this points to the fact that, while relative rates of (1) and (3) do not change greatly on substituting bromine for chlorine, reaction 1 is slightly favored by the presence of bromine in the 4-position.

If the above mechanism is correct, significant differences in structure should be observed between polymers produced by decomposition of copper complexes and polymers produced with classical free radical initiators. Some preliminary studies of poly(dibromophenylene oxide) show this to be the case. The results of that study will be published elsewhere.

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Observations by High-Resolution Carbon-13 Nuclear Magnetic Resonance of Cellulose I Related to Morphology and Crystal Structure

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ABSTRACT: High-resolution solid-phase ^{13}C NMR spectra were obtained on celluloses from cotton linters, ramie, hydrocellulose prepared from cotton linters, *Acetobacter xylinum*, and *Valonia ventricosa*. The spectra from cotton, ramie, and hydrocellulose are virtually identical. Peak positions for all peaks are the same for the cotton, *Acetobacter*, and *Valonia* celluloses although there are differences in resolution and in the intensity of two broad resonances attributed to C-4 and C-6. These differences are ascribed to differences in the morphology of the samples. The higher resolution obtained in the NMR spectra of *Acetobacter* and *Valonia* celluloses plus the improved resolution obtained at an applied field of 4.7 T relative to 1.4 T shows definite multiplicity in the resonances assigned to C-1 and C-4. It is argued that this multiplicity is higher than two and reflects the fact that there must be more than two anhydroglucose residues per unit cell in the crystal structure of cellulose I.

Introduction

Although there is no concensus regarding the structure and morphology of cellulose I, the majority of recent X-ray diffraction and computer modeling studies support a proposed structure with parallel cellulose chains.[1-5] Recent evidence for native cellulose is overwhelmingly in favor of elementary fibrils, 3.5 × 3.5 nm in cross section, which may aggregate into larger microfibrils.[6-13] The exact size and structure of the microfibrils found in nature is somewhat dependent upon the biological source of the cellulose and the electron microscopic techniques used to observe the microfibrils. Cotton microfibrils apparently range in size from 10 to 50 nm with an average around 25 nm, bacterial cellulose (*Acetobacter xylinum*) occurs in ribbonlike structures with an average crystallite size of 7 nm, and algal cellulose (*Valonia ventricosa*) is found in highly uniform coherent ribbons 10 × 20 nm in cross section.[11] Little detailed crystallographic or morphological information exists regarding bacterial cellulose but it is thought that one of the major differences between different celluloses lies in the perfection of the packing of elementary fibrils into microfibrils, with *Valonia* cellulose representing a high degree of regularity and order, cotton much less regularity and order,[11] and *Acetobacter* an intermediate degree of lateral order.[12] This regularity of *Valonia* cellulose is underscored by the fact that X-ray fiber diffraction is usually done on it.[1-4]

The solid-state NMR spectrum of cellulose has been reported in earlier publications.[14,15] In our earlier work we concluded that the various resonance regions showed rather uniform chain mobilities; thus we suggested that the morphology of the sample might affect the appearance of the NMR spectrum.[15] The work reported here supports this conclusion by comparing the solid-state NMR spectra of several morphologically different samples of cellulose I.

Experimental Section

Several preparations of cellulose will be discussed below. Cotton linters which were washed, bleached, and double cut (chemical cotton) were obtained from Hercules Chemical Co.[16] A sample of hydrocellulose was prepared from the chemical cotton by the methods of Rowland et al.,[17] which involved boiling the sample for 40 min in 2.5 M HCl. A sample of ramie was obtained from Fischer, Dottikon, Switzerland.[18] Algal cellulose was obtained from the cell walls of 10–15 *Valonia ventricosa* cells which had been purified and dried.[19]

Bacterial cellulose was obtained by growing *Acetobacter xylinum* on a liquid glucose medium in static culture.[20] The pellicle of cellulose and bacteria which floated on the surface of the media after 4 days of culture was washed in distilled water and then boiled in 3% (w/v) NaOH for 10 min, followed by washing in distilled water and drying by manual pressure between absorbent towelling.

The NMR spectra reported were obtained on two spectrometers. One is a home-built spectrometer operating at a ^{13}C resonance frequency of 15.08 MHz (1.4 T) and using an iron core magnet. The second is a Bruker CXP-200[18] with a superconducting solenoid magnet operating at a ^{13}C frequency of 50.03 MHz (4.7 T). The spectra discussed were all obtained by cross polarization from the spin-locked protons[21] with the Hartmann–Hahn[22] matching condition met followed by high-power proton decoupling. For the high-field (50 MHz) data, amplitudes of the radio-frequency fields expressed in frequency units, $\gamma B_1/2\pi$, for both the carbons and the protons were approximately 40 kHz; at low field (15 MHz) the radio-frequency amplitudes were 56 kHz. Spectra were obtained using magic-angle sample spinning.[23,24] At 50 MHz the samples were hand packed into deuterated poly(methyl methacrylate) (PMMA) rotors, which were spun at approximately 3200 Hz. For low-field spectra the samples were packed in poly(chlorotrifluoroethylene) rotors and spun at ca. 2200 Hz. Both the algal and bacterial samples were quite small in volume so the excess volume in the rotor was filled with poly(tetrafluoroethylene) spacers, which produce no signal in the experiment as performed. A small piece of linear polyethylene was inserted in all rotors to give a chemical shift reference peak at 33.6 ppm for the crystalline polyethylene peak.[25,26] The width of this reference peak was also used as an indicator of the accuracy of the magic-angle setting and of applied static field homogeneity and stability. The polyethylene resonance consists of two NMR peaks, a narrow crystalline resonance peak at 33.6 ppm and a broad shoulder due to noncrystalline carbons at higher field.[25] The CXP-200 uses quadrature phase detection while the home-built spectrometer has only a single-phase detection. In all spectra, temperature-inversion techniques were used to minimize base line noise and roll.[27]

Results and Discussion

Crystallinity. In any study of the physical and chemical behavior of various preparations of cellulose, the question of crystallinity effects must be addressed. In earlier studies of the solid-state NMR of microcrystalline cellulose, transverse relaxation times for the ^{13}C NMR signals were measured.[15] These measurements indicated a rather uniform molecular mobility for all anhydroglucose units even though nonuniformity was implied by the multiplicity of spectral features. Therefore, if there exist noncrystalline regions of cellulose I analogous to those in synthetic polymers,[25] then molecules in those regions are most likely hydrogen bonded to an extent that renders them nearly as immobile as crystalline regions. Thus crystallinity in cellulose I implies more packing regularity rather than less mobility.

Figure 1 addresses the question of crystallinity in a different manner. Three spectra of samples of cellulose I derived from cotton and ramie are shown. Cotton, ramie, and hydrocellulose are all highly crystalline preparations, with crystallinities as measured by X-ray methods between 80 and 96%. The reported crystallinities for various preparations of cellulose vary with measurement technique.[28] In fact, the definition of crystallinity in cellulose is not entirely straightforward since one must consider the degree of order along the cellulose chain as well as the degree of lateral order or the chain packing. X-ray diffractograms and solvent absorption studies are common techniques for measuring crystallinity in cellulose.[28,29] Since these techniques depend on different physical phenomena, the absolute crystallinities obtained may differ. Nuclear magnetic relaxation measurements differentiate crystalline from noncrystalline regions when molecular mobilities are different. The spectra in this paper, however, suggest a definition of crystallinity based on structural uniformity. In general, each technique can often be used to rank the crystallinity of a series of similar samples irrespective of the absolute numbers obtained. For the samples whose spectra are presented in Figure 1, cotton is the least crystalline followed by ramie and then hydrocellulose.[30] The spectra in Figure 1 are virtually identical, with only minor differences in resolution due to instrumental effects. The peaks assigned to C-4,[14,15] a narrow resonance at 90 ppm and a broader one at 86 ppm, do not change in relative intensity among the spectra in Figure 1. More importantly, a measurement of the intensities of the broad and narrow C-4 resonances yields 55–60% narrow component, which is far smaller than most other estimates of crystallinity for these celluloses. Our current understanding of line widths in ^{13}C NMR of solids leads to the expectation that crystalline regions of polymers will give narrow resonances while noncrystalline or paracrystalline regions will give broader resonances.[31] The broadness in the resonances of noncrystalline regions is due to a greater disorder of molecular packing, a distribution of molecular conformations, and/or higher molecular mobility in these regions. Since we have previously demonstrated that the NMR spectrum of cellulose I does not reflect motional differences, the broad features of C-4 and C-6 in Figure 1 are likely to reflect disorder in packing, hydrogen bonding, or conformational differences within a chain or between chains. Similarly the ratio of the narrow peak at 66 ppm and its upfield shoulder <64 ppm (both assigned to C-6[14,15]) does not change with presumed change in crystallinity. The overlap of these two C-6 peaks precludes an accurate measurement of intensities but the narrow peak is on the order of 60% as was seen for C-4.

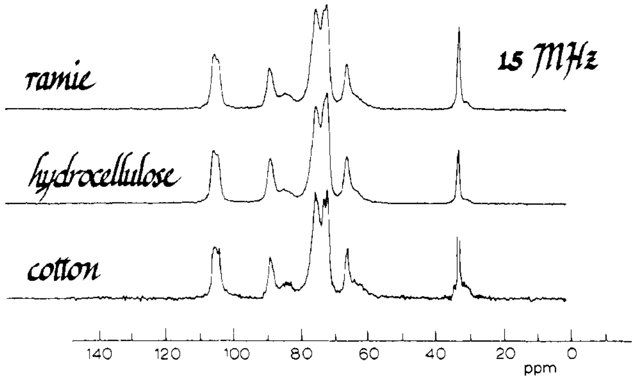

Figure 1. CPMASS spectra of cotton, hydrocellulose, and ramie obtained at 1.4 T. The high-field peak at 33.6 ppm is due to a piece of linear polyethylene used as a chemical shift reference. The ramie spectrum is the result of 12 562 scans with a 6-ms cross polarization time and 4.2 s between successive scans. The hydrocellulose spectrum was obtained from 10 890 scans with a 2-ms contact time and 5.6 s between scans. Both spectra result from 1K points of free induction decay (FID) followed by 7K of zero filling to improve digital resolution. The spectrum of cotton is the result of 5700 scans with a contact time of 1 ms and 4.5-s repetition time. It is the transform of 2K of data in the FID followed by 6K of zero filling. The FID's for the above spectra were taken with a 50-μs dwell time and a 50-μs delay between the fall of the cross polarization pulse and the start of data acquisition. No digital filtering was applied prior to Fourier transformation.

One further feature of note in Figure 1 is the fact that, aside from differences in resolution, the region containing C-2, C-3, and C-5 (70–80 ppm) is identical for the three samples.

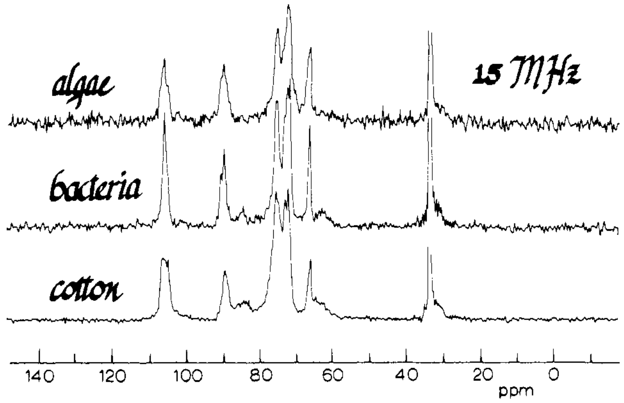

Figure 2. CPMASS spectra of cellulose I derived from cotton, bacteria, and algae obtained at an applied field of 1.4 T. The high-field peak at 33.6 ppm is due to a piece of linear polyethylene used as a shift reference. The algae spectrum is the result of 12020 scans, the bacterial cellulose spectrum is from 12300 scans, and the cotton spectrum is from 5700 scans. All spectra were taken with a 1-ms cross polarization contact time, 4.5 s between sequential scans, with 50-μs dwell per data point, and 50-μs delay between the end of cross polarization and the onset of data acquisition. The spectra are all the result of 2K of data in the FID followed by 6K of zero filling with no exponential filtering prior to Fourier transformation.

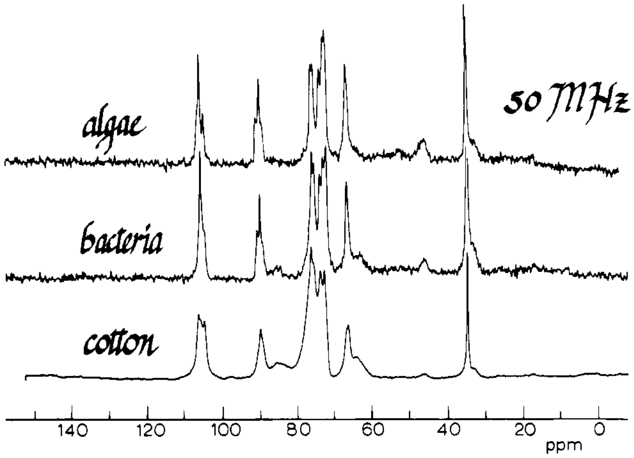

Figure 3. CPMASS spectra of cellulose I derived from cotton, bacteria, and algae obtained at an external field of 4.7 T. The samples are the same ones as in Figure 2. The peak at 33.6 ppm is due to a piece of linear polyethylene in the sample for a chemical shift reference. The number of scans per spectrum was 6912 for algae, 3072 for the bacteria, and 8192 for cotton. All spectra were taken with a 1-ms contact time, 6–10-s repetition time, 50-μs dwell per point, and 50-μs delay between the cross polarization and the start of data acquisition. There were 1000 data points in the FID's followed by zero filling to a total of 8K for improved digital resolution. There was no digital filtering applied prior to Fourier transformation. The bump in the base line at 46.8 ppm is due to the deuterated PMMA rotor, which contains sufficient protons to give a small cross polarization signal. There are no PMMA resonances in the region between 60 and 120 ppm to interfere with the cellulose spectra.

Morphology. In order to evaluate experimentally the question of order–disorder, we obtained samples of cellulose I from different biological sources. Figure 2 shows the ^{13}C cross polarization–magic-angle sample spinning (CPMASS) spectra of cellulose I obtained from cotton, *Acetobacter xylinum*, and *Valonia ventricosa* obtained at an applied magnetic field of 1.4 T. Figure 3 contains the ^{13}C CPMASS spectra of the same samples obtained at a higher applied field (B_0 = 4.7 T). A discussion of the differences in resolution as a function of B_0 is deferred until later.

It should be pointed out that the samples whose spectra are shown in Figures 2 and 3 were selected for differences in morphology but the distinction between morphology and crystallinity is difficult to make. The microfibrils of *Valonia* show a greater degree of lateral order than those of cotton but this morphological difference implies higher crystallinity in *Valonia* than in cotton cellulose. In fact, the range of crystallinities represented by the samples in Figures 2 and 3 is greater than in Figure 1.

There are several striking differences between the spectra in Figure 3. The most outstanding is the disappearance of the broad peaks at 64 and 86 ppm in going from cotton to algal cellulose. Approximate integrals of the broad and narrow peaks due to C-4 in the cotton spectrum yield 40 ± 10% broad component. The improved resolution for the C-6 peak at high field allows an estimation of 47 ± 10% broad shoulder for the 50-MHz cotton spectrum. These numbers agree with the results reported for the 15-MHz spectra in Figure 1. An examination of the proposed structure for native cellulose elementary fibrils[10,11] reveals that 49% of the glucose units are located on the surface of the elementary fibril. The logical assignment for the peaks in the cotton spectrum is that the narrow C-4 and C-6 peaks (at 66 and 90 ppm) are due to the 51% of anhydroglucoses buried in the elementary fibril and the broad resonances are due to the 49% of glucoses on the surface of the elementary fibril. Those surface monomers will not be constrained by packing and hydrogen bonding in as regular an array as anhydroglucoses in the center of the elementary fibril and thus they will exhibit a dispersion of chemical shifts and a possible shift in peak position. Further argument in support of this is given by the fact that the measured T_2 for the shoulder on C-6 is considerably shorter than the T_2 for all other resonances,[15] which indicates that C-6 atoms represented by the shoulder are more mobile. If these carbons are on the outside of the elementary fibril, then they are no longer hydrogen bonded into a rigid position and may rotate about the C-5–C-6 bond, giving a shorter T_2.

The proposed crystal structure for native cellulose includes a twofold screw axis proceeding down the cellulose chain.[1-4] This implies that if cellulose is in the extended-chain configuration in the elementary fibril as proposed, then each alternate C-6 or C-4 in a surface monomer would point into the center of the elementary fibril. The expectation would be of two different types of surface monomers and one type of central monomer in ratios of approximately 1:1:2. Thus C-6 should give three peaks with 1:1:2 ratios. Such is not the case in the ^{13}C NMR spectrum, presumably because the significant magnetic inequivalencies are between the inside and the outside of the elementary fibril. Differences between surface anhydroglucose units, such as the 180° rotation, will only contribute to the width of the resonance line observed.

It is also unclear why C-4 should exhibit a broadened and shifted resonance in cotton cellulose while C-1 does not. Since C-1 and C-4 are equally involved in the glycosidic bond, one might expect similar spectral changes in the two peaks. The peak shape for C-6, on the other hand, is qualitatively what one would expect if C-6's on the surface of an elementary fibril would assume various rotational conformations while those in the center are required to remain in the tg position proposed. Chemical shift dependences on conformation are well-known and could largely explain the appearance of the shoulder.[25,32]

The interpretation of the broad resonances in cotton cellulose being due to anhydroglucose units on the surface of elementary fibrils is strongly supported by morphological studies of *Valonia* and *Acetobacter* celluloses. X-ray evidence suggests that the organization of algal cellulose is such that the crystallite widths are between 17 and 20 nm.[11,33] Furthermore, electron microscopy identifies *Valonia* microfibrils to be 10 × 20 nm in cross section.[11] In contrast, recent X-ray studies on *Acetobacter* have determined the average crystallite width to be 7 nm, while accompanying electron microscopic measurements have identified microfibrils which average 6 × 6 nm in cross section.[12] These dimensions lead to percentages of "surface" anhydroglucose monomers of 25–28% in *Acetobacter* and 10–14% in *Valonia.* Implicit in this calculation is the assumption that if the 3.5 × 3.5 nm elementary fibrils are present in these two materials, the cellulose chains at the associated interfaces within the microfibril are not differentiated by ^{13}C NMR from other interior crystalline chains. The fact that algal, bacterial, and cotton celluloses all show 3.5-nm elementary fibrils under the electron microscope when sonicated or stretched on mylar film[10,34,35] does not require their existence before such treatment.

The spectra of Figures 2 and 3 clearly show diminishing intensities in both the 64- and 86-ppm regions proceeding from cotton, to *Acetobacter*, to *Valonia.* The C-4 wing at 86 ppm appears to be less than 25% of the total C-4 intensity in *Acetobacter*, while the shoulder of C-6 at 64 ppm is diminished relative to the cotton spectrum. The signal-to-noise ratio is not good enough for accurate comparison. The small amplitude of the broad peaks and the poor signal-to-noise ratio in the *Valonia* spectrum make it impossible to quantitate the relative peak intensities, yet a qualitative agreement between surface anhydroglucose percentages and wing intensities is certainly evident.

There are no studies of the morphology of cotton cellulose which show that the arrangement of elementary fibrils into microfibrils is such that the anhydroglucose units on the surface of the elementary fibrils will be disordered, although this assumption underlies our interpretation of the broad resonances for C-4 and C-6 in cotton.

Crystal Structure. The improved resolution in Figure 3 combined with the inherently better resolution due to the greater regularity of the *Valonia* and *Acetobacter* celluloses reveals multiplicities in the ^{13}C resonances that were not reported earlier.[14,15] A careful comparison of the chemical shifts of corresponding sharp peaks in the three spectra of Figure 3 gives shifts consistent within 0.5 ppm. This number is an upper limit on the reproducibility since no correction was made to account for shifts due to peak overlaps. This correspondence implies that the anhydroglucoses associated with the interior of the crystal occupy the same set of sites in the three samples. The peak for C-6 is a singlet for all three samples whereas the region containing the resonances for C-2, C-3, and C-5 is split into at least five peaks. These peaks have not been assigned to the different carbons; neither have we attempted to deconvolute them and obtain integral areas since exact peak shapes are not known. Although the resolution is not sufficient to characterize unequivocally the line shape of the C-4 resonance at 90 ppm in bacterial and algal cellulose, it crudely has the shape of a 1:2:1 triplet. Finally the C-1 peak is clearly split into what appears to be overlapping peaks of uncertain intensities, but a crude estimation of the ratios is again 1:2:1. The NMR data do not permit a determination of bond lengths and angles but they call into question some of the details of presently accepted crystal structures obtained by X-ray and packing analyses.[1-4] The accepted structures include two anhydroglucose monomers per symmetry-related unit and a twofold screw axis relating those units in the unit cell.[1-5] Strictly interpreted, the bond angles and distances to adjacent atoms are equivalent in the two symmetry-related units; thus there should be no more than two NMR peaks for each carbon. Atalla et al.[14] have interpreted their NMR results in terms of two different types of glycosidic linkages. Thus anhydrocellobiose is their monomeric unit for cellulose.

Fiber X-ray diffraction structures are necessarily less exact than corresponding structures obtained with molecular single crystals because of the limited data set obtainable from the fibers. In the X-ray data for *Valonia*, there is an indication of eight chains in the asymmetric unit, which includes too many atoms for the data set and refinement methods used.[3] At present, the body of NMR data in solids is insufficient to establish the magnitude of chemical shifts that might be expected due to skeletal variations or hydrogen bonding. A relaxation of the symmetry of the twofold screw axis would satisfy the approximate multiplicities of C-1 and C-4 by giving a unit cell with four inequivalent anhydroglucoses and two different types of glycosidic linkage. This is one possible explanation for the NMR data reported in this paper which is not in conflict with the X-ray data[1-5] and which also substantially agrees with Atalla et al.'s hypothesis.[14] It is hoped that as CPMASS NMR in solids becomes more mature, we will be able to estimate the chemical shift produced by small structural changes.

The small splittings in the peaks due to C-1 and C-4 may arise from effects other than inequivalences in the unit cell. However, as will be shown in the arguments below, other sources of splittings in cellulose are highly unlikely. Solid-state ^{13}C NMR peak positions and widths are affected by interactions such as anisotropic local molecular or bulk magnetic susceptibilities. These long-range effects are most prominent in aromatic materials because of the large anisotropic magnetic susceptibility of the conjugated rings.[31] In addition, shoulders can be produced by errors in setting the magic angle in CPMASS experiments.[31] The narrow resonance obtained for the polyethylene peak in Figures 1–3 and the comparable chemical shift anisotropies for cellulose and polyethylene carbons eliminate magic-angle errors as sources of the splitting. Furthermore such errors would not produce two sharp shoulders on an NMR resonance unless the samples have bimodal overall orientation, which these samples should not have.

With respect to the question of possible anisotropic magnetic susceptibility effects, the most important fact is that cellulose is not expected to have significant local or bulk magnetic anisotropies since chemical structures which produce large magnetic anisotropies, e.g., aromatic rings, are absent. A rough estimate of magnetic anisotropy effects can be made in analogy to sucrose, for which all of the bulk magnetic susceptibility tensor components lie within 5% of the mean value.[36] On the other hand, hexamethylbenzene has a large bulk magnetic susceptibility anisotropy.[36] Since the magnetic anisotropy broadening of hexamethylbenzene is known,[31] the magnetic anisotropy broadening for cellulose can be estimated. It is approximately 0.1 ppm, which is much smaller than the splitting observed. Another argument against long-range effects being the origin of the splitting of C-1 and C-4 is that the observed line shape should vary with changes in morphology; yet no changes in chemical shift were observed between the three different samples in Figure 3.

NMR Spectral Resolution in Cellulose. As an aside, a few comments will be made concerning the improvement in resolution in the 50-MHz spectra relative to the 15-MHz spectra. First of all cellulose is an ideal sample for high-field (4.7 T) CPMASS experiments. The small ^{13}C chemical shift anisotropies and spectral range ensure that spinning side bands will be small and outside the spectral region of interest. The modest density of protons and small distribution of proton chemical shifts for protons directly bonded to ^{13}C nuclei imply that there should be no net loss in resolution at high field.[31] Our earlier work[15] on the relaxation times (T_2) for microcrystalline cellulose showed that the homogeneous contribution to most of the line widths is about 4 ± 1 Hz, which means that 0.2 ppm of resolution might be gained in going from 1.4 to 4.7 T. The improvement in resolution due to the higher stability of the superconducting solenoid is about 0.25 ppm. The combination of these effects is sufficient to make a visible difference between Figures 2 and 3. Our earlier work[15] also gave a T_2 contribution of 14 Hz for the shoulder on C-6, which translates at 4.7 T to a line width decrease of 0.65 ppm which is sufficient to cause what is clearly only a shoulder in the cotton spectrum in Figure 2 to separate into an overlapping but separate peak in the cotton spectrum in Figure 3.

Conclusions

A comparison of the solid-state CPMASS ^{13}C NMR spectra of native celluloses from ramie, cotton linters, *Acetobacter xylinum*, and *Valonia ventricosa* shows spectral features related to the morphology and crystal structure of the cellulose.

The spectra from cotton linters, hydrocellulose prepared from cotton linters, and ramie show little difference. It was concluded, from both the relative intensities and the invariance in appearance of the broad peaks in the spectra, that these broad features do not originate from paracrystalline regions of the samples as identified by other methods of crystallinity estimation in cellulose. These broad peaks are attributed to anhydroglucoses on the surface of cellulose elementary fibrils. The rationale for this conclusion is based upon the observation that the broad resonances are, respectively, attenuated and missing in celluloses obtained from *Acetobacter xylinum* and *Valonia ventricosa*. Since the elementary fibrils in bacterial and algal cellulose are known to have a higher degree of lateral organization than cotton elementary fibrils, the resulting decrease in surface-to-volume ratio decreases the intensity of the broad peaks.

The spectra of *Valonia* and *Acetobacter* celluloses show splittings which had not been reported in earlier work.[14,15] These splittings are interpreted to be due to inequivalences of anhydroglucose units in the unit cell. The accepted crystal structure for native cellulose includes two magnetically inequivalent anhydroglucoses per unit cell but the NMR spectra show splittings which can only be explained with more than two inequivalent anhydroglucose moieties. Although CPMASS NMR data do not give direct structures, there is a suggestion in the splittings of the peaks assigned to the C-1 and C-4 carbons that the assymmetric unit ought to include at least four anhydroglucose units.

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