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Determination of the Distribution of the Substituent Group in Cellulose Acetate by Full Assignment of All Carbonyl Carbon Peaks of $^{13}C\{^1H\}$ NMR Spectra

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ABSTRACT: An attempt was made to assign a number of peaks, observed in carbonyl region (168—171 ppm) of $^{13}C\{^1H\}$ NMR spectra of cellulose acetate (CA), to twelve magnetically different acetyl groups existing in 8 kinds of glucopyranose units constituting CA. CA samples with total degree of substitution 《F》 ranging 0.43 to 2.46 were prepared by acid-hydrolysis of CA with 《F》=2.92 dissolved in acetic acid. $^{13}C\{^1H\}$ NMR measurements were made on these ten CA samples in deuterated dimethylsulfoxide and 14 peaks in total were observed definetely over the whole range of 《F》 from 2.92—0.43. From the spectra of CA samples with highest 《F》 (2.92) and lowest (0.43) carbonyl carbon peaks at C_6, C_3, and C_2 positions in trisubstituted and monosubstituted glucopyranose units were successfully assigned. Deducing the effect of an acetyl group on the chemical shift of other acetyl groups, and vise versa, based on an empirical rule, 6 carbonyl carbon peaks in 3 kinds of disubstituted glucopyranose units were assigned. Eight average molar fractions $《f_{lmn}》$ (l=1 or 0 means that a hydroxyl group attached directly at C_2 position is or is not substituted and m=1 or 0 means that C_3 position is or is not substituted, and n corresponds to C_6 position) [*i.e.*, $《f_{000}》$, $《f_{100}》$, $\cdots$, $《f_{111}》$] were evaluated from integrated intensity ratios. The probability of substitution at C_k position $《f_k》$ (k=2, 3, and 6) and 《F》, both evaluated from the above $《f_{lmn}》$ values, fairly agree with those estimated by the conventional NMR method, indicating the validity of the assignment in this paper.

KEY WORDS Cellulose Acetate / ^{13}C NMR / Carbonyl Carbon / Degree of Substitution / Distribution of Substituent Groups /

Cellulose is a linear chain molecule with the chemical structure illustrated in Figure 1. Recently studies were actively carried out, in particular by Kamide *et al.*,[1-5] on the distribution of the existing probability of substituent groups at three different hydroxyl groups (C_2, C_3, and C_6 positions) in glucopyranose units constituting cellulose derivative molecules and its correlation with the physical and physiological properties. In these studies, only the degrees of substitution at C_2, C_3, and C_6 positions $《f_k》$ (k=2, 3, and 6) were evaluated by NMR method. Note that $《f_k》$ values are averaged over all molecules contained in the sample, as well as all glucopy-

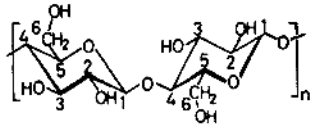

Figure 1. Chemical structure of cellulose: numbers (1—6) denote carbon positions.

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ranose units constituting an individual polymeric chain, in other words, $\langle\langle f_k \rangle\rangle$ is a doubly-averaged quantity.[1-5] Thus, the total degree of substitution was also expressed by $\langle\langle F \rangle\rangle$. In addition, Kamide *et al.*[6] showed by thin-layer chromatography (TLC) that CA sample consists of molecules with different average total degrees of substitution $\langle F \rangle$ [here, F is the degree of substitution of a given glucopyranose unit and $\langle F \rangle$ is obtained by averaging F over all glucopyranose units constituting a single molecule] and evaluated the distribution of $\langle F \rangle$, denoted as $g(\langle F \rangle)$ (see Figure 2). However, when we see the possible substituted glucopyranose units of cellulose derivative, there are 8 kinds of unsubstituted and partially or fully substituted glucopyranose units as shown in Figure 3: single trisubstituted, three disubstituted, three monosubstituted and one unsubstituted glucopyranose units. Molar fractions of these glucopyranose units ($\langle\langle F_{lmn} \rangle\rangle$, defined later in detail) are an effective measure of the distribution of the substitutent group within a glucopyranose unit, enabling us to also judge whether the reaction is homogeneous or not. Also, an accurate evaluation of these fractions is paramountly important in order to understand on a molecular basis the solubility of cellulose derivatives against various solvents and the physiological properties. Since the 1950's, the separation and quantitative determination of tri-, di-, mono-substituted and unsubstituted glucopyranose units have been exclusively performed by applying distillation and chromatographic techniques to chemically decomposed cellulose derivatives, particulaly sodium cellulose xanthate.[7] However, the decomposition of cellulose derivative molecules into glucose units is extremely difficult without desubstituting reactions in spite of the numerous attempts to prevent such reactions. In fact, experimental results reported on $\langle\langle F_{lmn} \rangle\rangle$ of cellulose xanthate differ depending on researchers who carried out, unexceptionally, the conversion of the xanthate group into a more stable form through very complicated chemical reactions.[3]

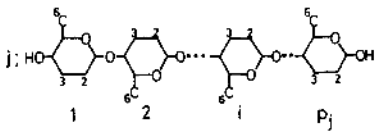

Figure 2. Schematic representation of the jth cellulose molecule having the degree of polymerization, p_j.

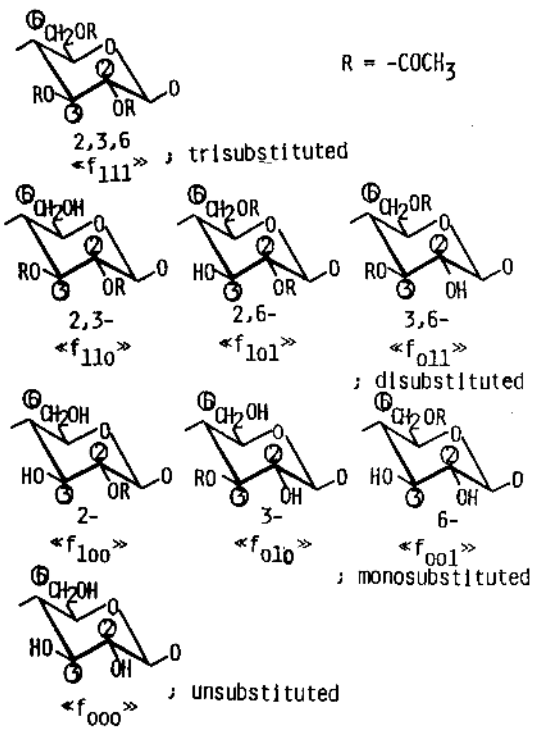

Figure 3. Substituted and unsubstituted glucopyranose units in CA molecules: $\langle\langle f_{lmn} \rangle\rangle$ represents molar fraction of the unit.

Wu,[8] and Clark and Stephenson[9] proposed methods for estimating molar fractions of 2,3,6-tri-, 2,6-di-, 3,6-di-, and 6-mono-substituted glucopyranose units of cellulose nitrates, whose C_6 position was fully substituted (*i.e.*, $\langle\langle f_6 \rangle\rangle = 1$) from their ^{13}C NMR spectra. However their methods cannot be applied to cellulose nitrate whose hydroxyl group at the C_6 position is not fully substituted.

Kamide and Okajima[10] were the first to give

peak assignments to three roughly separated carbonyl carbon peaks of ^{13}C NMR spectra of CA in solution as the substitutent (acetyl) group at C_3, C_2, and C_6 positions from higher magnetic field and showed that $\langle\langle f_k \rangle\rangle$ could be evaluated from the intensitity ratio of these three peaks. Later, Miyamoto *et al.*[11] gave the reverse assignment on C_2 and C_3 positions using a principally similar method as Kamide and Okajima. Applying the low-power selective spin decoupling method to almost completely substituted CA ($\langle\langle F \rangle\rangle = 2.92$), Kowsaka *et al.*[12] assigned, from the lower magnetic field, the carbonyl carbon peaks to the acetyl groups at C_6, C_3, and C_2 positions and simultaneously demonstrated that there are not only 3 but also many peaks in the carbonyl carbon region of ^{13}C NMR spectra of partially substituted CA ($\langle\langle F \rangle\rangle = 2.46$ and 0.68), insisting that these peaks correspond to 12 carbonyl carbon peaks, originating from magnetically different all ($=12$) –OR groups existing in 8 pyranose rings as shown in Figure 3. In order to evaluate $\langle\langle f_k \rangle\rangle$ for not-fully substituted CA with good accuracy, the reliable assignment of the above-mentioned 12 peaks is very prerequisite because rough classification of these peaks into three parts (carbonyl at C_2, C_3, and C_6) made by Kamide and Okajima[10] and Miyamoto *et al.*[11] is too oversimplified, ignoring possible peak overlapping from different types of substituted glucopyranose units.

Both Miyamoto *et al.*[13] and Kamide and his collaborators[5] came to the same conclusion that CA samples with the same $\langle\langle F \rangle\rangle$, prepared under different conditions, had different degrees of water solubility, and discussed the relations between $\langle\langle f_k \rangle\rangle$ by NMR method and the water solubility for CA: CA having $\langle\langle f_2 \rangle\rangle \simeq \langle\langle f_3 \rangle\rangle \simeq \langle\langle f_6 \rangle\rangle$ (*i.e.*, CA with uniformly distributed acetyl groups) dissolves almost completely in water at room temperature in the range of $\langle\langle F \rangle\rangle$ from 0.6 to 1.0. In contrast to this, CA having $\langle\langle f_6 \rangle\rangle \gg \langle\langle f_2 \rangle\rangle + \langle\langle f_3 \rangle\rangle$ (*i.e.*, CA with acetyl groups highly selectively located at C_6 position) is little better than insoluble in water over the entire $\langle\langle F \rangle\rangle$ range. In addition, Kamide *et al.*[5] demonstrated that CA having $\langle\langle f_2 \rangle\rangle \simeq \langle\langle f_3 \rangle\rangle \simeq \langle\langle f_6 \rangle\rangle$ easily dissolves completely in dimethylacetamide (DMAC) at room temperature irrespective of $\langle\langle F \rangle\rangle$, if $\langle\langle F \rangle\rangle$ is at least larger than 0.6, and CA having $\langle\langle f_6 \rangle\rangle \gg \langle\langle f_2 \rangle\rangle + \langle\langle f_3 \rangle\rangle$ dissolves only partly in DMAC even at 80°C in the same $\langle\langle F \rangle\rangle$ range. Now, we conclude that neither $\langle\langle F \rangle\rangle$, $\langle\langle f_2 \rangle\rangle$, $\langle\langle f_3 \rangle\rangle$ nor $\langle\langle f_6 \rangle\rangle$ control individually the solubility. Kamide *et al.*[5] obtained experimental evidence suggesting that the relative content of 3-monosubstituted glucopyranose units controls water solubility, but this needs fur-

Table I. Degree of substitution, weight- and viscosity-average molecular weight and peak chemical shift in carbonyl region of CAs

Sample code	$\langle\langle F \rangle\rangle$	$\bar{M}_w$, $(\bar{M}_v)/10^5$	Chemical shift/ppm (±0.02 ppm)		
Ca-0	2.92	2.32[a]	169.94	169.40 (169.17) 169.11	168.93 168.78
CA-1	2.46	1.05[a]	170.02 169.95 169.82	169.41 169.11	168.91 168.78 168.73
CA-2	1.75	0.82[b]	170.04 169.97 169.83	169.43 169.17 169.14 169.11	168.93 168.80
CA-3	1.23	0.80[b]	170.06 170.00 169.97 (169.85)	(169.61) 169.45 169.21 169.16	168.92 168.79 (168.72)
CA-4	1.06	0.64[b]	170.04 169.96 169.87	169.58 169.46 169.34 169.22 169.10	168.92 168.77
CA-5	0.95	0.47[b]	170.00 168.87	169.61 169.48 169.36 169.22	168.93 168.87
CA-6	0.77	0.36[b]	170.04 (168.88)	169.59 169.47 169.36 169.18	168.93 168.75
CA-7	0.69	0.33[b]	170.04 169.98 (168.89)	169.61 169.46 169.34	168.93 (168.71)
CA-8	0.54		170.02 169.97 (169.90)	169.59 (169.43) 169.34 169.09	168.92 168.79
CA-9	0.43		170.04 (169.99) 169.90 169.78	169.61 169.34 (169.10)	168.94

[a] $\bar{M}_w$, from light scattering.
[b] $\bar{M}_v$, from $[\eta]$ in DMAC at 25°C.

ther experimental study.

This article assigns all peaks in the carbonyl carbon region of ^{13}C NMR spectra of CA and provides a firm basis for estimating $\langle\langle f_k \rangle\rangle$ and molar fractions of 8 kinds of glucopyranose units of CA by NMR alone.

EXPERIMENTAL

A cellulose triacetate (CTA) whole polymer with $\langle\langle F \rangle\rangle = 2.92$ (sample code CA-0) and 9 incompletely substituted CA samples, prepared by acid hydrolysis of sample CA-0 in acetic acid (sample code CA-1—CA-9) were used. The detailed preparing procedures are described in previous papers.[14,15]

Table I collects the weight-average molecular weight $\bar{M}_w$ of sample code CA-0 and 1, determined by light scattering in DMAC, and the viscosity-average molecular weight $\bar{M}_v$ of sample code CA-2—7, determined from the limiting viscosity number in DMAC solution.[14-16]

Proton noise-decoupled ^{13}C NMR ($^{13}C\{^1H\}$ NMR) spectra of these CA solutions in deuterated dimethylsulfoxide (DMSO-d_6) were recorded on a FX-200 FT-NMR spectrometer (JEOL, Japan) at a resonance frequency of 50.18 MHz at 90°C. The detailed operating conditions were almost the same as those in the previous paper.[12] Tetramethylsilane (TMS) was the internal reference. Integrated peak intensity was determined from an integral curve. $\langle\langle F \rangle\rangle$ was evaluated from the integrated intensity ratio of peaks in acetyl methyl carbon region (20—22 ppm) and peaks in C_1 carbon region (91—105 ppm).

The second column of Table I compiles the $\langle\langle F \rangle\rangle$ of these CA samples.

RESULTS AND DISCUSSION

Figure 4(a)—(j) show the carbonyl carbon region of $^{13}C\{^1H\}$ NMR spectra of samples CA-0—9 in DMSO-d_6. These spectra were recorded at a spectral width of 1 kHz (4096 data points) in order to attain high degital resolution. The chemical shift from TMS as an internal reference was determined from the spectra obtained independently at a spectral width of 10 kHz (8192 data points). The degital resolution of these spectra was estimated to be about 0.01 ppm and the relative error of chemical shifts was less than 0.02 ppm. In the spectrum of sample code CA-0 ($\langle\langle F \rangle\rangle = 2.92$) in Figure 4(a), three main peaks were observed as reported in the previous paper,[12] originating from trisubstituted glucopyranose

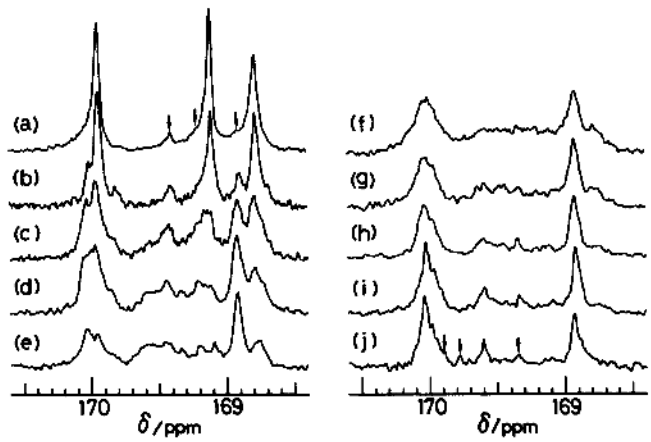

Figure 4. Carbonyl carbon region of $^{13}C\{^1H\}$ NMR spectra of cellulose acetates: (a), CA-0; (b), CA-1; (c), CA-2; (d), CA-3; (e), CA-4; (f), CA-5; (g), CA-6; (h), CA-7; (i), CA-8; (j), CA-9.

unit, and these peaks are assigned, from lower magnetic field, to three carbonyl carbons at C_6, C_3, and C_2 positions, respectively. In the same spectrum, small peaks or shoulders, observed at 169.4, 169.2, and 168.9 ppm (as denoted by arrows in the figure) may possibly have originated from disubstituted glucopyranose units. In the spectrum of sample CA-9 ($\langle\langle F \rangle\rangle = 0.43$) in Figure 4(j), three peaks (170.0, 169.6, and 168.9 ppm) due to three monosubstituted glucopyranose units are observed and in addition, a group of small peaks (as denoted by arrows in the figure), considered to have originated from disubstituted glucopyranose units, are detected at 169.9, 169.8, and 169.3 ppm. $^{13}C\{^1H\}$ NMR spectra for CA samples having intermediate $\langle\langle F \rangle\rangle$ (1.75—0.77) show very complicated patterns, possibly due to superposition of the above mentioned peak groups and of peaks from disubstituted units.

In and after the fourth column of Table I are summerized the chemical shifts of all peaks (in 0.01 ppm unit) in carbonyl carbon region of $^{13}C\{^1H\}$ NMR spectrum of each CA sample. In the table, chemical shifts of shoulders are shown in parentheses. The table indicates that for each CA sample 6—11 peaks or shoulders in total are observed in the carbonyl carbon region. Considering the relative error (± 0.02 ppm) of chemical shifts, the peaks observed commonly for two or more CA samples are carefully chosen as listed in Table II. Here, the peaks are numbered from the lower magnetic field. As previously described, the total number of carbonyl carbon peaks, expected previously and theoretically as due to substituted, is 12, but from actual experiments, 14 peaks are observed (Table II).

Table II. Peak assignments in the carbonyl carbon region of CAs

Peak No.	δ/ppm	Carbon position	Glucopyranose unit	
1	170.04	6	6-Mono-	
2	170.00	6	2,6-Di-	
3	169.94	6	2,3,6-Tri-	
4	169.89	6	3,6-Di-	
5	169.83	6	3,6-Di-	
6	169.60	3	3-Mono-	
7	169.46	3	3,6-Di-	
8	169.41	3	3,6-Di-	
9	169.35	3	3,6-Di-	
10	169.22	3	2,3-Di-	
11	169.11	3 (2)	2,3,6-Tri-	(2,6-Di-)
12	168.93	2	2-Mono-	2,6-Di-
13	168.79	2	2,3,6-Tri-	2,3-Di-
14	168.71	2	2,3-Di-	

Peaks No. 3, 11, and 13 are the main peaks in the spectrum of highly substituted CA ($\langle\langle F \rangle\rangle = 2.92$) as shown in Figure 4(a) of the 2,3,6-trisubstituted glucopyranose unit and are assigned to the carbonyl carbons at C_6, C_3, and C_2 carbon positions in the 2,3,6-trisubstituted unit, respectively. This mutual positional order in the tri-substituted unit can be postulated to be maintained in low substituted CA ($\langle\langle F \rangle\rangle = 0.43$); thus, the main peaks in Figure 4(j) No. 1, 6, and 12 can be assigned to the carbonyl carbons in 6-, 3-, and 2-monosubstituterd glucopyranose units, respectively. The assignment for these 6 peaks are collected in columns 3 and 4 (in part) of Table II.

Generally, the degree of change in chemical shift due to the introduction of a specific substitution group into polymer is the maximun at the nucleus, to which the substituent group is directly combined (*i.e.*, α-position) and is smaller at the nucleus which is combined indirectly with and separated through a large number of nuclei from the substituent group. For example, the chemical shift of the carbonyl carbon at C_3 position is significantly influenced by whether the C_2 position is substituted or not, but is not so much affected by the C_6 position. Thus, it is reasonably expected that the chemical shifts of C_3 carbonyl carbons of 3-mono- and 3,6-disubstituted glucopyranose units are relatively close and C_3 carbonyl carbons of 2,3-di- and 2,3,6-trisubstituted units give similar chemical shifts. According to this expectation, we can assign the No. 10 peak in the vicinity of

peak No. 11 to the C_3 carbonyl carbon in the 2,3-disubstituted glucopyranose unit and peaks No. 7, 8, and 9 in vicinity of peak No. 6 to C_3 carbonyl carbons in the 3,6-disubstituted glucopyranose unit, respectively. Accordingly, peak No. 14 near peak No. 13 is assigned to the C_2 peak in the 2,3-disubstituted glucopyranose unit. Since no peak exists near the position of peak No. 12, which is assigned already to the C_2 carbonyl carbon in the 2-monosubstituted unit, then the C_2 carbonyl carbon in the 2,6-disubstituted unit is considered to be heavily superposed with that of the 2-monosubstituted unit. In a similar manner, peaks No. 4 and 5, observed in the vicinity of peak No. 3, is assigned to the C_6 carbonyl carbon in the 3,6-disubstituted unit and peak No. 2 in the vicinity of peak No. 3 to the C_6 carbonyl carbon in the 2,6-disubstituted unit. The above assignments are also shown in columns 3, 4, and 5 (in part) of Table II.

Table III collects the integrated peak intensities of carbonyl carbon peaks No. 1—14, I_n (n is the peak No. and $n=1$—14), for samples CA-0, 1, 2, 3, 8, and 9. Here, I_n is normalized with total integrated intensity ($\sum_{n=1}^{14} I_n$ ($\equiv\langle\langle F\rangle\rangle$)). Several peaks overlap each other, and some I_n cannot be estimated separately. The overlapping peaks are shown with underlined and their total peak intensities are shown under the main peak (I_n) in the table. Inspection of Tables II and III leads us to the conclusion that in CA with $\langle\langle F\rangle\rangle > 2.4$ (CA-0 and CA-1) a large amount of the 2,3,6-trisubstituted (from peak No. 3, 11, and 13) unit coexists with a small amount of 2,6- and 3,6-disubstituted (from peak No. 5, 8, and 12) glucopyranose units, and CAs with $\langle\langle F\rangle\rangle < 0.6$ (CA-8 and CA-9) are mainly constituted by 2-, 3-, and 6-monosubstituted glucopyranose (from peak No. 1, 6, and 12) units with a small amount of 3,6-disubstituted glucopyranose unit (from peak No. 9). Then, we can conclude that the highly substituted CA consists mainly of trisubstituted and disubstituted glucopyranose units and low substituted CA consists predominantly of monosubstituted units. Note that in this study, all not-fully substituted CA samples were prepared by acid hydrolysis, which is expected to occur randomly (or homogeneously), and the above conclusion is consistent with what is expected in the case of random acid hydrolysis of CA. Kamide *et al.*[6] pointed out from TLC analysis on CTA that "CTA" with $\langle\langle F\rangle\rangle$ of 2.92 is a mixture of CTA with $\langle\langle F\rangle\rangle = 3$ (*i.e.*, trisubstituted unit) and not-fully substituted CA. This finding was repeatedly and more firmly ascertained in this NMR study. Although peak No. 11 (C_3 carbonyl carbon in trisubstituted glucopyranose units) was observed for CA sampled CA-8 and CA-9 (low substituted CA's), the corresponding C_6, C_2 carbonyl carbons for the trisubstituted one (peak No. 3 and 13, respectively) were not found. Then, in this case peak No. 11

Table III. Integrated peak intensity I_n of carbonyl carbon peaks of CA samples

Sample code	$\langle\langle F\rangle\rangle$	I_n													
		$n=1$	2	3	4	5	6	7	8	9	10	11	12	13	14
CA-0	2.92			0.97		0.02	0.00		0.06	0.00		0.95	0.01	0.91	0.00
CA-1	2.46			0.77		0.09	0.00		0.16	0.00		0.66	0.21	0.57	
CA-2	1.75			0.56			0.00	0.23		0.00	0.40		0.23	0.33	0.00
CA-3	1.23		0.40				0.00	0.20		0.03	0.17		0.24	0.18	
CA-8	0.54	0.22			0.00	0.00	0.08	0.03		0.04		0.02	0.13	0.02	
CA-9	0.43	0.18			0.00	0.00	0.08	0.00		0.05	0.00	0.01	0.11	0.00	0.00

should be assigned to the C_2 carbonyl carbon of the 2,6-disubstituted glucopyranose unit. On the other hand, the same carbonyl carbon (*i.e.*, the C_2 carbonyl carbon at the 2,6-disubstituted unit) peak is observed as peak No. 12 for highly substituted CA samples (CA-0 and CA-1), indicating that change in $\langle\langle F \rangle\rangle$ from 2.5—2.9 to ~0.5 brings about a shift of the chemical shift of the C_2 carbonyl carbon peak in the 2,6-disubstituted unit by ~0.14 (±0.04) ppm. Similar variation in the chemical shift with $\langle\langle F \rangle\rangle$ was noticed on other peaks. One or two peaks, observed over the range of 169.34—169.48 ppm, are considered due to the C_3 carbonyl carbon of the 3,6-disubstituted unit. Its location varies depending on $\langle\langle F \rangle\rangle$. We already assigned peak No. 14 to the C_2 carbonyl carbon of the 2,3-disubstituted unit, but the low substituted CA No. 13 peak, in place of No. 14 peak, should be assigned to the above carbonyl carbon. A tentative explanation of these phenomena would be a possible long-range (accordingly, weak) effect from the substituent group in the nearest neighbouring glucopyranose unit on the carbonyl carbon in question. That is, the peak position of the chemical shift of the C_2 carbonyl carbon in the 2,6-disubstituted unit may change depending on whether the substituent group is existing or not probably at the C_3 position, which is the nearest the β-glucoside linkage, in the neighbouring glucopyranose unit. This long-range effect will become remarkable for the disubstituted units, existing in a wide range of $\langle\langle F \rangle\rangle$. Then, peaks due to either C_3 or C_2 carbonyl carbon are expected to be located at the position of peak No. 11. Additional assignments of peaks No. 11 and 13, determined from integrated peak intensity, are also shown in Table II. Figure 5(a) and (b) demonstrate carbonyl carbon region of $^{13}C\{^1H\}$ NMR sepctra of samples CA-1 and CA-8 with full assignment.

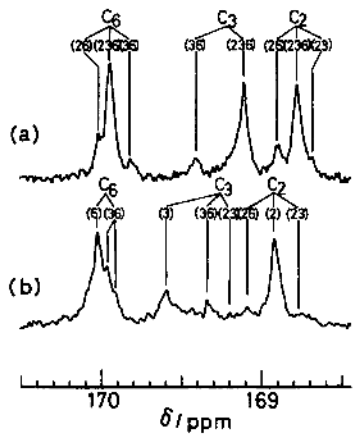

Figure 5. Peak assignments of the carbonyl carbon peaks of cellulose acetates: (a), CA-1; (b), CA-8.

Using the assignment for all the peaks in carbonyl carbon region as given in Table II we can derive equations to give $\langle\langle f_k \rangle\rangle$:

$$\langle\langle f_2 \rangle\rangle = \langle\langle F \rangle\rangle \left(\sum_{j=12}^{14} I_j \right) \Big/ \left(\sum_{j=1}^{14} I_j \right) \quad \text{(for CA with } \langle\langle F \rangle\rangle \geqq 2) \tag{1}$$

$$\langle\langle f_2 \rangle\rangle = \langle\langle F \rangle\rangle \left(\sum_{j=11}^{14} I_j \right) \Big/ \left(\sum_{j=1}^{14} I_j \right) \quad \text{(for CA with } \langle\langle F \rangle\rangle < 2) \tag{2}$$

$$\langle\langle f_3 \rangle\rangle = \langle\langle F \rangle\rangle \left(\sum_{j=6}^{11} I_j \right) \Big/ \left(\sum_{j=1}^{14} I_j \right) \quad \text{(for CA with } \langle\langle F \rangle\rangle \geqq 2) \tag{3}$$

$$\langle\langle f_3 \rangle\rangle = \langle\langle F \rangle\rangle \left(\sum_{j=6}^{10} I_j \right) \Big/ \left(\sum_{j=1}^{14} I_j \right) \quad \text{(for CA with } \langle\langle F \rangle\rangle < 2) \tag{4}$$

$$\langle\langle f_6 \rangle\rangle = \langle\langle F \rangle\rangle \left(\sum_{j=1}^{5} I_j \right) \Big/ \left(\sum_{j=1}^{14} I_j \right) \tag{5}$$

Table IV collects $\langle\langle f_k \rangle\rangle$ of all CA samples calculated using eq 1—5 from the data in Table III. Evidently, it is confirmed for CA with $\langle\langle F \rangle\rangle \geqq 0.7$, that $\langle\langle f_2 \rangle\rangle \simeq \langle\langle f_3 \rangle\rangle \simeq \langle\langle f_6 \rangle\rangle$. In the previous paper,[5] Kamide *et al.* reported for CA synthesized by a two-step method a similar tendency ($\langle\langle f_2 \rangle\rangle \simeq \langle\langle f_3 \rangle\rangle \simeq \langle\langle f_6 \rangle\rangle$), although in their study, determination of $\langle\langle f_k \rangle\rangle$ was made by rough approximation. The relations between $\langle\langle f_k \rangle\rangle$ of CA and its physical properties

will be described elsewhere.[17] In addition, from I_n ($n=1$—14) data, for any CA sample we can evaluate the distribution of seven different substituted glucopyranose units as shown in Figure 3. We denote molar fractions of eight (seven substituted and single unsubstituted) glucopyranose units with $\langle\langle f_{lmn}\rangle\rangle$, where l, m, and n mean the existence of the acetyl group at the C_2, C_3, and C_6 positions, respectively, and they can take the value of 0 (unsubstituted) or 1 (substituted). According to this notation, the doubly-averaged molar fraction of the trisubstituted unit is $\langle\langle f_{111}\rangle\rangle$ and those of disubstituted units are $\langle\langle f_{110}\rangle\rangle$, $\langle\langle f_{101}\rangle\rangle$, and $\langle\langle f_{011}\rangle\rangle$ and that of unsubstituted unit is $\langle\langle f_{000}\rangle\rangle$ and so on (see Figure 3). The summation of all $\langle\langle f_{lmn}\rangle\rangle$ is unity:

$$\sum_{l=0}^{1}\sum_{m=0}^{1}\sum_{n=0}^{1}\langle\langle f_{lmn}\rangle\rangle=1 \tag{6}$$

According to this definition, $\langle\langle f_k\rangle\rangle$ and $\langle\langle F\rangle\rangle$ can be expressed in terms of $\langle\langle f_{lmn}\rangle\rangle$ as follows:

$$\langle\langle f_2\rangle\rangle=\sum_{m=0}^{1}\sum_{n=0}^{1}\langle\langle f_{1mn}\rangle\rangle \tag{7}$$

$$\langle\langle f_3\rangle\rangle=\sum_{l=0}^{1}\sum_{n=0}^{1}\langle\langle f_{l1n}\rangle\rangle \tag{8}$$

$$\langle\langle f_6\rangle\rangle=\sum_{l=0}^{1}\sum_{m=0}^{1}\langle\langle f_{lm1}\rangle\rangle \tag{9}$$

$$\langle\langle F\rangle\rangle=\langle\langle f_2\rangle\rangle+\langle\langle f_3\rangle\rangle+\langle\langle f_6\rangle\rangle \tag{10}$$

Based on the assignment of all carbonyl carbon peaks in Table II and neglecting peak overlapping, we can calculate $\langle\langle f_{lmn}\rangle\rangle$ from I_n through the following equations:

$$\langle\langle f_{111}\rangle\rangle=I_3=I_{13}+I_{14}-\langle\langle f_{110}\rangle\rangle \tag{11}$$

$$\langle\langle f_{111}\rangle\rangle=I_{11}=I_{13}\quad\text{(for CA with }\langle\langle F\rangle\rangle\geqq 2) \tag{12}$$

$$\langle\langle f_{111}\rangle\rangle=I_{11}-\langle\langle f_{101}\rangle\rangle\quad\text{(for CA with }\langle\langle F\rangle\rangle<2) \tag{13}$$

$$\langle\langle f_{110}\rangle\rangle=I_{10}=I_{13}+I_{14}-\langle\langle f_{111}\rangle\rangle \tag{14}$$

$$\langle\langle f_{101}\rangle\rangle=I_2=I_{11}2+I_{12}-\langle\langle f_{111}\rangle\rangle-\langle\langle f_{100}\rangle\rangle \tag{15}$$

$$\langle\langle f_{101}\rangle\rangle=I_{12}\quad\text{(for CA with }\langle\langle F\rangle\rangle\geqq 2) \tag{16}$$

$$\langle\langle f_{101}\rangle\rangle=I_{11}\quad\text{(for CA with }\langle\langle F\rangle\rangle<2) \tag{17}$$

$$\langle\langle f_{011}\rangle\rangle=I_4+I_5=I_7+I_8+I_9 \tag{18}$$

Table IV. Total degree of substitution $\langle\langle F\rangle\rangle$ and distribution of degree of substitution $\langle\langle f_k\rangle\rangle$ of CA samples

Sample code	$\langle\langle F\rangle\rangle$	$\langle\langle f_2\rangle\rangle$	$\langle\langle f_3\rangle\rangle$	$\langle\langle f_6\rangle\rangle$
CA-0	2.92	0.92	1.01	0.99
CA-1	2.46	0.79	0.82	0.85
CA-2	1.75	0.56	0.63	0.56
CA-3	1.23	0.42	0.40	0.40
CA-4	1.06	0.33	0.42	0.31
CA-5	0.95	0.29	0.33	0.33
CA-6	0.77	0.26	0.24	0.27
CA-7	0.69	0.20	0.23	0.26
CA-8	0.54	0.17	0.15	0.22
CA-9	0.43	0.12	0.13	0.18

Table V. $\langle\langle f_{lmn}\rangle\rangle$, $\langle\langle f_k\rangle\rangle_{calc}$, $\langle\langle F\rangle\rangle_{calc}$, $\langle\langle f_k\rangle\rangle_{con}$, and $\langle\langle F\rangle\rangle_{con}$ values of CA samples

Sample code	$\langle\langle f_{111}\rangle\rangle$	$\langle\langle f_{110}\rangle\rangle$	$\langle\langle f_{101}\rangle\rangle$	$\langle\langle f_{011}\rangle\rangle$	$\langle\langle f_{100}\rangle\rangle$	$\langle\langle f_{010}\rangle\rangle$	$\langle\langle f_{001}\rangle\rangle$	$\langle\langle f_{000}\rangle\rangle$
CA-1	0.48	0.18	0.21	0.16	0[a]	0[a]	0[a]	0[a]
CA-8	0[a]	0.02	0.02	0.07	0.13	0.08	0.13	0.45
CA-9	0[a]	0.00	0.01	0.05	0.11	0.08	0.12	0.63

Sample code	$\langle\langle f_2\rangle\rangle_{calc}$	$\langle\langle f_3\rangle\rangle_{calc}$	$\langle\langle f_6\rangle\rangle_{calc}$	$\langle\langle F\rangle\rangle_{calc}$	$\langle\langle f_2\rangle\rangle_{con}$	$\langle\langle f_3\rangle\rangle_{con}$	$\langle\langle f_6\rangle\rangle_{con}$	$\langle\langle F\rangle\rangle_{con}$
CA-1	0.87	0.82	0.85	2.54	0.79	0.82	0.85	2.46
CA-8	0.17	0.17	0.22	0.56	0.17	0.15	0.22	0.54
CA-9	0.12	0.13	0.18	0.43	0.12	0.13	0.18	0.43

[a] Approximated previously.

$$\langle\langle f_{100}\rangle\rangle = I_{12} \quad \text{(for CA with } \langle\langle F\rangle\rangle < 2) \tag{19}$$

$$\langle\langle f_{010}\rangle\rangle = I_6 \tag{20}$$

$$\langle\langle f_{001}\rangle\rangle = I_1 \tag{21}$$

The peaks in $^{13}C\{^1H\}$ NMR spectra of CA samples, except for CA-0, are quite broad indeed and overlap significantly as shown in Figure 4. Then, an accurate evaluation of $\langle\langle f_{lmn}\rangle\rangle$ for CA is quite difficult.

Since for the sample code CA-8 peaks No. 6, 11, and 12 are separetely observed without overlapping with other peaks, $\langle\langle f_{010}\rangle\rangle$, $\langle\langle f_{100}\rangle\rangle$, and $\langle\langle f_{101}\rangle\rangle$ for the sample can be evaluated using the I_n data of these peaks (Table III) from eq 20, 19, and 17, respectively. The fraction $\langle\langle f_{110}\rangle\rangle$ for the sample can also be determined from eq 14 using data on $I_{13}+I_{14}$ in Table III and assuming $\langle\langle f_{111}\rangle\rangle = 0$, because (1) peaks No. 13 and 14 overlap with each other, but not with other peaks, enabling an estimate of $I_{13}+I_{14}$ and (2) $\langle\langle F\rangle\rangle$ for this sample is low (0.54). $\langle\langle f_{011}\rangle\rangle$ for the sample can also be evaluated using data on $I_7+I_8+I_9$ from eq 18, in the same manner. In addition, for this CA cample, peak No. 1 significantly overlaps with peaks No. 2, 3, 4, and 5 then, $\langle\langle f_{001}\rangle\rangle$ cannot be simply estimated from eq 21 using I_1 data. An alternative way of estimating $\langle\langle f_{001}\rangle\rangle$ is given by the equation:

$$\langle\langle f_{001}\rangle\rangle = \sum_{j=1}^{5} I_j - \langle\langle f_{101}\rangle\rangle - \langle\langle f_{011}\rangle\rangle - \langle\langle f_{111}\rangle\rangle \tag{22}$$

Combination of eq 5 and 10 leads to eq 22. Neglecting $\langle\langle f_{111}\rangle\rangle$ in eq 22 for sample CA-8, $\langle\langle f_{001}\rangle\rangle$ can be estimated roughly from $\langle\langle f_{101}\rangle\rangle$ and $\langle\langle f_{011}\rangle\rangle$ data previously determined by eq 16 and 18.

Equation 6 can be rearranged as follows:

$$\langle\langle f_{000}\rangle\rangle = 1 - (\langle\langle f_{111}\rangle\rangle + \langle\langle f_{110}\rangle\rangle + \langle\langle f_{101}\rangle\rangle + \langle\langle f_{011}\rangle\rangle + \langle\langle f_{100}\rangle\rangle + \langle\langle f_{010}\rangle\rangle + \langle\langle f_{001}\rangle\rangle) \tag{23}$$

Then, eq 23 enables us to estimate $\langle\langle f_{000}\rangle\rangle$ from already known $\langle\langle f_{lmn}\rangle\rangle$ data.

The values of $\langle\langle f_{lmn}\rangle\rangle$ for sample CA-8 thus determined are listed in Table V, where the corresponding data for samples CA-1 and CA-9, both obtained in a similar manner are also included. Here, for sample CA-1, it is assumed that the molar fractions of mono- and unsubstituted glucopyranose units are neglected ($\langle\langle f_{100}\rangle\rangle = \langle\langle f_{010}\rangle\rangle = \langle\langle f_{001}\rangle\rangle = \langle\langle f_{000}\rangle\rangle = 0$).

From $\langle\langle f_{lmn}\rangle\rangle$ data shown in Table V, $\langle\langle f_k\rangle\rangle$ and $\langle\langle F\rangle\rangle$ can be directly evaluated using eq 7—9 and eq 10. The results are also summarized in 10th—14th columns in Table V. Here, $\langle\langle f_k\rangle\rangle$ and $\langle\langle F\rangle\rangle$ thus obtained are denoted by $\langle\langle f_k\rangle\rangle_{calc}$ and $\langle\langle F\rangle\rangle_{calc}$, respectively. $\langle\langle f_k\rangle\rangle$ and $\langle\langle F\rangle\rangle$ estimated previously by the conventional method for these samples through application of eq 1—5 and eq 10, are denoted as $\langle\langle f_k\rangle\rangle_{con}$ and $\langle\langle F\rangle\rangle_{con}$, and are also compiled in the 15th—18th columns in Table V. $\langle\langle f_k\rangle\rangle_{calc} \simeq \langle\langle f_k\rangle\rangle_{con}$ ($k=2$, 3, and 6) and $\langle\langle F\rangle\rangle_{calc} \simeq \langle\langle F\rangle\rangle_{con}$ thus appear to be confirmed.

Summarizing, all peaks observed in the carbonyl carbon regionin ^{13}C NMR spectra of fully- and not-fully-substituted CA were unambigously assigned and a method proposed for evaluating the molar fractions of 8 kinds of glucopyranose units such as 2,3,6-tri-, 2,3-di-, 2,6-di-, 3,6-di-, 2-mono-, 3-mono-, and 6-monosubstituted units and an unsubstituted unit (denoted here as $\langle\langle f_{lmn}\rangle\rangle$), based on these assignment. It was confirmed for three CA samples that $\langle\langle f_k\rangle\rangle$ and $\langle\langle F\rangle\rangle$, calculated from $\langle\langle f_{lmn}\rangle\rangle$, coincide approximately with those by conventional methods, supporting the reliability of the present method of assignment.

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