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Solid-state CP/MAS ^{13}C-NMR analysis of cellulose and tri-*O*-substituted cellulose ethers

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Highly crystalline tri-*O*-substituted cellulose ethers having ethyl, *n*-propyl, *n*-butyl, allyl, and methallyl substituents were prepared from low-molecular weight cellulose (DP = 15). Influences of conformational and packing effects on solid-state ^{13}C-NMR spectra were studied by using X-ray diffraction and solid- and solution-state ^{13}C-NMR analyses of the cellulose derivatives. Unit-cell sizes tentatively obtained from X-ray diffraction patterns of the cellulose derivatives indicated that conformations and packing states of cellulose chains and alkyl chains of substituents were different between the derivatives. Solid- and solution-state ^{13}C-NMR spectra of cellulose allomorphs, and effects of hydrogen bonds present in celluloses I, II, and III on chemical shifts of their solid-state ^{13}C-NMR spectra were proposed.

INTRODUCTION

In a previous paper (Isogai *et al.*, 1989), the authors reported X-ray diffraction and solid-state CP/MAS ^{13}C-NMR analyses of highly crystalline and pure cellulose allomorphs, celluloses I, II, III_I, III_{II}, and IV_{II}. Chemical shifts of C4 and C6 resonances in solid-state ^{13}C-NMR spectra were different between the cellulose allomorphs, indicating that these resonances were directly related to the appearance of each cellulose allomorph. However, since the relationship between chemical shifts of solid-state ^{13}C-NMR spectra of compounds and either primary, secondary or tertiary structures, such as conformations, hydrogen bonds, or packing effects, has not been established yet, the solid-state ^{13}C-NMR spectra of cellulose allomorphs could not be interpreted in terms of the above aspects.

Horii *et al.* (1983, 1987) proposed the hypothesis that all chemical shifts of C1, C4, and C6 resonances were governed by conformations of C1–O–C4 in the glucosidic linkage and of C5–C6–O; the torsion angles, Φ, Ψ and χ for C1–O, O–C4, and C5–C6, respectively. However, few examples were used in the experiments, and there seemed to be some exceptions to the above rules in polysaccharides (Atalla, R.H., pers. comm.). Kamide *et al.* (1984, 1985) proposed that chemical shifts of C4 resonances in solid-state ^{13}C-NMR spectra of celluloses were governed by the presence of an intramolecular hydrogen bond between C3–OH and the C5 oxygen atom of the adjacent anhydroglucose ring. Their hypothesis, however, seems to be speculative at this point. In solution-state ^{13}C-NMR, conformational effects of cyclohexane derivatives on chemical shifts have been reported, and the effects of torsion angles are lower than 5 ppm (Anet *et al.*, 1971; Dalling & Grant, 1972). Thus, it is significant to establish the relationship

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between chemical shifts of solid-state ^{13}C-NMR spectra and solid-state structures, for application of solid-state ^{13}C-NMR analyses to cellulose allomorphs.

Solid-state ^{13}C-NMR analyses of many cellulose derivatives have been reported (Patterson *et al.*, 1985; Doyle *et al.*, 1985; Doyle *et al.*, 1986; Hoshino *et al.*, 1989). However, since the reported cellulose ethers had heterogeneous structures, i.e. degrees of substitution (DS) were lower than 2·5 and hydroxyalkyl groups were formed as branches on cellulose chains, these derivatives probably had low crystallinities.

In previous papers, about 20 kinds of tri-*O*-substituted cellulose ethers were prepared by using a non-aqueous cellulose solvent system, SO_2-diethylamine-dimethylsulfoxide, under non-aqueous and homogeneous conditions (Isogai *et al.*, 1984*a*, *b*, 1985*a*, 1986). Most of these derivatives prepared from microcrystalline cellulose powder had crystalline structures, although the crystallinities were not so high (Isogai *et al.*, 1984*a*, 1985*b*, 1986). If highly crystalline tri-*O*-substituted cellulose ethers having various substituents can be prepared, they must have various unit-cell sizes and cellulose chain conformations, depending on substituents. The effects of conformations and packing states of cellulose chains and substituents on chemical shifts of solid-state ^{13}C-NMR spectra may be obtained by using these derivatives. Since low-molecular weight cellulose samples with degrees of polymerization (DPn) of 7 and 15 were prepared from usual cellulose samples by homogeneous hydrolysis in phosphoric acid (Isogai & Usuda, 1991), tri-*O*-substituted cellulose ethers having high crystallinities may be prepared from the low-molecular weight celluloses.

In this paper, therefore, tri-*O*-substituted cellulose ethers were first prepared from low-molecular weight cellulose with a DPn of 15 under non-aqueous and homogeneous conditions (Isogai *et al.*, 1984*a*, *b*, 1986). The unit cell sizes and conformations of the derivatives were obtained from X-ray diffraction data. Then, their solid- and solution-state ^{13}C-NMR spectra were measured, and the effects of conformations and packing states on chemical shifts of solid-state ^{13}C-NMR spectra were studied. On the basis of the results of cellulose derivatives thus obtained, solid-state ^{13}C-NMR spectra of cellulose allomorphs were examined in terms of secondary and tertiary structures of cellulose molecules in crystals.

MATERIALS AND METHODS

Materials

Low-molecular weight celluloses, which had degrees of polymerization of 7 and 15, were prepared from microcrystalline cellulose powder by using phosphoric acid (Isogai & Usuda, 1991). The cellulose sample with a DPn of 7 was used for solid-state ^{13}C-NMR as a highly crystalline cellulose II sample and for solution-state ^{13}C-NMR by dissolving it in dimethylsulfoxide-d_6. Amorphous cellulose was prepared from microcrystalline cellulose powder, according to the reported method (Isogai & Atalla, 1991).

Preparation of tri-*O*-substituted cellulose ethers

The cellulose sample with a DPn of 15 was dissolved in the SO_2-diethylamine–dimethylsulfoxide system, and was etherified with powdered NaOH and an etherifying reagent, such as ethyl iodide, *n*-propyl iodide, *n*-butyl iodide, allyl chloride, or methallyl chloride, under non-aqueous and homogeneous conditions (Isogai *et al.*, 1984*a*, *b*, 1986). Chloroform-extracts from the reaction mixture were evaporated, and methanol was added to the syrup. Regenerated cellulose derivatives were sufficiently washed with methanol by centrifugation. Tri-*O*-substituted cellulose ethers thus prepared were isolated as white powders by drying *in vacuo*, and their yields were more than 80%.

Analyses

X-Ray diffraction patterns of cellulose derivates were recorded on a JEOL JDX-5B diffractometer equipped with both reflection- and transmission-type goniometers, using Ni-filtered CuK_α radiation. Radiation conditions were 30 kV and 25 mA, and the scanning rate was 0·5° of 2θ per minute.

Solid-state ^{13}C-NMR spectra were recorded on a JEOL JNM-GX 270 spectrometer (^{13}C frequency = 67·8 MHz) with a CP/MAS unit at room temperature. The spinning rate and the contact time were 3·6–3·8 kHz and 2 ms, respectively. A bullet type Kel-F rotor contained about 300 mg of a sample. The recycle time of the pulse was 5 s. The spectrum was accumulated 80–100 times. The signal of the CH of adamantane was used as an external reference to determine chemical shifts (Isogai *et al.*, 1989). Solution-state ^{13}C-NMR spectra were collected on a Bruker AC-300 spectrometer (^{13}C frequency = 75·5 MHz). Spectra were accumulated using 5 μs of the pulse width, 18·5 kHz of the sweep width, and 2 s of the pulse interval. Dimethylsulfoxide-d_6 (DMSO-d_6) and chloroform-d_1 were used for NMR solvents of the cellulose sample with a DPn of 7 and tri-*O*-substituted cellulose ethers, respectively.

RESULTS AND DISCUSSION

Unit-cell sizes of tri-*O*-substituted cellulose ethers

Figure 1 shows X-ray diffraction patterns of tri-*O*-substituted cellulose ethers prepared from low-molecular weight cellulose with a DPn of 15. Although these

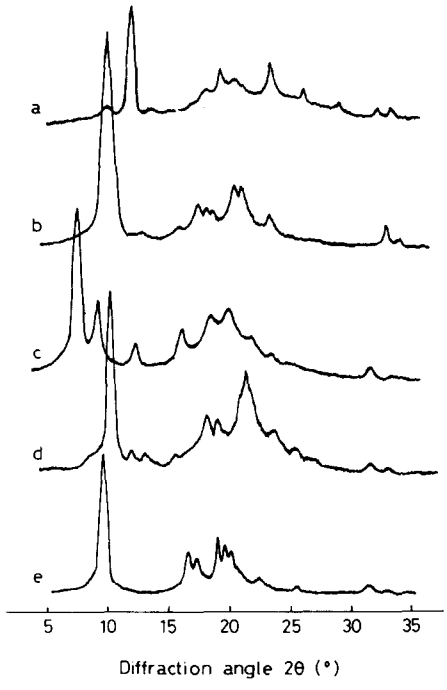

Fig. 1. X-Ray diffraction patterns of tri-*O*-substituted cellulose ethers: (a) tri-*O*-ethylcellulose; (b) tri-*O*-*n*-propylcellulose; (c) tri-*O*-*n*-butylcellulose; (d) tri-*O*-allylcellulose; (e) tri-*O*-methallylcellulose.

derivatives had patterns similar to those of the corresponding tri-*O*-substituted cellulose ethers prepared from microcrystalline cellulose powder with a viscosity average degree of polymerization (DP_v) of 200, the samples prepared in this study had much higher crystallinities (Isogai *et al.*, 1985*b*, 1986). Furthermore, transmission electron micrographic analysis showed that all these derivatives formed single crystals (Sugiyama, J., Isogai, A. & Okano, T., unpublished). As shown in Fig. 1, each derivative had a diffraction peak with relatively high intensity around 7–12° of 2θ, and the diffraction angle increased with an increase in the size of substituents.

Table 1 summarizes the unit-cell sizes of the cellulose derivatives, tentatively obtained from the X-ray diffraction data, and most of the diffraction peaks in Fig. 1 corresponded well to Miller indexes calculated from the proposed unit-cell sizes. This shows that these cellulose derivatives had various unit-cell dimensions, *a* and *b* values, depending on substituents. Furthermore, the *c* values (fiber axis) of the unit-cell dimensions of tri-*O*-*n*-propylcellulose and tri-*O*-allylcellulose were 1·5 nm, whereas the others were 1·0 nm. This observation indicates that in the former two derivatives the cellulose chains have three-fold helices, whereas in the latter the cellulose chains have two-fold helices as in the cellulose allomorphs, celluloses I–IV. Thus, the torsion angles Φ and Ψ for C1–O and O–C4 bonds, respectively, in the β-1,4-glucosidic linkage probably differ in the various cellulose derivatives. Also the packing states of cellulose chains in unit cells must be different between the samples. Furthermore, packing states and conformations of substituents must be different between those substituted at C2, C3 and C6.

Therefore, these X-ray diffraction analyses of the highly crystalline cellulose derivatives indicate that: (1) conformations differ among the tri-*O*-substituted cellulose ethers; (2) the cellulose chains show different packings, depending on substituents; and (3) conformations and packing states of substituents vary according to substituents at C2, C3 and C6. However, it should be noted that these unit-cell sizes were tentatively obtained, and further experiments are necessary for determination of the definite unit-cell sizes of the derivatives.

Solid- and solution-state ^{13}C-NMR spectra of tri-*O*-substituted cellulose ethers

Figure 2 shows solution-state ^{13}C-NMR spectra of tri-*O*-substituted cellulose ethers in $CDCl_3$. Chemical shifts of six carbons of anhydroglucose residues were almost equal between the cellulose derivatives; 102–103 ppm for C1, 82–83 ppm for C2, 83–85 ppm for C3, 77–78 ppm for C4, 75–77 ppm for C5, and 68–70 ppm for C6. Resonances of the substituent-carbons, which are the nearest to glucose residues, appeared around 59–76 ppm, depending on the primary structures of substituents, and some of the resonances overlapped with those of anhydroglucose residues.

Figure 3 shows solid-state ^{13}C-NMR spectra of the highly crystalline tri-*O*-substituted cellulose ethers. Although the resonances in solid state were broader than those in solution state, each resonance in solid state corresponded well to that in solution state. Furthermore, the chemical shifts of carbons of anhydroglucose residues and substituents in solid state were almost

Table 1. Unit-cell sizes of tri-*O*-substituted cellulose ethers

Substituent	*a* (nm)	*b* (nm)	*c* (nm)	γ (degrees)	Type
$-CH_2CH_3$	0·91	0·91	1·04	120	Hexagonal
$-CH_2CH_2CH_3$	0·98	0·98	1·52	120	Hexagonal
$-CH_2CH_2CH_2CH_3$	1·08	1·81	1·03	90	Orthorhombic
$-CH_2CH{=}CH_2$	0·96	0·96	1·52	120	Hexagonal
$-CH_2C(CH_3){=}CH_2$	1·03	1·03	1·04	120	Hexagonal

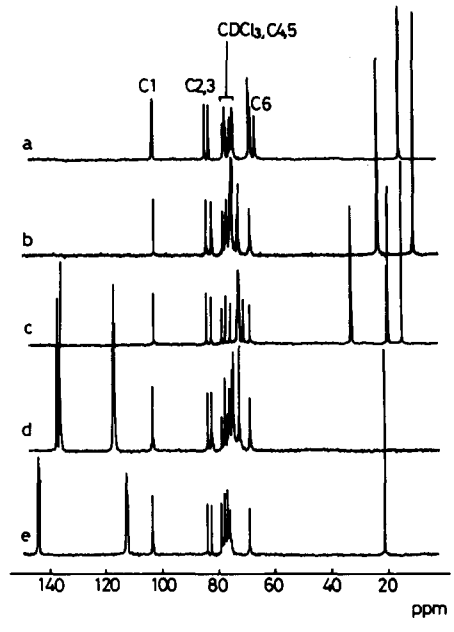

Fig. 2. Solution-state ^{13}C-NMR spectra of tri-*O*-substituted cellulose ethers: (a) tri-*O*-ethylcellulose; (b) tri-*O*-*n*-propylcellulose; (c) tri-*O*-*n*-butylcellulose; (d) tri-*O*-allylcellulose; (e) tri-*O*-methallylcellulose.

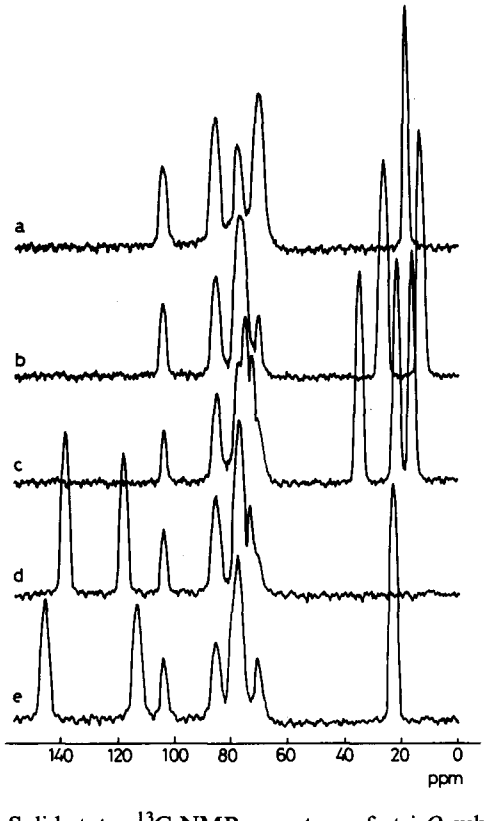

Fig. 3. Solid-state ^{13}C-NMR spectra of tri-*O*-substituted cellulose ethers: (a) tri-*O*-ethylcellulose; (b) tri-*O*-*n*-propylcellulose; (c) tri-*O*-*n*-butylcellulose; (d) tri-*O*-allylcellulose; (e) tri-*O*-methallylcellulose.

equal to or a little higher than those in solution state. Thus, the chemical shifts in solid-state ^{13}C-NMR were substantially and predominantly influenced by the primary structures of compounds. The differences of chemical shifts between solid and solution states were lower than 5 ppm.

As described in the above section, X-ray diffraction analyses of the highly crystalline tri-*O*-substituted cellulose ethers indicated that the carbons of anhydroglucose residues have different conformations and packing states between cellulose derivatives, and also that the carbons in the same substituents must have different conformations and packing states between the substituted positions, C2, C3 and C6. These X-ray diffraction and NMR analyses show that the conformational or packing effects of crystalline cellulose derivatives on solid-state ^{13}C-NMR spectra appear as at most 5 ppm differences of chemical shifts. Namely, the effects of secondary and tertiary structures such as conformations and packing states, respectively, on chemical shifts of solid-state ^{13}C-NMR spectra were found to be at most 5 ppm.

Solid-state ^{13}C-NMR spectra of cellulose

The above results were applied to solid-state ^{13}C-NMR spectra of crystalline and amorphous celluloses (Isogai *et al.*, 1989). Table 2 shows approximate chemical shifts of cellulose allomorphs and amorphous cellulose in solid state, and those of cellulose with a DPn of 7 dissolved in DMSO. As shown in this table, the differences of chemical shifts between solid and solution states were lower than 5 ppm for C1 and C6 resonances of all cellulose allomorphs and amorphous cellulose. On the other hand, C4 resonances of celluloses I, II, and III had 8–9 ppm downfield shifts when compared to that of cellulose dissolved in DMSO, whereas C4 resonances of cellulose IV and amorphous cellulose had only 4 ppm downfield shifts. The large downfield shifts of C4 resonances for celluloses I, II, and III cannot be explained simply in terms of the conformational or packing effects.

In the previous paper (Isogai & Usuda, 1992), crystallization to cellulose III from celluloses I and II,

Table 2. Chemical shifts (ppm) of C1, C4 and C6 of celluloses in solid and solution states

Cellulose	C1; Δ^a	C4; Δ^a	C6; Δ^a
Cellulose I	106; 3	89; 9	66; 5
Cellulose II	106; 3	89; 9	64; 3
Cellulose III	106; 3	88; 8	63; 2
Cellulose IV	106; 3	84; 4	64; 3
Amorphous cellulose	105; 2	84; 4	63; 2
Cellulose in DMSO	103; 0	80; 0	61; 0

[a]Difference of chemical shift (ppm) between solid and solution states.

and amorphous cellulose with liquid ammonia was studied by X-ray diffraction and solid-state ^{13}C-NMR analyses. Crystallinity of cellulose III increased with an increase in the temperature of the ammonia treatment, and the crystallization was clearly associated with the downfield shifts of C4 resonances from about 84 ppm to about 88 ppm. Thus, the crystallization to cellulose III and the downfield shifts of C4 resonances seem to be correlated, and this relationship is probably applicable to other cellulose allomorphs, celluloses I and II. In other words, the chemical shifts of C4 resonances in solid-state ^{13}C-NMR spectra may be key factors in the crystallization of cellulose molecules to celluloses I, II or III (Isogai *et al.*, 1987).

Since the conformational and packing effects are excluded from explanations of the large downfield shifts of C4 resonances, as described in the previous section, the effect of hydrogen bonds may be one of the plausible explanations for the above observations.

The large downfield shifts of C4 resonance appeared on cellulose II and cellulose oligomers with molecular weights higher than that of cellotetraose, and cellotriose and cellobiose had C4 resonances at 84 ppm (Dudley *et al.*, 1983). Thus, it is noteworthy that some intra- or inter-molecular hydrogen bonds, which bring about the large downfield shifting of C4 in celluloses I, II and III, may be absent in cellobiose or cellotriose. However, the hypothesis about the relationship between chemical shifts of, particularly, C4 in solid-state ^{13}C-NMR spectra and some hydrogen bond formation in cellulose allomorphs should be further studied.

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