O.; Schaefer, J.; Waugh, J. S. Magic-angle spinning and polarization transfer in proton-enhanced NMR. *J. Magn. Reson.* **1977**, *28*, 105.

Vanderhart, D. L.; Atalla, R. H. Studies of microstructure in native celluloses using solid-state ^{13}C NMR. *Macromolecules* **1984**, *17*, 1465.

Van Soest, P. J. *Nutritional Ecology of the Ruminant*; Cornell University Press: Ithaca, NY, 1982; Chapter 7, p 108.

Van Soest, P. J.; Robertson, J. B. *Analysis of Forages and Fibrous Foods*; Cornell University Press: Ithaca, NY, 1985.

Wooten, J. B. ^{13}C CPMAS NMR of bright and burley tobaccos. *J. Agric. Food Chem*. **1995**, *43*, 2858.

Zhang, M.; Maciel, G. E. Built-in carbon-13 intensity reference for solid-state analysis by magic-angle-spinning nuclear magnetic resonance spectrometry. *Anal. Chem*. **1989**, *61*, 2582.

Received for review September 18, 1997. Revised manuscript received January 26, 1998. Accepted January 29, 1998.

JF970813F