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CP/MAS ^{13}C NMR Study of Cellulose and Cellulose Derivatives. 2. Complete Assignment of the ^{13}C Resonance for the Ring Carbons of Cellulose Triacetate Polymorphs

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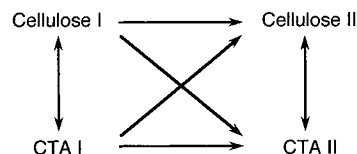
Abstract: Complex ring ^{13}C resonance lines of the cross-polarization/magic angle spinning (CP/MAS) ^{13}C NMR spectra of cellulose triacetate (CTA) I and CTA II were completely assigned, for the first time, by ^{13}C-enriched CTA allomorphs. The ^{13}C-enriched CTA I was prepared by heterogeneous acetylation of bacterial cellulose which was biosynthesized by *Acetobacter xylinum* (*A. xylinum*) ATCC10245 from culture medium containing D-(2-^{13}C)-, D-(3-^{13}C)-, or D-(5-^{13}C)glucose as a carbon source, while CTA II samples were obtained by solution acetylation of the ^{13}C-enriched bacterial celluloses. From comparison of the spectra of normal CTA prepared from ramie with those of the enriched CTA samples, it was revealed that all carbons composed of CTA I appeared as a singlet, while those of CTA II except for C1 were shown as equal-intensity doublets in the CP/MAS ^{13}C NMR spectrum. This finding suggests that CTA I is made up of one kind of glucopyranose residue while there are two magnetically inequivalent sites in the unit cell of CTA II in the same population.

Introduction

The well-known polymorphism in cellulose science is also found in cellulose derivatives. Among them, the most famous case is the cellulose triacetate (CTA) system where the CTA I and CTA II reflect the following reversible and nonreversible crystalline transformations (Scheme 1). This scheme was clearly established by Sprague et al.[1] and despite a few controversial reports[2−4] is now widely accepted. According to this scheme, CTA I is obtained by heterogeneous acetylation of cellulose I, whereas CTA II is produced by homogeneous acetylation or by heterogeneous acetylation from cellulose II. At the supermolecular level, CTA I and CTA II were found to have specific morphologies closely related to those of cellulose I and cellulose II, respectively. This indicated that the chain polarity was preserved in going from cellulose I to CTA I or cellulose II to CTA II. Many researchers, therefore, support the parallel arrangement[5] for cellulose I and CTAI and the antiparallel arrangement[6,7] for cellulose II and CTA II. Although many

Scheme 1. Polymorphism Relationships between Cellulose and CTA



crystallographic studies of the CTA polymorphs by employing X-ray[5,6] or electron diffraction[7,8] have been carried out to elucidate the complete structure of the CTA allomorphs in three dimensions, the discussion regarding the crystal structures of the CTA and cellulose allomorphs are currently in progress because there are no reliable methods for the preparation of single crystals.

Besides the diffraction techniques, high-resolution solid-state ^{13}C NMR spectroscopy has been employed as a powerful tool to gain a deeper insight into the crystal structures of the CTA polymorphs. Authors in our group[9] reported that spectral differences between CTA I and CTA II can be observed in the regions of carbonyl, methyl, and ring carbons, and suggested that the spectral differences prove the differences between CTA I and CTA II in the chain conformation and lattice packing. VanderHart et al.[10] and Kono et al.[11] previously reported that

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^{13}C NMR spectra of CTA oligomers prepared from homogeneous acetylation of the cellodextrins showed similar spectral characteristics of CTA I. With respect to the differences between CTA I and CTA II in the ring carbon region, VanderHart et al.[10] also reported that notable contrasts appear at the both ^{13}C signals of C1 and C6; CTA I exhibits a resonance for C1 at 102 ppm and CTA II possesses one at 100 ppm. Resonances at C6 are distinctive with a singlet appearing at 62 ppm for CTA I and a doublet displayed by CTA II at 65 and 67 ppm. In addition, since the number of resonances associated with C2−C5 in the CTA II spectrum is also observed to be larger than that in the CTA I spectrum, they suggested that magnetic inequivalence within the unit cell of CTA II is greater than for CTA I, which indicated a lower symmetry in the CTA II unit cell. However, the ^{13}C signals for C2−C5 of both CTA allomorphs have not been assigned because a number of resonance lines for these carbons overlap in narrow regions of the spectra.

In this study, we have carried out the assignment of the ^{13}C resonances for anhydroglucopyranose ring carbons of both CTA I and CTA II composing backbone structures of these allomorphs, which had not been assigned before except for C1 and C6, to elucidate the structures of the both CTA crystals by their solid-state ^{13}C NMR spectra. For the assignment of these ^{13}C signals, ^{13}C-enriched cellulose was biosynthesized from D-(2-^{13}C)glucose, D-(3-^{13}C)glucose, or D-(5-^{13}C)glucose as a carbon source by *A. xylinum* and converted to CTA I and CTA II crystals. The redistribution of the specific glucose label to other ring carbon sites was monitored by using quantitative ^{13}C intensities from CTA solution spectra. These relative intensities were used to interpret and assign resonances in the solid-state ^{13}C spectra of both CTA I and CTA II. From the chemical shifts and splittings of the ^{13}C signals of ring carbons in CTA allomorphs, structures of the CTA I and CTA II previously proposed by X-ray analysis were reevaluated, as described herein.

Experimental Section

Preparation of ^{13}C-Enriched Cellulose. ^{13}C-enriched cellulose was biosynthesized by *A. xylinum* ATCC10245 from a Hestrin and Schramn medium[12] containing 1% (v/v) of ethanol and 7.5% of D-(2-^{13}C)glucose, D-(3-^{13}C)glucose, or D-(5-^{13}C)glucose (Cambridge Isotope Laboratories, Inc., MA, the isotropic purity of all the labeling compounds is 99%) in unlabeled glucose. Biosynthesis was carried out at 28 °C for 7 days under static conditions.

Preparation of CTA Allomorphs. CTA I was prepared from ramie by heterogeneous acetylation using the method of Tanghe et al.[13] Preparation of CTA II was carried out by the solution acetylation as follows; the ramie (0.5 g) was suspended in 2 mL of acetic acid for 24 h. A mixture of acetic acid (0.4 mL), acetic anhydride (0.4 mL), and 71% (w/v) perchloric acid (10 μL) was added to the suspension. The suspension was stirred at room temperature for an appropriate time prior to termination by the addition of water (200 mL) to the mixtures. The product was washed with 500 mL of water and 200 mL of methanol and was dried under vacuum. The ^{13}C-enriched CTA I and CTA II were prepared from the ^{13}C-enriched celluloses by the same methods as for the preparation of CTA I and CTA II from ramie, respectively. After these CTA samples were cut with a scissors into small pieces, their crystallinity was improved by an annealing treatment[4,10,11] at 210 °C for 15 min under nitrogen (CTA has a glass transition temperature in the range of 175−189 °C[14,15]). The degree of substitution of each sample was determined according to the usual titration method.[16]

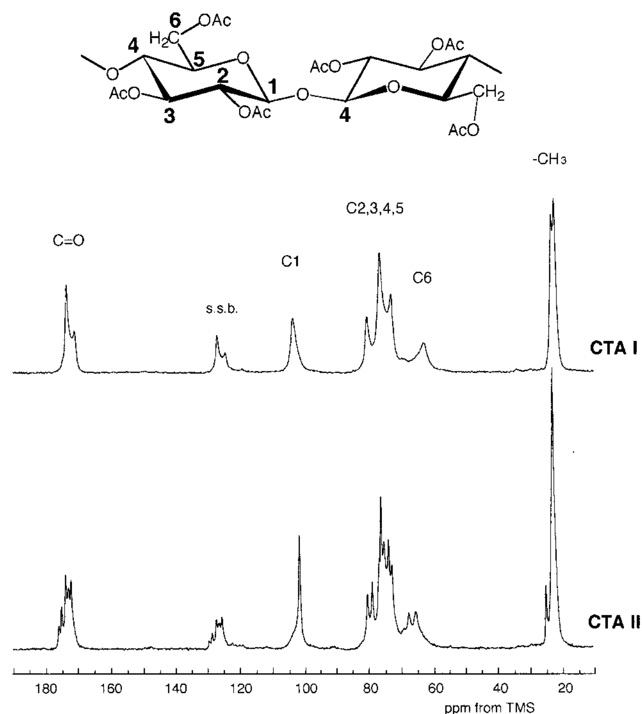

Figure 1. CP/MAS ^{13}C NMR spectra (75 MHz) of CTA I (top) and CTA II (bottom). The abbreviation "s.s.b" in this figure indicates spinning sideband.

General Methods. X-ray diffraction patterns of samples were recorded on a Rigaku Rint-2000 diffractometer equipped with a refraction-type goniometer and a pulse-height discriminator system, using Ni-filtered Cu Kα radiation. Radiation conditions were 40 kV and 25 mA, and the scanning rate was 1° of 2θ per minute. ^{13}C NMR analysis of CTA in solution was performed at 75.4 MHz by means of a Bruker MSL-300 spectrometer at 23 °C in $CDCl_3$. For the ^{13}C quantitative analysis, a pulse-repetition time of 10 s with 2000−2500 transients was acquired by using inverse gated-decoupling and a flip angle of 45°. The spectrum width was 20000 Hz with 32 K data points. Chemical shifts were converted to ppm from tetramethylsilane by assigning the center peak of the triplet resonance of $CDCl_3$ to be 77.0 ppm. CP/MAS ^{13}C NMR measurements and the line-fitting analysis of the solid-state NMR spectra using Nonlinear least-squares methods were performed according to the method described previously.[17]

Results and Discussion

Differences between CP/MAS ^{13}C NMR Spectra of CTA I and That of CTA II. CTA I and CTA II samples were prepared by heterogeneous acetylation of cellulose I and by solution acetylation of cellulose, respectively. The CTAs were subsequently exposed to thermal treatment at 210 °C for 15 min to enhance their crystallinities.[4,10,11] The crystallinities and allomorphs of the samples were confirmed by X-ray diffraction before the CP/MAS ^{13}C NMR measurements.

Figure 1 shows the CP/MAS ^{13}C NMR spectra of the CTA I and CTA II from ramie. This figure shows general features of the spectra of highly crystallized CTAs. As previously reported,[9−11] the spectrum of CTA I was completely different

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from that of CTA II in all regions of glucosidic ring, methyl, and carbonyl carbons, which was also confirmed in this figure: In the carbonyl carbon regions, there was a doublet splitting for CTA I, whereas there was a complex quintet splitting for CTA II. In the case of the methyl carbon region, the sharper doublet for the methyl carbon of CTA I with a 1.0 ppm splitting was observed, while that for the CTA II spectrum has a wider doublet (2.4 ppm) whose downfield peak has much less intensity in comparison with that of CTA I. The most notable difference between the spectrum of CTA I and that of CTA II was observed in the ring carbon region. CTA I shows a resonance for C1 at 102 ppm and CTA II possesses one at 100 ppm. The C6 signal of CTA I was shown as a singlet appearing at 63 ppm and that of CTA II was a doublet at 65 and 67 ppm. In the C2−C5 region of these spectra, the predominant signals can be counted as three for CTA I in contrast to seven for CTA II. Since the backbone structure of CTA was constructed by these carbons, it is important to assign the ^{13}C signals for the elucidation of the notable differences in the spectra of CTA I and CTA II. Therefore, all resonance lines for the ring carbons were assigned by using the site-specifically ^{13}C-enriched CTA I and CTA II, respectively, described below in detail.

Characterization of ^{13}C-Enriched CTA I and CTA II. For the assignment of the resonances for C2−C5 carbons of CTA allomorphs, ^{13}C-enriched cellulose was biosynthesized by *A. xylinum* ATCC10245 from culture media containing D-(2-^{13}C)-, D-(3-^{13}C)-, or D-(5-^{13}C)glucose as labeling sources, followed by their transformation to CTA I and CTA II by heterogeneous and solution acetylation of the enriched celluloses, respectively. Figure 2 shows the ring carbon region of the inverse gated-decoupling ^{13}C NMR spectrum of CTA I prepared from ramie and those of ^{13}C-enriched CTA I samples prepared from D-(2-^{13}C)-, D-(3-^{13}C)-, and D-(5-^{13}C)glucose, and Table 1 summarizes the integral ratio (I.R.) of ^{13}C resonance lines for each carbon in the solution-state NMR spectra of these CTA I samples. In the CTA I prepared from D-(2-^{13}C)glucose, according to this table, the ^{13}C labeling is mainly found at the C2 carbon (40.7%) in addition to a small amount of labeling at C1 (16.2%), C3 (14.6%), and C5 (12.3%). In the case of CTA I from D-(3-^{13}C)-glucose, ^{13}C labeling was observed at C1 (13.8%), C2 (24.7%), and C5 (10.3%) except for C3 (36.3%). In the case of CTA I from D-[5-^{13}C]glucose, most of ^{13}C labeling (55.7%) was observed in the original position and transfers of labeling to C2 (9.0%) and C3 (11.1%) were also occurred. The dilution of the labeling of the original position in the introduced glucose as the carbon source and the redistribution of the label from the original position to other positions during the biosynthesis of cellulose by *A. xylinum* have been investigated by similar experiments with ^{14}C[18−22]- and ^{13}C[23−25]-enriched glucoses. The

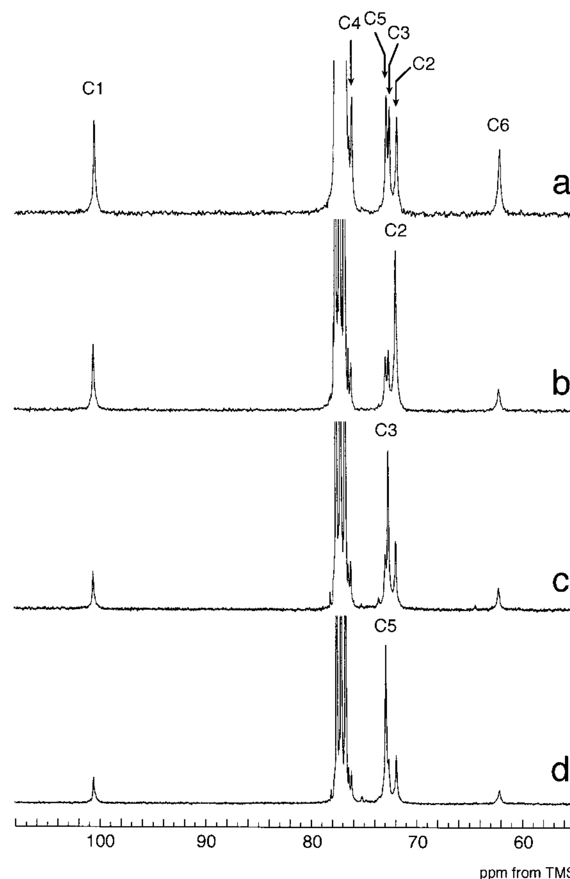

Figure 2. Quantitative-mode ^{13}C NMR spectra of CTA I samples in $CDCl_3$ prepared from different celluloses: (a) ramie; (b) *Acetobacter* cellulose biosynthesized from D-(2-^{13}C)glucose; (c) *Acetobacter* cellulose biosynthesized from D-(3-^{13}C)glucose; and (d) *Acetobacter* cellulose biosynthesized from D-(5-^{13}C)glucose.

dilution and redistribution of the label was explained by the glycolysis and/or isomerization process during the glucose-metabolism of the strain such as the pentose-phosphate cycle, the Embden−Meyerhof, and the Entner−Doudoroff pathways.[18−25] The integral ratio of each carbon of the ^{13}C-enriched CTA II samples prepared from ^{13}C-labeled glucoses should be identical with those of the corresponding CTA I samples summarized in Table 1 because ^{13}C-enriched CTA polymorphs were prepared from the same ^{13}C-enriched cellulose. From these findings, since the intensity ratio of each carbon in the solid-state NMR spectra of these ^{13}C-enriched CTA I should be in complete agreement with the I.R. of the corresponding carbon as shown in Table 1, it is expected that the intensities of the ^{13}C signals at C2, C3, and C5 would fluctuate accordingly in the CP/MAS ^{13}C NMR spectra of CTA samples prepared from D-(2-^{13}C)-, D-(3-^{13}C)-, or D-(5-^{13}C)glucose.

Assignment of ^{13}C Resonance for Ring Carbons of CTA Allomorphs in the Solid State. Figure 3a shows the ring carbon region of the CP/MAS ^{13}C NMR spectrum of CTA I from ramie. The results of line-fitting analysis of the spectrum by using a nonlinear least-squares method, where each resonance is assumed to be Lorenzian, are shown in Figure 3b, and Table 2 summarized the results of the line-fitting analysis. In the line-fitting analysis, ^{13}C signals from the noncrystalline part of CTA

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Table 1. ^{13}C Integral Ratio (I.R.) for Each Carbon in CTA I Prepared from ^{13}C-Enriched Cellulose

sample	carbon (chem shift/ppm)	C1 (100.4)	C2 (71.7)	C3 (72.5)	C4 (76.0)	C5 (72.7)	C6 (61.9)
CTA I from ramie	I.R.[a]	16.3	17.4	16.4	17.6	17.3	15.0
CTA I from D-(2-^{13}C)glucose	I.R.[a]	16.2	40.7	14.6	8.3	12.3	7.9
CTA I from D-(3-^{13}C)glucose	I.R.[a]	13.8	24.7	36.3	7.3	11.0	6.9
CTA I from D-(5-^{13}C)glucose	I.R.[a]	9.4	9.0	11.1	7.4	55.7	7.4

[a] These value were determined from the line-fitting analyses of the NMR spectra of these samples in $CDCl_3$, which were shown in Figure 2. The sum of the I.R. values over all carbons is 100 for each sample.

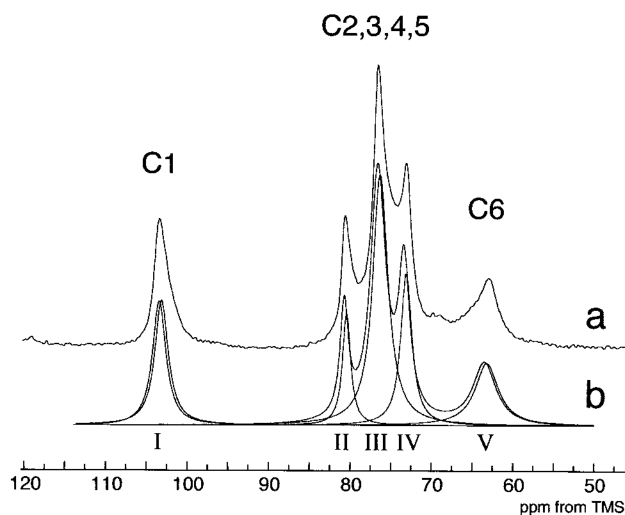

Figure 3. Line-fitting analyses for ring carbon regions of the CP/MAS ^{13}C NMR spectrum of CTA I prepared from ramie: (a) original spectrum and (b) individual fit lines and the synthetic spectrum.

Table 2. Chemical Shifts (δ), Line Widths ($\Delta\nu$), and Integrated Fractions (*I*) of the Respective Lines for Ring Carbon Signals of CTA Allomorphs

allomorph	lines[a]	carbon	δ/ppm	$\Delta\nu$/Hz	I[b]
CTA I	I	C1	102.4	146	2.10
	II	C4	80.3	92	1.70
	III	C2, 3	76.2	165	4.15
	IV	C5	72.9	118	2.20
	V	C6	62.5	252	1.85
CTA II	I	C1	100.4	73	1.75
	II	C4	80.1	69	1.00
	III	C4	78.7	57	0.94
	IV	C2	76.6	56	0.94
	V	C3, 5	75.8	60	1.92
	VI	C3	74.8	67	1.13
	VII	C2	73.5	67	1.08
	VIII	C5	72.5	74	1.18
	IX	C6	65.2	131	1.01
	X	C6	67.3	134	1.05

[a] Line numbers for CTA I and CTA II were indicated in Figures 3b and 5b, respectively. [b] The total value of *I* of each line observed in respective spectrum was 12.

must be separated from these spectra for the precise assignment of ^{13}C signals of crystalline parts. However, the ^{13}C line shape of the noncrystalline part has not been completely established although the spectral features of almost noncrystalline CTA were reported.[10] In this experiment, therefore, separation of the noncrystalline line shape from the solid-state NMR spectra of CTA samples was not carried out. As can be clearly seen in Figure 3b, the synthetic spectrum reproduced by five Lorentian lines I−V was in agreement with the experimental spectrum (Figure 3a), indicating that the line-fitting method used in this experiment could be adequate. In the C2−C5 region of the

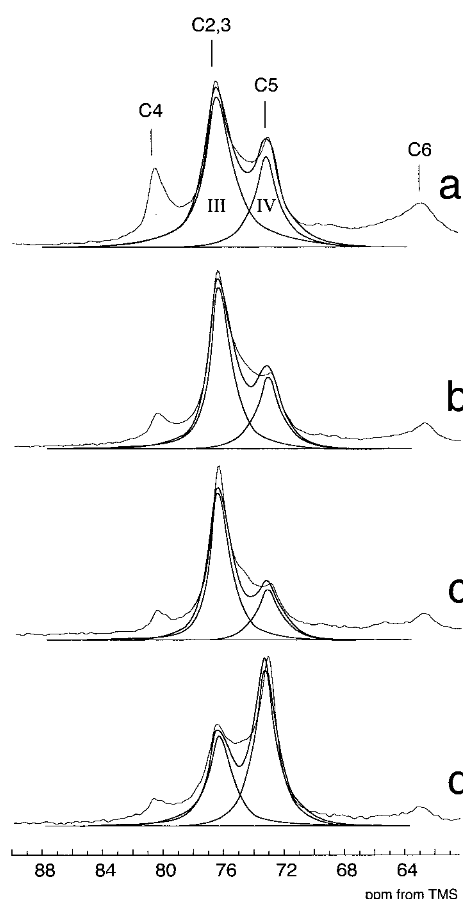

Figure 4. CP/MAS ^{13}C NMR spectra of CTA I samples prepared from different celluloses: (a) ramie; (b) *Acetobacter* cellulose biosynthesized from D-(2-^{13}C)glucose; (c) *Acetobacter* cellulose biosynthesized from D-(3-^{13}C)-glucose; and (d) *Acetobacter* cellulose biosynthesized from D-(5-^{13}C)glucose.

spectrum of CTA I, three lines II−IV were observed at 80.3, 76.2, and 72.9 ppm with the intensity ratio ca. 1:2:1, respectively. Since the four carbon atoms of CTA should appear in this region, resonances for two carbon atoms among C2−C5 of CTA I were expected to be overlapped at 76.2 ppm. Figure 4 shows the C2−C5 regions of CP/MAS ^{13}C NMR spectra of ^{13}C-enriched CTA I samples prepared from D-(2-^{13}C)-, D-(3-^{13}C)-, and D-(5-^{13}C)glucose. In this figure, the ^{13}C resonance at 80.3 ppm (line II) was missing in all spectra of ^{13}C-enriched samples, indicating that this resonance could be assigned to C4 of CTA I. Line-fitting analysis of the ^{13}C spectra of the enriched spectra was performed for the assignments of lines III and IV assuming that the 85−65 ppm region of these spectra was represented by three Lorenzian lines II−IV. Figure 4 shows the Lorenzian lines III and IV obtained by the line-fitting analysis of the spectra, and the integral values of both lines were summarized in Table 3. In this table, the sum of the integral

Table 3. Integral Values of Lines III and IV Obtained by the Line-Fitting Analysis of the ^{13}C CP/MAS Spectra of CTA I Samples

sample		lines[a] III	IV	total[b]
CTA I from ramie	calcd[c]	33.8	17.3	51.1
	obsd[d]	33.4	17.7	51.1
CTA I from D-(2-^{13}C)glucose	calcd[c]	55.3	12.3	67.6
	obsd[d]	49.0	18.6	67.6
CTA I from D-(3-^{13}C)glucose	calcd[c]	61.0	11.0	72.0
	obsd[d]	54.8	17.2	72.0
CTA I from D-(5-^{13}C)glucose	calcd[c]	20.1	55.7	75.8
	obsd[d]	26.4	49.4	75.8

[a] Line numbers are indicated in Figure 4. [b] These values are the sum of the I.R. values of C2, C3, and C5 of each sample that is described in Table 1. [c] The integral value of each line was calculated on the basis of the assumption that these lines were assigned as follows. The lines of III and IV were (C2 + C3) and C5, respectively. [d] These values were determined by the line-fitting analysis of the ^{13}C CP/MAS spectra of CTA I samples, which are shown in Figure 4a−d. The sum of the integral values of lines III and IV is that of I.R. of C2, C3, and C5 of each sample that is described in Table 1.

values of lines III and IV of each spectrum was represented to be that of I.R. values of C2, C3, and C5 of the corresponding CTA sample (Table 1) in order to make direct comparison of the integral value of each line in the solid-state NMR spectra of CTA with relative ^{13}C occupancy for each carbon of the samples. In the spectrum of CTA I prepared from D-(2-^{13}C)-glucose, the integral value of line III (49.0%) almost agrees with the sum of I.R. values of C2 and C3 (55.3%). This suggested that line III could be assigned to be C2 and C3 signals of CTA I, which was confirmed by close agreement between the integral value of line III (54.8%) and the sum of I.R. values of C2 and C3 (61.0%) in the spectrum of CTA I prepared from D-(3-^{13}C)glucose. Thus, the left line IV could be assigned to C5 of CTA I, which was proven by the finding that the integral value of line IV (49.4%) in the spectrum of CTA I prepared from D-(5-^{13}C)glucose agreed approximately with the I.R. of C5 (55.7%) of the corresponding sample.

In the case of CTA II, as shown in Figure 5, it was confirmed that the C2−C5 region of spectrum was constructed by using the seven Lorentzian lines II−VIII at the chemical shift of 80.1, 78.7, 76.6, 75.8, 74.8, 73.5, and 72.5 ppm with the intensity ratio of ca. 1:1:2:1:1:1:1 (Table 2), respectively, from the results of line-fitting analysis. Figure 6 shows the C2−C5 regions of ^{13}C NMR spectra of ^{13}C-enriched CTA II samples prepared from D-(2-^{13}C)-, D-(3-^{13}C)-, and D-(5-^{13}C)glucose. Two resonance lines II and III observed at 80.1 and 78.7 ppm were missing in all the spectra of enriched samples, which indicated that both lines were assigned to C4 signals of CTA II. Table 4 summarizes the integral values of lines IV−VIII obtained by the line-fitting analysis of the 68−85 ppm region of these samples. Integral values of lines IV and VII in the each spectrum agreed approximately with half the I.R. of C2 of the corresponding sample, suggesting that both lines could be assigned to be C2 of CTA II. Since the integral value of line IV is approximately equal to that of line VII in all samples, C2 signals was revealed to be a doublet of equal intensity. In addition, it was confirmed that the integral values of lines VI and VIII almost agreed with half of the I.R. of C3 and that of C5, respectively, and that the integral value of line V was in good agreement with half of the sum of I.R. values of C3 and C5 in all samples. This finding indicated that C3 signals of CTA II were assigned to be a

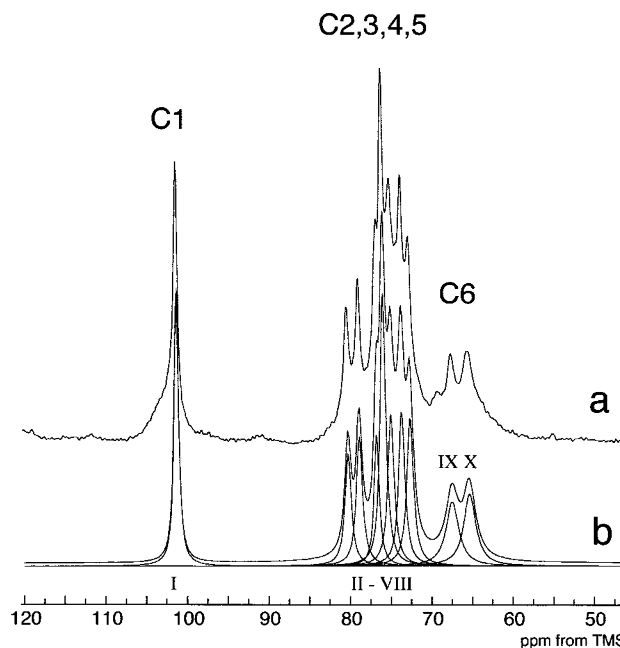

Figure 5. Line-fitting analyses for ring carbon regions of the CP/MAS ^{13}C NMR spectrum of CTA II prepared from ramie: (a) original spectrum and (b) individual fit lines and the synthetic spectrum.

doublet of lines V and VI and that C5 signals were assigned to be a doublet of lines V and VIII. In the spectrum of nonlabeled CTA II, it was therefore considered that line V has twice the intensity of the other signals due to the overlap between doublet components associated with both C3 and C5. Through the analysis of the CP/MAS ^{13}C NMR spectra of the ^{13}C-enriched CTA samples, the resonance lines for each ring carbon of CTA allomorphs were completely assigned. The assignment of ring carbons of CTA I and CTA II is indicated in Figures 4 and 6, respectively, and specified in Table 2.

Crystal Structures of CTA Allomorphs. In this experiment, it was revealed that each ring carbon resonance of CTA I appeared as a singlet in the CP/MAS ^{13}C NMR spectrum and that all ring carbons of CTA II are shown to be doublets except for the C1 signal. This finding suggests that CTA I is made up of one kind of glucopyranose residue while there are two magnetically inequivalent sites in the unit cell of CTA II. It is also deduced that CTA II contains the two magnetically inequivalent sites in the same proportion because doublet intensities were approximately equal. These results are in agreement with the previous proposal.[10]

The crystal structure of CTA I proposed by Stipanovic and Sarko,[5] based on a combined X-ray and stereochemical model analysis, is generally accepted. According to this model, the structure packs in a two-parallel chain, orthorhombic unit cell with dimensions a = 23.63 Å, b = 6.27 Å, and c = 10.43 Å, but with $P2_1$ symmetry. They also indicated that the chain locations were at x, y, z = 0, 0, 0 and a/2, 0, 0 and that the second chain is moved slightly in the y-direction from the positions at a/2, 0, 0. This movement should destroy the equivalence of the two chains and that is not supported by our data for CTA I. In the case of CTA II, the structural model derived from X-ray and electron diffraction analysis, which was proposed by Roche et al.,[7] is widely accepted. The unit cell of CTA II is considered to be orthoronbic with space group

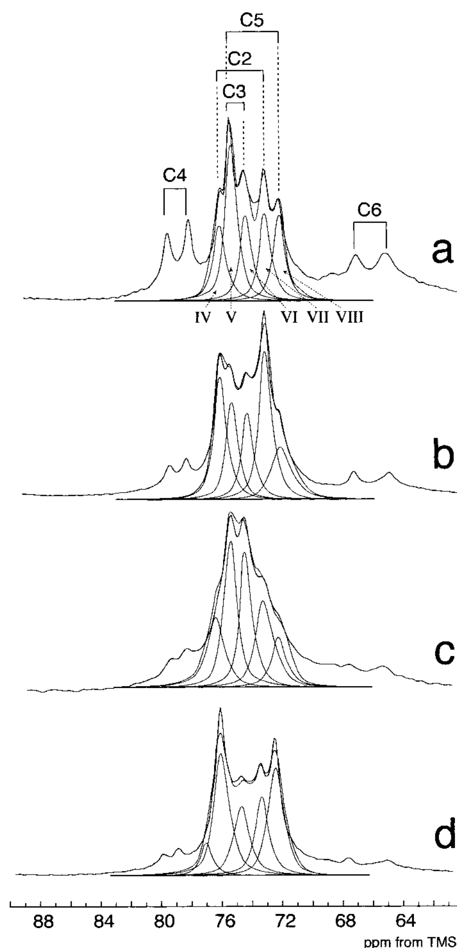

Figure 6. CP/MAS ^{13}C NMR spectra of CTA II samples prepared from different celluloses: (a) ramie; (b) *Acetobacter* cellulose biosynthesized from D-(2-^{13}C)glucose; (c) *Acetobacter* cellulose biosynthesized from D-(3-^{13}C)-glucose; and (d) *Acetobacter* cellulose biosynthesized from D-(5-^{13}C)glucose.

Table 4. Integral Values of Lines IV−VIII Obtained by the Line-Fitting Analysis of the ^{13}C CP/MAS Spectra of CTA II Samples

sample		lines[a]					
		IV	V	VI	VII	VIII	total[b]
CTA II from ramie	calcd[c]	8.7	16.9	8.2	8.7	8.6	51.1
	obsd[d]	7.7	15.7	9.3	8.8	9.6	51.1
CTA II from	calcd[c]	20.4	13.5	7.3	20.4	6.2	67.6
D-(2-^{13}C)glucose	obsd[d]	16.4	9.8	8.9	23.2	9.3	67.6
CTA II from	calcd[c]	12.4	23.7	18.2	12.4	5.5	72.0
D-(3-^{13}C)glucose	obsd[d]	11.4	21.0	17.4	14.8	7.4	72.0
CTA II from	calcd[c]	4.5	33.4	5.6	4.5	27.9	75.8
D-(5-^{13}C)glucose	obsd[d]	4.0	29.1	11.9	9.8	21.0	75.8

[a] Line numbers are indicated in Figure 6. [b] These values are the sum of the I.R. values of C2, C3, and C5 of each sample that is described in Table 1. [c] The integral value of each line was calculated on the basis of the assumption that these lines were assigned as follows. The lines of IV, V, VII, VIII, and VIII were C2/2, (C3+C5)/2, C3/2, C2/2, and C5/2, respectively. [d] These values were determined by the line-fitting analysis of the ^{13}C CP/MAS spectra of CTA II samples, which are shown in Figure 6a−d. The sum of the integral values of lines IV−VIII is that of I.R. of C2, C3, and C5 of each sample, which is described in Table 1.

$P2_12_12_1$ and dimensions $a = 24.68$ Å, $b = 11.52$ Å, and $c = 10.54$ Å. The unit cell has 4 chains: 2 parallel and 2 antiparallel. The 2 chains in each parallel and antiparallel pair are related via 2_1 screw axes positioned outside of the chains. In the suggested structure, all glucopyranose residues in the unit cell of CTA II have an identical conformation; moreover, the bond geometries of the glucopyranose residues of CTA II are posited to be exactly those of the central residue of cellotriose undecaacetate.[26] In this experiment, it was obviously revealed that ring carbons except for C1 experienced magnetic inequivalence and appeared as doublets in the CP/MAS ^{13}C NMR spectra of CTA II. Therefore, it could be considered that chains in CTA II can have the same conformations but vary in intermolecular interactions and thus experience shift of the resonance lines of carbons except for C1.

Besides backbone conformations for CTA chains, conformations of the exocyclic C5−C6 bonds are an important factor for determining the chain packing in the unit cell. Conformations for the exocyclic bonds of both CTA allomorphs are generally accepted to be in *gauche−gauche* (*g−g)* conformation by the X-ray and electron diffraction studies.[5−7] With respect to exocyclic C5−C6 bonds, Horii et al.[27] studied the relationships between chemical shifts of C6 of various carbohydrates in the solid-state ^{13}C NMR spectra and the O5−C5−C6−O6 dihedral angle of crystal structures of the carbohydrates determined by X-ray diffraction patterns. They concluded that chemical shifts of C6 fall into three groups of 62, 64, and 66 ppm, which are related to *g−g*, *gauche−trans* (*g−t*), and *trans−gauche* (*t−g*) conformations, respectively. On the basis of this finding, they also suggested that the hydroxymethyl groups of cellulose I and II are in the *t−g* and *g−t* conformation, respectively, which were supported by recent diffraction analysis.[28−31] In the CP/MAS ^{13}C NMR spectra of CTA I and CTA II, the chemical shifts of C6 differ strongly. The C6 signal of CTA I appears at 62.5 ppm, while the C6 doublet of CTA II is observed at 67.3 and 65.2 ppm. Because the C6 signal of cellulose is shifted downfield by the acetylation of hydroxyl groups,[15] the correspondence between chemical shift and conformation proposed by Horii is not directly applicable. However, the chemical shift differences observed are large enough to suggest a conformational difference in the exocyclic bonds between CTA I and CTA II. With respect to the conformations of exocyclic bonds of CTA II, Roche et al.[7] reported that the acetate groups connected to the C6 carbon may have contrasting geometries in alternating residues down each chain on the basis of the X-ray analysis of CTA II. VanderHart et al.[10] also suggested that there are different conformations of methyl groups bound to C6 of CTA II on the basis of the detailed analysis of the chemical shifts of the methyl carbon. It is therefore considered that the large difference in the chemical shifts of the C6 doublet supports these suggestions although there are no absolute proofs.

In conclusion, this study provided, for the first time, the complete assignment of all resonances for ring carbons of CTA polymorphs through the detailed analysis of the CP/MAS ^{13}C NMR spectra of CTA polymorphs in which certain carbons were selectively ^{13}C enriched. The results presented here indicated that the glucopyranose rings in the backbone structure of CTA I are magnetically equivalent and, therefore, experience the same

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conformation and crystal packing form. In contrast, magnetic inequivalence was observed in CTA II because ring carbons of CTA II except for C1 were shown as equal-intensity doublets in the CP/MAS ^{13}C NMR spectrum, which is consistent with the proposed X-ray structure[7] that has four chains, pairwise antiparallel, and a 2-fold screw axis lying off the chain axis. In addition, conformational differences in the exocyclic bonds between CTA I and CTA II were suggested by the noticeable difference in their C6 chemical shifts.

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