tionally defined analogues of norfenfluramine (also in the benzonorbornene system) have shown dramatic differences in pharmacology between the trans-anti-periplanar (Figure 10, structures **2** and **4**) and the gauche (structures **3** and **5**) conformations.[32] Furthermore, a dramatic difference between the two meta rotamers (5- vs. 7-(trifluoromethyl)-2-aminobenzonorbornene, Figure 10, **2** vs. **4**) was noted. Both the 5- and 7-trifluoromethyl rotamers approximate the global minimum found in these calculations; $\tau_1 = 270°$ and 90°. That the rigid analogues of these two equivalent minimum energy conformations show different pharmacological effects suggests a steric or electrostatic role for the $CF_3$ group rather than a conformational effect about $\tau_1$.

**Acknowledgment.** This work was supported by U.S. Public Health Service Research Grants DA 01990 and GM 22988. We thank Dr. H. J. R. Weintraub (Eli Lilly Research Laboratories) for providing us with the CAMSEQ program and for assistance in making it operational on the University of Kansas Honeywell 66/60 system; the skillful assistance of James Wiley in the latter endeavor is also gratefully acknowledged. Figure 2a was produced photographically by Charles Andrew (Graphic Arts, Center for Research, Inc., University of Kansas). Figures 2b–d and 9 were drawn by Professor Thomas Creese (Department of Mathematics, University of Kansas), who also provided extensive assistance in the handling and interpretation of the four-variable data.

**Registry No.** Fenfluramine, 458-24-2.

(33) Grunewald, G. L.; Reitz, T. J.; Hallett, A.; Rutledge, C. O.; Vollmer, S.; Archuleta, J. M., III; Ruth, J. A. *J. Med. Chem.* **1980**, *23*, 614–620.
(34) Grunewald, G. L.; Borchardt, R. T.; Rafferty, M. F.; Krass, P. *Mol. Pharmacol.* **1981**, *20*, 377–381.

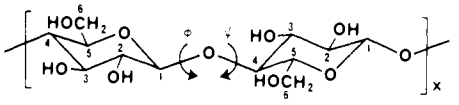
# High-Resolution $^{13}C$ CP/MAS NMR Spectra of Solid Cellulose Oligomers and the Structure of Cellulose II

**R. L. Dudley,[†] C. A. Fyfe,*[†] P. J. Stephenson,[†] Y. Deslandes,[‡] G. K. Hamer,[‡] and R. H. Marchessault*[‡]**

*Contribution from The Guelph Walterloo Centre for Graduate Work in Chemistry, Department of Chemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada, and Xerox Research Centre of Canada, Mississauga, Ontario L5L 1J9, Canada.* *Received September 28, 1982*

**Abstract:** High-resolution solid-state $^{13}C$ NMR spectra of cellulose II samples and of the complete series of solid cellulose oligomers up to cellohexaose have been obtained by using cross-polarization/magic-angle spinning (CP/MAS) techniques. Comparison of the spectra of the solid oligomers with those of cellulose II preparations indicates that the characteristic features of the cellulose II structure appear in the cellotetraose spectrum and are fully developed in those of cellopentaose and of the higher oligomers. Thus a single-crystal X-ray structure determination on one of the higher oligomers should reveal the details of the cellulose II structure that the $^{13}C$ NMR data suggest is made up of two independent chains. A comparison of the chemical shifts for samples of cellulose I, II, and IV shows characteristic displacements of C-4 and C-6 carbons for each polymorph.

Cellulose, a major constituent of plant cell walls, is the β-1,4-polymer of anhydroglucose (**1**). In the solid state it exists



1

in at least four distinct polymorphic forms, the most common of which are named cellulose I and cellulose II.[1] Cellulose I is the natural (native) polymorph and invariably occurs with a high degree of crystallinity; depending on the source,[2] the crystallinity can range from 60% to 90%. The cellulose II polymorph is obtained by mercerization or regeneration of cellulose from solution. If regeneration is carried out at high temperatures, cellulose IV can be produced. All regenerated celluloses have a much lower degree of crystallinity (e.g., ~40%) than the native form.

Several attempts at elucidating the three-dimensional structures of cellulose I and II have been made by using modern fiber diffraction methodology and sophisticated computer modeling.[3] The important conclusion of these studies was that native cellulose with its microfibrillar texture had a parallel chain polarity, while the chain arrangement in cellulose II was antiparallel. Since the needle-like microfibrils of cellulose I are thought to be nascent (i.e., the product of a simultaneous polymerization and crystallization), the occurrence of a polymorph with antiparallel chain polarity confirms that native cellulose is a metastable form that is the consequence of a biosynthetic imperative. Biopolymer specialists would welcome a physical technique that would provide evidence of chain polarity in the crystal. High-resolution $^{13}C$ cross-polarization/magic-angle spinning (CP/MAS) NMR promises to be such a technique since crystal symmetry can lead to multiple signals from carbon atoms that are chemically identical but crystallographically inequivalent.

The techniques of cross-polarization and magic-angle spinning have been developed in recent years to yield high-resolution NMR spectra of dilute nuclei (e.g., $^{13}C$) in the solid state.[4] The chemical shift values of these spectra are the isotropic values (for the solid state), which are similar to those obtained in solution and may be used for structural elucidation in terms of both molecular and crystal structures. In the particular case of cellulose, spectra have

[†] University of Guelph.
[‡] Xerox Research Centre of Canada.
(1) Bikales, N. S.; Segal, L. "Cellulose and Cellulose Derivatives—Part V, High Polymers" 2nd edn.; Wiley-Interscience: London, 1971.
(2) Warwicker, J.; Jeffries, R. *Shirley Inst. Pam.* **1966**, *No. 93*.
(3) (a) Gardner, K. H.; Blackwell, J. *Biopolymers* **1974**, *13*, 1975–2001. (b) Sarko, A.; Muggli, R. *Macromolecules* **1974**, *7*, 486–494.
(4) (a) *Philos. Trans. R. Soc. London, Ser. A* **1981**, *299*, 1452. (b) Lyerla, J. R. *Contemp. Top. Polym. Sci.* **1979**, *3*, 143–213. (c) Fleming, W. W.; Fyfe, C. A.; Kendrick, R. D.; Lyerla, J. R.; Vannie, H.; Yannoni, C. S. *ACS Symp. Ser.* **1980**, *No. 142*, 193–217. (d) Yannoni, C. S. *Acc. Chem. Res.*, in press. (e) Fyfe, C. A.; Lyerla, J. R.; Yannoni, C. S. *Ibid.*, in press. (f) Wasylishen, R. E.; Fyfe, C. A. *Annu. Rep. NMR Spectrosc.*, in press.

0002-7863/83/1505-2469$01.50/0 © 1983 American Chemical Society

Table I. Characterization of Cellulose Oligomers. $^{13}C$ Solution NMR Chemical Shifts[a] (ppm Relative to External $Me_4Si$) and Melting Point Ranges (°C)

| oligomer | residue | chemical shifts C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | melting point range |
|---|---|---|---|---|---|---|---|---|
| cellotriose | reducing end α | 92.7 | 72.1 | 72.1 | 79.5 | 71.0 | 60.9 | 207–211 |
| | reducing end β | 96.6 | 75.1 | 74.7 | 79.3 | 75.7 | 60.9 | |
| | internal residues | 103.2 | 73.8 | 74.8 | 79.3 | 75.7 | 60.8 | |
| | nonreducing end | 103.4 | 74.0 | 76.4 | 70.3 | 76.8 | 61.4 | |
| cellotetraose | reducing end α | 92.6 | 72.1 | 72.1 | 79.5 | 72.0 | 60.9 | 250–253 |
| | reducing end β | 96.6 | 75.1 | 75.0 | 79.4 | 75.6 | 60.9 | |
| | internal residues | 103.2 | 73.7 | 75.0 | 79.3 | 75.6 | 60.9 | |
| | nonreducing end | 103.4 | 74.0 | 76.3 | 70.3 | 76.8 | 61.4 | |
| cellopentaose | reducing end α | 92.6 | | | 79.3 | | 60.7 | 265–268 |
| | reducing end β | 96.6 | 75.1 | 74.8 | 79.2 | 75.7 | 60.7 | |
| | internal residues | 103.2 | 73.8 | 74.8 | 79.1 | 75.7 | 60.7 | |
| | nonreducing end | 103.4 | 74.0 | 76.3 | 70.3 | 76.8 | 61.4 | |
| cellohexaose | reducing end α | | 72.1 | 72.1 | | | | 275–278 |
| | reducing end β | 96.6 | | 74.8 | 79.3 | 75.6 | 60.7 | |
| | internal residues | 103.2 | 73.7 | 74.8 | 79.0 | 75.6 | 60.7 | |
| | nonreducing end | 103.4 | 73.9 | 76.3 | 70.3 | 76.8 | 61.4 | |

[a] Measured in $D_2O$ solution at 100.6 MHz.

Table II. $^{13}C$ Chemical Shifts (ppm Relative to $Me_4Si$) from the 22.6-MHz CP/MAS Spectra of Solid Cellulose Polymorphs

| | C-1 | C-2, C-3, C-5 | C-4 | C-6 |
|---|---|---|---|---|
| cellulose I | 105.0 | 74.1, 72.0 | 90.0 | 67.0 |
| cellulose II | 107.9, 106.2 | 77.7, 76.0, 73.4 | 88.9 | 64.0 |
| cellulose IV | 103.3 | 72.3 | 82.2 | 62.3 |

been reported by Earl and VanderHart[5] and by Atalla and co-workers[6] that clearly indicate the utility of the technique. Both groups reported characteristic splittings for C-1 in these spectra due to solid-state effects. In their study of cellulose II, Atalla and co-workers[6] interpreted the existence of two peaks for C-1 in terms of a nonequivalence of alternate glycosidic linkages (i.e., $\phi, \psi$ dihedral angle pairs, see **1**) along the molecular chain. This interpretation requires that dimeric anhydrocellobiose rather than anhydroglucose be considered the basic repeat unit of the cellulose II crystalline structure. The multiplicity of the C-1 peak in spectra of the cellulose I polymorph is less clear; more recent measurements by Earl and VanderHart[7] indicate a number of partially resolved peaks of different intensities, suggesting a complex unit cell for this polymorph.

The purpose of this work was to carry out a systematic examination of the solid-state $^{13}C$ CP/MAS spectra of a complete series of cellulose oligomers together with those of higher molecular weight cellulose II samples, firstly, to determine as accurately as possible those features of the cellulose II spectra that are characteristic of this polymorph and, secondly, to ascertain the point at which these characteristic features appeared in the spectra as the oligomer chain length was increased, thus establishing a link between the cellulose II structure and that of a *crystallizable* cellooligosaccharide that might be directly and accurately determined by single-crystal X-ray diffraction studies.

## Experimental Section

The samples of cellulose II examined in this study were from several different sources: microcrystalline rayon, tire-cord rayon, and mercerized ramie fiber. A highly crystalline, low-DP cellulose II sample was prepared according to Atalla[6] by dissolving Avicel cellulose powder in 85% $H_3PO_4$ at room temperature and precipitating the filtered acid solution in a 10-fold excess of water after 11 days. A special commercial rayon provided a regenerated sample that was 100% cellulose IV. Data for the cellulose I polymorph was obtained from a purified sample of *Valonia ventricosa*. All samples were hydrolysed in boiling 2.5 N HCl for 15 min and washed to neutrality before characterization.

The cellulose oligomers were prepared according to the methods of Whistler[8] by using direct acid hydrolysis of Whatman cellulose powder followed by chromatographic separation on carbon–Celite columns using ethanol–water eluants. The samples were then freeze-dried over 24 h. Improvements in sample crystallinity were achieved by adding a drop of water to the material or by redissolving and freeze-drying over a longer period. An independently prepared series of oligomers was also kindly loaned by the Shirley Institute.

High-resolution $^{13}C$ solution NMR spectra were recorded at 100.6 MHz on a Bruker WH-400 spectrometer. The chemical shifts were measured relative to a small amount of dioxane added to the $D_2O$ solution and converted to $Me_4Si$ as reference by adding 67.4 ppm.[9] The CP/MAS $^{13}C$ spectra were obtained at 22.6 MHz on a Bruker CXP-100 spectrometer using a home-built probe and room-temperature spinning apparatus. Spinning rates of ~3 kHz and spin-locking and decoupling fields of ~50 kHz were used. Spectra were referenced to external HMDS (by substitution) and converted to the $Me_4Si$ scale by adding 2.1 ppm to the measured chemical shifts.

Powder X-ray measurements were made on a Philips Model 1050/80 automated X-ray powder diffractometer equipped with a graphite monochromator and proportional counter. Copper Kα radiation was used ($\lambda$ = 0.1541 nm), and all measurements were made at ambient temperatures. Because of the limited amounts of sample, the cellulose oligomers were characterized via film measurements recorded in a Debye–Scherrer camera. The observed *d* spacings for the oligomers were in reasonable agreement with published data.[10]

## Results and Discussion

**Sample Characterization.** The cellulose oligomers were characterized by their high-resolution $^{13}C$ solution NMR spectra, melting points (Table I), and powder X-ray diffraction spacings. The $^{13}C$ NMR spectra recorded at 100.6 MHz (Figure 1) have sufficient resolution to identify clearly and assign the peaks due to a majority of the carbon atoms in the oligosaccharide structure (**2**). The spectra are in close agreement with those reported by Gast, Atalla, and McKelvey[9] and Heyraud and co-workers,[11] and the ratio of peak intensities of the reducing-end unit to the internal residues is consistent with the assigned dp.

(5) Earl, W. L.; VanderHart, D. L. *J. Am. Chem. Soc.* **1980**, *102*, 3251–3252.
(6) Atalla, R. H.; Gast, J. C.; Sindorf, D. W.; Bartuska, V. J.; Maciel, G. E. *J. Am. Chem. Soc.* **1980**, *102*, 3249–3251.
(7) Earl, W. L.; VanderHart, D. L. *Macromolecules* **1981**, *14*, 570–574.
(8) (a) Whistler, R. "Methods in Carbohydrate Chemistry"; Academic Press: New York, 1963; Vol. 1. (b) Miller, G. L.; Blum, R. *Archiv. Biochem. Biophys.* **1960**, *91*, 21–26.
(9) Gast, J. C.; Atalla, R. H.; McKelvey, R. D. *Carbohydr. Res.* **1980**, *84*, 137–146.
(10) Williams, D. G. *J. Poly. Sci., Polym. Phys. Ed.* **1970**, *8*, 637–642.
(11) Heyraud, A.; Rinaudo, M.; Vignon, M.; Vincendon, M. *Biopolymers* **1979**, *18*, 167–185.

Table III. $^{13}C$ Chemical Shifts (ppm Relative to $Me_4Si$) from the 22.6-MHz CP/MAS Spectra of Solid Cellulose Oligomers and Cellulose II

| | C-1 | C-1′[a] | C-2, C-3, C-5 | C-4 | C-4″[b] | C-6 |
|---|---|---|---|---|---|---|
| cellobiose | 104.6 | 97.5 | 79.7, 78.7, 76.5 | 85.0 | 72.4 | 63.8 |
| cellotriose | 107.4 | 96.2 | 78.9, 76.7 | 85.4 | 72.4 | 64.2 |
| cellotetraose | 108.3, 106.5 | 97.5 | 77.6, 75.9, 74.1 | 89.7 | | 64.3 |
| cellopentaose | 108.7, 106.5 | 97.9 | 77.6, 75.9, 73.7 | 89.7 | | 63.8 |
| cellohexaose | 108.3, 105.8 | | 77.6, 75.5, 73.8 | 89.3 | | 64.3 |
| cellulose II[c] | 107.9, 106.2 | | 77.7, 76.0, 73.4 | 88.9 | | 64.0 |

[a] Reducing-end residue (see 2). [b] Nonreducing-end residue (see 2). [c] $H_3PO_4$-hydrolyzed cotton.

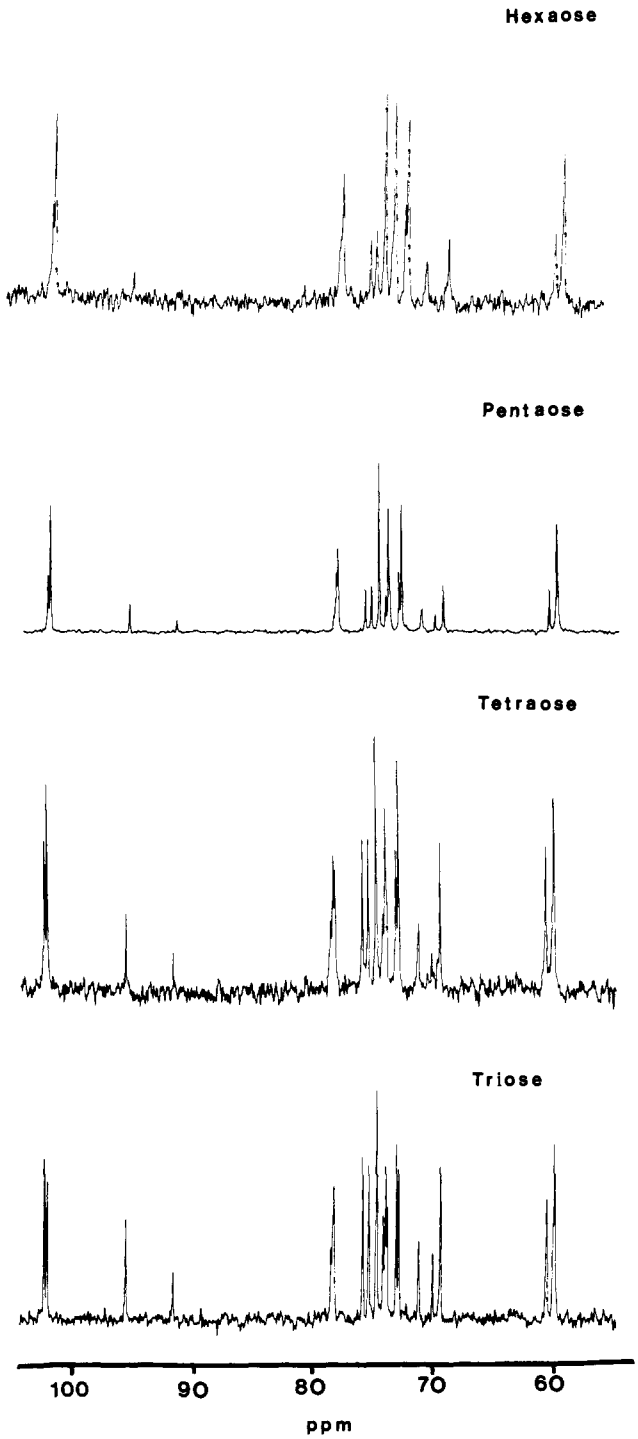



Figure 1. High-resolution $^{13}C$ NMR spectra of cellulose oligomers recorded in $D_2O$ solution at 100.6 MHz. Chemical shifts are in parts per million relative to $Me_4Si$.

The various cellulose samples were shown by X-ray diffraction to be pure polymorphs. Both the cellulose I and $H_3PO_4$-hydrolysed

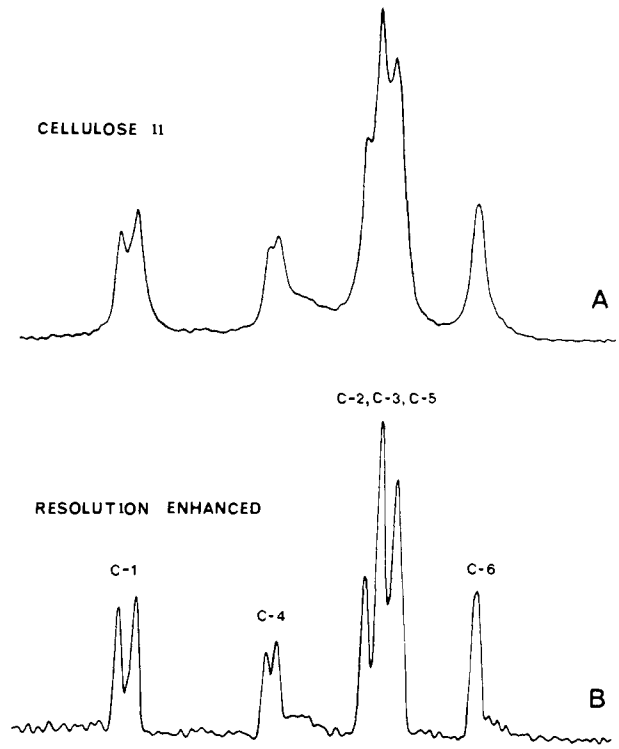
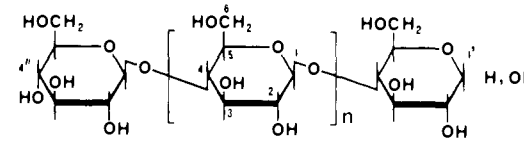



Figure 2. CP/MAS $^{13}C$ NMR spectrum of cellulose II (microcrystalline rayon) recorded at 22.6 MHz by using a 1-ms contact time and a 1-s delay (A) and the same spectral data resolution enhanced by using a −25-Hz Gaussian multiplication (B). Chemical shifts are in parts per million relative to $Me_4Si$.




2

cellulose II samples had an estimated degree of crystallinity of 90% or more. The crystallinity of the cellulose II microcrystalline rayon and cellulose IV polymorphs was ~40–50%, while that of the tire cord and mercerized ramie samples was slightly higher.

**$^{13}C$ CP/MAS Spectra of Cellulose II Samples.** The characteristic features of the cellulose II spectrum reported by Atalla and co-workers[6] have been confirmed and examined in more detail. In a variety of samples ranging from microcrystalline rayon to mercerized ramie fibers, C-1 always appears as a doublet, i.e., two peaks of equal intensity (Figure 2A). A similar but much smaller splitting is observed for C-4 in very crystalline samples and may be observed in most samples if resolution enhancement is used (Figure 2B). The intensity ratio of the two peaks in both C-1 and C-4 resonances is always ~1:1 over a wide range of cross-polarization contact times (1–5 ms) and delay times (1–10 s). This insensitivity to experimental conditions shows clearly that the peak doubling arises from differences in the solid-state environment of these carbon atoms due to the symmetry of the unit cell rather than arising from different regions of the sample (e.g.,

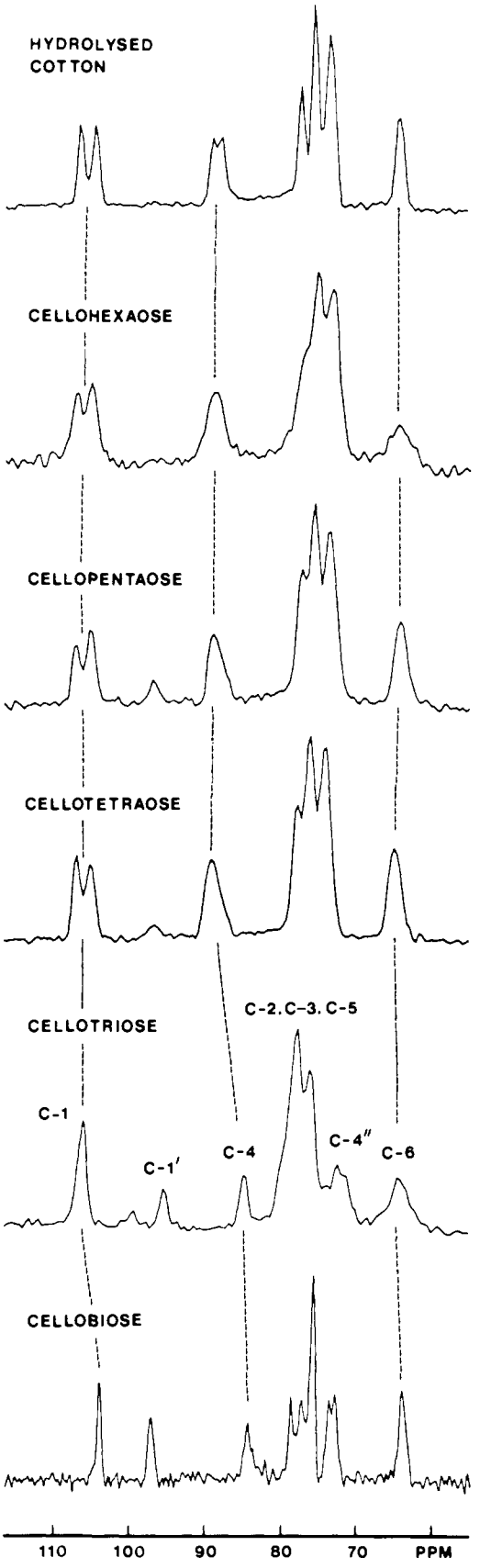



**Figure 3.** CP/MAS $^{13}C$ NMR spectra of cellulose oligomers and of a low-dp cellulose II sample obtained by $H_3PO_4$ hydrolysis of cotton (top). All spectra were recorded at 22.6 MHz by using a 1-ms contact time and a 1-s delay. Chemical shifts are in parts per million relative to $Me_4Si$.

bulk/surface, crystalline/amorphous, etc.). In the latter case, discrimination between the peaks of the doublet should be observed due to expected differences in relaxation behaviour.

The carbon chemical shifts themselves are characteristic of each polymorph (Table II). The position of the C-4 resonance clearly differentiates between celluloses I and II and cellulose IV. In addition there is a distinct shift in the position of the peak due to C-6 on conversion of cellulose I to cellulose II.

It may thus be concluded that splitting of the C-1 and C-4 resonances and the chemical shifts of C-4 and C-6 are diagnostic of the solid-state structure characterized as cellulose II and can therefore form a basis for comparison with the solid-state $^{13}C$ spectra of the cellulose oligomers.

**CP/MAS $^{13}C$ Spectra of Cellulose Oligomers.** The CP/MAS $^{13}C$ NMR spectra of a series of cellulose oligosaccharides (cellobiose to cellohexaose) are shown in Figure 3 together with the spectrum of a highly crystalline cellulose II sample (top spectrum); the appropriate chemical shifts are summarized in Table III. The resonances are identified in the triose spectrum with reference again to the basic oligosaccharide structure (**2**).

Although the cross-polarization sequence, by its nature, does not yield completely reliable quantitative spectral intensities, the same contact and delay times were used for all samples (which are themselves structurally similar) and consequently it is felt that general comparisons between oligomer spectra are valid. As can be seen in Figure 3, the relative intensities of the reducing-end C-1 peaks (~97 ppm) generally decrease as expected with increasing chain length. Further, the chemical shifts of the carbons of the internal residues converge to those of cellulose II (Table III). Most importantly, the shift of C-4 converges to exactly that in cellulose II and the doublet splitting of C-1 becomes clearly observable. The transformation of the oligosaccharide spectra to the spectrum characteristic of cellulose II is definitely established for cellotetraose and complete for cellopentaose and higher oligomers. Thus the CP/MAS spectra form a bridge between cellulose II itself and crystallizable model oligomer systems. Although the NMR spectra alone cannot be used to unambiguously assign a structure, as there may be a number of possible reasons why C-1 and C-4 should lie at inequivalent lattice positions, it can be inferred with some certainty that a full, single-crystal X-ray structure determination of one of these oligosaccharides will in fact yield the cellulose II structure without ambiguity. In this regard, it may be noted that an X-ray structure of cellotetraose has been published;[12] however, the results are considered inconclusive,[13] due to the small size of the crystal that yielded only limited diffraction data.

At this time, the close similarity of the oligosaccharide and cellulose II spectra suggests an explanation different from that proposed by Atalla and co-workers[6] to account for the doublet structure of the C-1 and C-4 resonances of cellulose II. In cellotetraose, where there are only three interglycosidic linkages, alternation of $\phi$, $\psi$ dihedral angle pairs should lead to a 2:1 intensity ratio for the C-1 doublet, rather than the ~1:1 ratio observed. Thus, a structure for cellotetraose (and by extension, cellulose II) that invokes two independent chains in the unit cell seems more plausible.

**Acknowledgment.** We acknowledge the kind loan of a series of cellulose oligomers by Dr. R. Jeffries of the Shirley Institute, Didsbury, Manchester, England. We also acknowledge the financial assistance of the Natural Sciences and Engineering Research Council of Canada in the form of an Operating Grant (C.A.F.). The high-resolution $^{13}C$ NMR spectra were obtained by using the facilities of the South Western Ontario NMR Centre. The X-ray powder diffraction data were provided by Dr. T. L. Bluhm of Xerox Research Centre of Canada.

**Registry No.** Cellulose, 9004-34-6; cellotriose, 33404-34-1; cellotetraose, 38819-01-1; cellopentaose, 2240-27-9; cellohexaose, 2478-35-5.

(12) Poppleton, B. J.; Matieson, A. M. *Nature (London)* **1968**, *219*, 1046–1048.
(13) Strahs, G. *Adv. Carbohydr. Chem. Biochem.* **1970**, *25*.