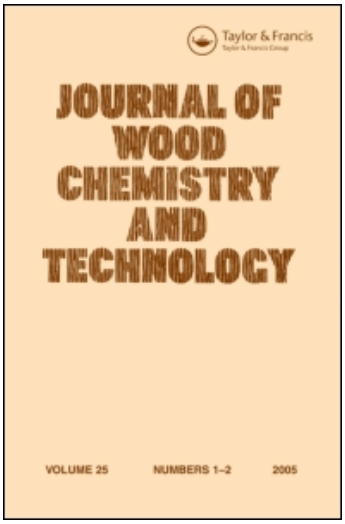

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CRYSTALLINE FORMS OF CELLULOSE IN SOFTWOODS AND HARDWOODS

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ABSTRACT

Resolution-enhanced ^{13}C CP/MAS NMR spectra were obtained for woods from 7 different tree species. Ratios of signal strengths at 90.2 and 88.6 ppm were used to estimate relative proportions of $I\alpha$ and $I\beta$ crystalline forms of cellulose. These ratios were distinctly higher for softwoods than for hardwoods. The NMR results therefore support published X-ray diffraction evidence for differences between crystalline forms of cellulose in softwoods and hardwoods. Resolution of signals at 84.0 and 84.9 ppm provided evidence for a high degree of molecular order on the surfaces of cellulose crystallites in wood.

INTRODUCTION

Native celluloses are believed to be composites of two crystalline forms, designated $I\alpha$ and $I\beta$.[1,2] A CP/MAS NMR spectrum has shown evidence for predominance of the $I\beta$ form in spruce kraft pulp,[2] but this observation does not provide a reliable indication of relative proportions of crystalline forms in wood because the $I\alpha$ form can be converted to the more stable $I\beta$ form by heat treatment.[3] The present work reports results of experiments in which sample preparation was minimized to avoid disturbing the relative proportions of crystalline forms. Several CP/MAS NMR

spectra of wood have been published, but the peaks are too broad for clear distinction of signals characteristic of either crystalline form. Spectra of various pure celluloses have been improved by digital resolution enhancement.[3-5] This technique is not as easily applied to CP/MAS NMR spectra of wood because it results in deterioration of a signal-to-noise ratio which is already depressed by dilution of the cellulose by other cell components, e.g., hemicelluloses and lignin. Signal-to-noise ratios can however be improved by lengthy data averaging. Each experiment reported here involved at least 30 hours of data averaging.

RESULTS AND DISCUSSION

Crystalline and non-crystalline components

It is possible to separate the CP/MAS NMR spectrum of a sample of wood into subspectra corresponding to crystalline and non-crystalline components.[6-8] Figure 1 illustrates results for *Pinus radiata* wood. The procedure exploits differences in proton rotating-frame relaxation time constants, $T_{1\rho}(H)$, which are longer for crystalline cellulose (Figure 1a) than for lignin and other non-crystalline components (Figure 1b). Most hemicellulose chains are disordered and therefore contribute signals to the latter subspectrum.[7] Figure 1b includes signals, labelled "h", resembling signals observed in spectra of solid hemicelluloses.[9,10] These overlap signals associated with lignin. A recent paper has discussed the possibility of a small proportion of hemicellulose chains being relatively well-ordered, with values of $T_{1\rho}(H)$ similar to those for cellulose.[8] Supporting evidence appears in Figure 1, which shows better resolution than was achieved in the earlier studies. A relatively weak signal at 102 ppm in Figure 1a is assigned to C-1 in well-ordered hemicelluloses such as glucomannans.[11] This signal is partly resolved from a signal at 105 ppm assigned to C-1 in cellulose. The relative area under the

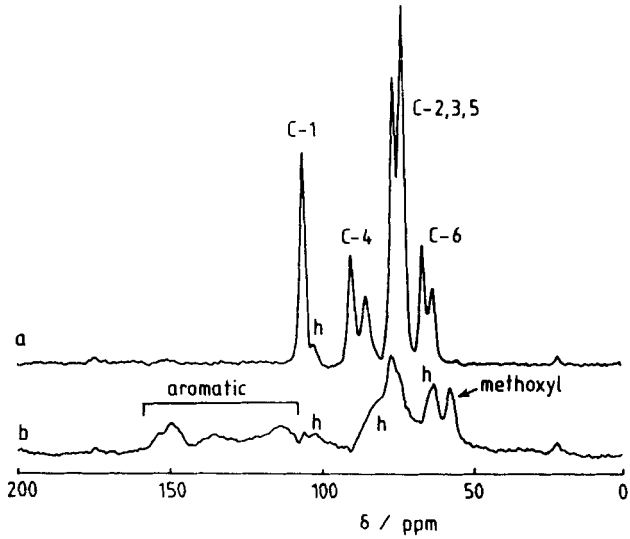

FIGURE 1 Subspectra separated from a ^{13}C CP/MAS NMR spectrum of *Pinus radiata* wood by exploiting differences in $T_{1\rho}(H)$: (a) $T_{1\rho}(H)$ = 10.0 ms, (b) $T_{1\rho}(H)$ = 4.7 ms. Hemicellulose signals are labelled "h".

peak at 102 ppm, multiplied by 6 to allow for other carbon atoms in each structural unit, provides an estimate of 4% of total carbon contained in well-ordered hemicelluloses.

The content of well-ordered hemicelluloses can also be estimated by a second procedure. Chemical analyses have shown that the sample contained 46% cellulose, 26% hemicelluloses and 28% lignin by dry weight of organic matter.[8] Typical carbon contents of components are 44% for carbohydrates and 65% for lignin,[8] so about 41% of the carbon is contained in cellulose. This is too small to account for the area contained in Figure 1a, relative to the area contained in Figure 1b. The difference of 5% of total carbon is attributed to well-ordered hemicelluloses.

The two procedures yield similar estimates for the content of well-ordered hemicelluloses, both estimates accounting for only a

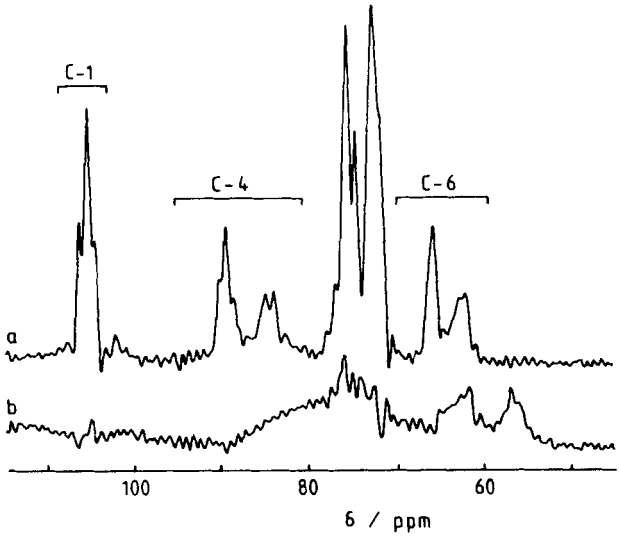

FIGURE 2 Portions of the subspectra from Figure 1 after resolution enhancement. Chemical shift ranges for C-1, C-4 and C-6 of cellulose are marked.

small fraction of the total content of hemicelluloses. In other words, NMR signals from well-ordered hemicelluloses are likely to be an order of magnitude weaker than signals from crystalline cellulose.

Resolution enhancement

Figure 2 shows portions of each subspectrum from Figure 1 after resolution enhancement. Fine structure has emerged from some signals in Figure 2a, but the broader bands in Figure 2b do not respond as well. This experiment required 5 days of signal averaging. Signal-to-noise ratios deteriorated during separation of subspectra. All further discussion of resolution-enhancement is therefore confined to spectra obtained without separation into subspectra. Relatively sharp signals are assigned to crystalline cellulose or well-ordered hemicelluloses, while broader background

TABLE 1

List of Samples.

Label	Species
	Softwoods:
PR	*Pinus radiata*
PM	*Pseudotsuga menziesii*
AA	*Agathis australis**
	Hardwoods:
CS	*Castanea sativa*
BT	*Beilschmiedia tawa**
ED	*Eucalyptus delegatensis*
QRA,QRB	*Quercus robur*

* species endemic to New Zealand

signals are assigned to lignin and disordered hemicelluloses. Chemical shift ranges assigned to C-1, C-4 and C-6 of cellulose are marked in Figure 2a. Each range will be considered, in turn, for samples listed in Table 1.

C-1 region

Figure 3 shows portions of spectra of six of the samples listed in Table 1. Three peaks are assigned to C-1 of crystalline cellulose. There were no detectable variations in chemical shifts between samples. The central signal at 105.5 ppm is assigned primarily to the Iα form, and the two outer signals at 104.4 and 106.4 ppm are assigned to the Iβ form.[2,3] Patterns of signal strengths in this region have been used to monitor variations in the Iα/Iβ ratio,[3,12] but only in samples with crystal dimensions so large that signals from surfaces can be ignored. That is not the case in the present work, as discussed below. The signal at 105.5 includes a significant component assigned to non-crystalline cellulose or chains on the surfaces of both crystal forms.[13] We cannot distinguish between variations in the Iα/Iβ ratio and

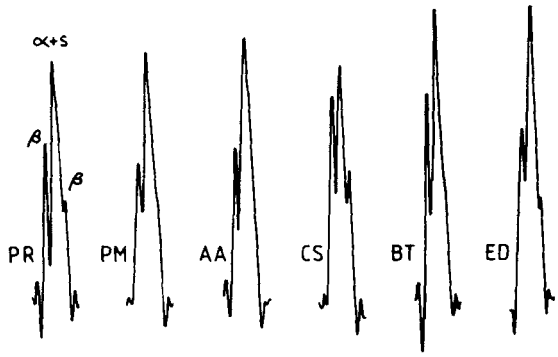

FIGURE 3 Portions of resolution-enhanced CP/MAS NMR spectra showing signals assigned to C-1 in the chemical shift range 103 to 108 ppm. Sample codes are shown in Table 1. Labels: α, β = crystal-interior chains of Iα and Iβ forms, s = surface.

variations in the surface/volume ratio without more detailed consideration of signals assigned to C-4 and C-6.

C-4 region

Figure 4 shows relevant portions of six spectra. A group of three signals at 88.6, 89.4 and 90.2 ppm is assigned primarily to C-4 in cellulose molecules fully enclosed within crystal interiors. A similar pattern has been interpreted by VanderHart and Atalla as an overlapping pair of doublets contributed by the Iβ form (88.6 and 89.4 ppm) and the Iα form (89.4 and 90.2 ppm).[2] This interpretation requires that the strength of the central component should equal the sum of the strengths of the outer components. Patterns of signal strengths observed for the three softwoods comply with this requirement, but patterns observed for the hardwoods do not. The possibility of interference from an unidentified component is discussed in more detail in a separate section.

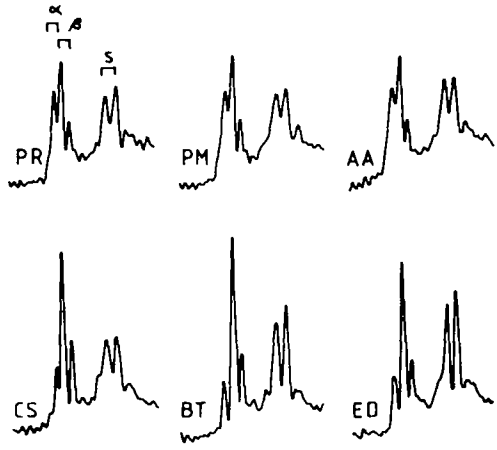

FIGURE 4 Portions of resolution-enhanced CP/MAS NMR spectra showing signals assigned to C-4 in the chemical shift range 80 to 95 ppm. Softwoods are grouped in the top row, hardwoods in the bottom row, with sample codes as shown in Table 1. Labels α, β and s as in Figure 3.

Ratios of signal strengths measured at 90.2 and 88.6 ppm indicate $I\alpha/I\beta$ ratios that are distinctly higher in softwoods than in hardwoods. This will be discussed in more detail below.

All spectra in Figure 4 show a pair of peaks of similar strength at 84.0 and 84.9 ppm superimposed on a broad background. There has been some debate over the assignment of signals in this region. Earl and VanderHart assigned a broad peak to C-4 in chains exposed on crystal surfaces.[14] Horii *et al.* suggested that the peak should be assigned primarily or exclusively to disordered cellulose.[13,15] The resolution-enhanced spectra generated in the present work are consistent with a combination of both assignments, i.e., narrow peaks assigned to well-ordered chains exposed on crystal surfaces and a broad background assigned to disordered cellulose along with hemicelluloses and lignin. The earlier assignment to disordered cellulose alone was based on

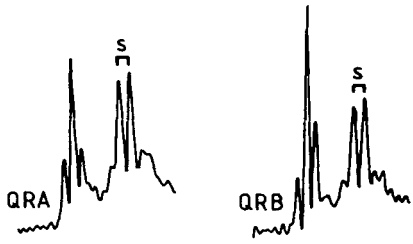

FIGURE 5 Portions of resolution-enhanced CP/MAS NMR spectra showing signals assigned to C-4 in the chemical shift range 80 to 95 ppm. The samples were taken from two different *Quercus robur* trees. Label: s = surface.

failure to resolve narrow signals.[13] Each sample of cellulose examined in that earlier work was dried under vacuum for 2 or 3 days before being packed in a rotor. Moisture has subsequently been found essential for good resolution in CP/MAS NMR studies of lignocellulosic materials.[2,16] Samples used in the present work were exceptionally moist (see Experimental).

Relative strengths of C-4 signals assigned to crystal-surface and crystal-interior cellulose showed variations between the 6 tree species represented in Figure 4. Two further samples were taken different trees of the same species, namely, *Quercus robur*. The results (Figure 5) show that the variations in patterns of NMR signals between individual trees can be as large as variations between species.

Published CP/MAS NMR spectra of crystalline mannans show signals in the range 81 to 83 ppm, assigned to C-4. Figures 4 and 5 show weak signals in this range, but the signal-to-noise ratios are not adequate to provide reliable evidence for mannose units in well-ordered chains.

C-6 region

Figure 6 shows a pair of peaks at 65.5 and 66.2 ppm, assigned to C-6 in crystal-interior cellulose. There are also peaks at

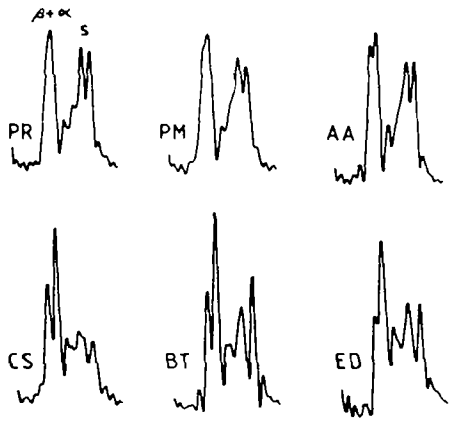

FIGURE 6 Portions of resolution-enhanced CP/MAS NMR spectra showing signals assigned to C-6 in the chemical shift range 59 to 70 ppm. Softwoods are grouped in the top row, hardwoods in the bottom row, with sample codes as shown in Table 1. Labels α, β, s as in Figure 3.

lower chemical shifts assigned to C-6 in chains exposed on crystal surfaces. Any $-CH_2OH$ groups within crystal interiors are locked into a rigid conformations by hydrogen bonds, but rotation becomes possible on crystal surfaces. Chemical experiments on native celluloses from different sources have shown that some of the surface $-CH_2OH$ groups are hydrogen bonded to oxygen atoms on the surface and some are available for interactions with chemical reagents.[17] Patterns of C-6 signals are distinctly different for softwoods and hardwoods. Signals at 65.5 ppm and 66.2 ppm are of similar strength in spectra of softwoods, but the signal at 65.5 ppm is stronger in spectra of hardwoods. Pairs of signals were recorded at 61.9 and 62.7 ppm in the spectra of softwoods, while the corresponding pairs were slightly more separated at 61.7 and 62.8 ppm in the spectra of hardwoods. The different separations suggest differences between the environments of C-6 on surfaces of softwood and hardwood cellulose crystallites.

The unidentified component

The assignments of peaks to Iα and Iβ crystalline forms of cellulose, as discussed above, do not fully account for signal strengths observed at 88.6 and 65.5 ppm in samples of hardwoods. These two peaks could perhaps be assigned to C-4 and C-6 in some other crystalline form, but the chemical shifts do not match data for known polymorphs of cellulose.[18]

An alternative explanation involves a revision of the current model describing crystal planes exposed on surfaces. Crystallites of native cellulose are generally believed to have cross-sections that are roughly rectangular.[19,20] The chains can be divided into two categories: crystal-interior chains with hydrogen bonds to two other chains, and crystal-surface chains with hydrogen bonds to just one other chain. Some authors have suggested hexagonal cross-sections.[21,22] Some of the crystal-surface chains would then be hydrogen-bonded to two adjacent chains, as in crystal interiors. It is conceivable that the chemical shifts for these chains could be similar to those for crystal-interior chains. Recent X-ray diffraction evidence seems to support rectangular cross sections,[23] but it should be remembered that this evidence is based on diffraction lineshapes interpreted without allowing for distortions caused by mixtures of the Iα and Iβ forms.

Cellulose families

Examples of cellulose I (native cellulose) isolated from different sources are commonly classified as one of two families, i.e., the family of *Valonia* and bacteria, in which the cellulose is quite rich in the Iα crystalline form, or the family of cotton and ramie in which it is the *Iβ* form that is dominant.[2,24] The NMR evidence presented in this study suggests that softwood celluloses are associated with the *Valonia*-bacteria family, while hardwoods are associated with the cotton-ramie family. The evidence is summarized in Table 2. Signal heights h(90.2) and h(88.6) were measured at 90.2 and 88.6 ppm, and the ratio was used as an indicator for relative proportions of the Iα and Iβ forms.

TABLE 2

Parameters Distinguishing Families of Native Celluloses.

Cellulose origin	NMR: h(90.2)/h(88.5)	Diffraction: "monoclinic angle"
softwoods	1.8	97.7
Valonia	1.9,1.7,1.5	97.0
bacteria	1.2,1.4	98.2
hardwoods	0.8	96.4
cotton	0.4	96.5
ramie	0.3	96.3

Ratios for non-wood celluloses are based on published results from curve-fitting experiments.[12] Two rows in Table 2 show additional ratios measured from published resolution-enhanced spectra of celluloses isolated from bacteria,[3] *Valonia ventricosa*[3,5] and *Valonia macrophysa*.[4] The two families can be distinguished by testing for ratios >1.0 or <1.0.

The NMR evidence is consistent with evidence from studies based on comparisons of X-ray diffraction patterns from 17 softwoods and 61 hardwoods.[25,26] These X-ray results were interpreted in terms of a monoclinic Iβ crystal structure with variable monoclinic angle. Mean values are shown in Table 2, along with a summary of literature values of "monoclinic angles" for non-wood celluloses collected by Okano *et al.*[25] The two families can be distinguished by testing for angles >96.8° or <96.8°.

Variation of the monoclinic angle has been attributed to lattice distortions arising from intimate contact between non-cellulosic substances and crystal surfaces.[26] This interpretation of the X-ray diffraction data is of course flawed by neglect of the Iα crystalline component. It seems more likely that the apparent variation in "monoclinic angle" simply represents the

effects of variations in proportions of crystalline forms. If the monoclinic angle was truly variable, the expected effect on the NMR spectrum would be variable chemical shifts rather than the constant chemical shifts and variable signal strengths described above.

VanderHart and Atalla found NMR evidence to support placement of a softwood kraft pulp in the cotton-ramie family.[2] That placement does not conflict with the present work. Debzi *et al.* have shown that the Iα form is metastable and can be converted to the more stable Iβ form by heating the specimen in a suitable medium.[3] Temperatures of about 260°C were required for *Valonia* and bacterial cellulose, but the authors noted that specimens of lower crystallinity are converted faster and more easily than those of higher crystallinity. Given the comparatively poor crystallinity of wood cellulose it seems reasonable to suppose that the proportions of crystalline forms could have been affected by the temperatures, typically 170°C, reached during kraft pulping. Results from NMR experiments in progress in this laboratory have provided clear evidence for an increase in the ratio h(90.2)/h(88.6) as pulping proceeds.

Michel placed a eucalypt wood pulp in the cotton-ramie family after examination of evidence from FT-IR spectra.[27] That placement is consistent with the present work, regardless of whether or not the cellulose was affected by processing.

The proposed placement of softwood cellulose in the *Valonia*-bacteria family conflicts with a published suggestion that the predominant crystalline form of cellulose depends on the geometry of the primary sites of biosynthesis.[28] The sites are arranged as linear arrays in the genus *Valonia* and as rosettes in the genus *Pinus*,[29] so different proportions of crystalline forms might have been expected.

CONCLUSION

Resolution-enhanced CP/MAS NMR spectra provide clear evidence for differences in ordering of cellulose molecules in softwoods

and hardwoods. The differences are best explained in terms of a mixture of crystalline forms, rather than a distortion of the lattice caused by intimate association with non-cellulosic substances. The results also show that the cross-sectional dimensions of cellulose crystallites can vary between samples of woods, even within one species.

EXPERIMENTAL

The *Pinus radiata* wood used for Figures 1 and 2 was slabwood Wiley-milled to 20 mesh. All other samples, including *Pinus radiata* wood used for Figures 3,4 and 6, were prepared from slices of wood about 1 mm thick sawn from air-dried timber. A sharp knife was then used to cut each slice into fragments with dimensions <1 mm. Mean weights per fragment ranged between 0.3 and 0.4 mg. Mechanical milling devices were avoided because of reported deterioration of the degree of cellulose crystallinity.[6] Dry samples were also avoided because of unacceptable NMR line widths. About 450 fragments were soaked in distilled water for at least 24 hours. Excess water was allowed to drain from the fragments and they were packed in a 7 mm diameter sapphire rotor and retained with Vespel end caps. Final water contents were about 50% by weight.

Rotors were spun at 4 kHz in a Doty Scientific magic-angle spinning probe for ^{13}C CP/MAS NMR spectroscopy at 50.3 MHz on a Varian XL-200 spectrometer. Each 7 μs 90° proton preparation pulse was followed by a 1 ms cross-polarization contact time, then 30 ms of data acquisition and a delay of 0.6 s before the sequence was repeated. Preliminary experiments showed that proton spin-lattice relaxation time constants were in the range 0.04 s to 0.22 s, so the delay was adequate for recovery of proton magnetization. The decoupler field strength was increased to values between 60 and 70 kHz for data acquisition. Preliminary experiments showed that field strengths >60 kHz were a prerequisite for success in resolving the signals discussed above.

Resolution-enhanced spectra were obtained by convoluting NMR data with a function of the form:

$$f(t) = \exp(at^n - bt^m) \quad [1]$$

If n = 1 and m = 2 this function corresponds to the Lorentzian-to-Gaussian transformation, commonly used in solid-state NMR studies of cellulose.[3-5] This function was originally designed for use in solution NMR.[30] It is not ideal for solid-state NMR spectra in which lineshapes are already better described by Gaussian rather than Lorentzian functions. A small improvement was found when the exponents were incremented to n = 2 and m = 3, so these values were used in generating Figures 2-5.

Chemical shifts were referenced relative to TMS, using the methyl signal of hexamethylbenzene as a secondary reference with a chemical shift of 17.4 ppm.

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