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^{13}C NMR study on peracetylated derivatives of cyclomaltoheptaose (β-cyclodextrin, β-CD) and its methylated derivative †

Yasuyuki Tezuka *, Irwan Hermawan

Department of Material Science and Technology, Nagaoka University of Technology, Kamitomioka, Nagaoka, Niigata 940-21, Japan

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Abstract

Peracetylated samples of cyclomaltoheptaose (β-cyclodextrin, β-CD) and its methylated derivative were studied by ^{13}C NMR. The acetyl carbonyl carbon signal in peracetylated β-CD was resolved into a triplet, and the three peaks were assigned by long-range C–H COSY and INAPT techniques. The individual carbonyl peak was found to be indicative of the location of the acetyl group on the 2, 3, and 6 position in the glucose residues. An acetylated derivative of a partly methylated β-CD was also subjected to ^{13}C NMR analysis to determine the distribution of acetyl and, subsequently, methyl groups on the glucose residues.

1. Introduction

Cyclodextrins (CDs) and their derivatives are of increasing interest in many areas of science and technology [1–3]. As CD derivatives are commonly produced by the reaction of hydroxyl groups at C-2, -3, or -6 of the anhydroglucose unit, a convenient and reliable analytical means to assess the distribution of substituents in CD derivatives is of significant importance.

We recently proposed a new analytical technique applicable for diverse polysaccharide derivatives, in particular cellulose ethers, in which their unsubstituted hydroxyl groups are acetylated and then subjected to ^{13}C NMR measurement

* Corresponding author.

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[4–8]. The following advantages are noted for this method. (*1*) The acetylation of cellulose ethers is a facile and simple pretreatment, and provides derivatives readily soluble in common NMR solvents over a wide range of degrees of substitution, thus facilitating comparison with model polymers. (*2*) The carbonyl carbon signal of acetyl groups is remarkably sensitive to its location on the glucose residue, allowing direct determination of the distribution of substituents. (*3*) Acetylation of the hydroxyl groups eliminates spectral complications caused by intra- and inter-molecular hydrogen bonds. (*4*) This procedure provides a convenient analytical method for cellulose and other polysaccharide derivatives through retaining their polymeric form, and thus can avoiding the sometimes troublesome hydrolysis or alcoholysis pretreatments required for conventional chromatographic processes.

We have thus far applied this technique for the structural analysis of a series of cellulose ethers, such as *O*-methyl [4], *O*-(hydroxyethyl) [5], and *O*-(2-hydroxypropyl)celluloses [6], as well as methylhydroxyalkylcelluloses possessing two different ether substituents [7,8]. We have also determined the distribution of two ester substituents in cellulose acetate butanoate [9]. Furthermore, a newly developed enterosoluble cellulose derivative having two types of ether [*O*-methyl and *O*-(2-hydroxypropyl)] and ester (acetyl and succinyl) substituents has been fully characterized [10].

As a further extension of the preceding studies, we now report a ^{13}C NMR study on peracetylated samples of β-CD and methylated β-CD in order to determine the distribution of methyl groups on the glucose residues. This study aims to provide a complementary analytical means to the conventional GLC technique, which requires a carefully controlled hydrolysis pretreatment.

2. Results and discussion

^{13}C NMR studies on acetylated derivatives of oligo- and poly-saccharides have demonstrated that the chemical shift of carbonyl carbon signals is remarkably sensitive to their position of substitution in the sugar residue, to the type of sugar residue, and to the mode of the linkage between them [11]. For peracetylated β-cyclodextrin (β-CD), the carbonyl carbon signal was observed as a triplet in various common NMR solvents (Fig. 1). In particular, a distinct separation of three peaks was achieved in $CDCl_3$.

Since a preceding ^{13}C NMR study on peracetylated β-CD failed to assign the individual carbonyl peaks [12], we utilized two independent NMR techniques, namely, long-range C–H COSY and the INAPT methods. In both procedures, the proton signal assignment for the 2, 3, and 6 positions in the glucose residue is prerequisite. And a H-H COSY measurement confirmed the previous assignment [13,14] by tracing the correlation peaks of the adjacent proton signals.

The results of the long-range C–H COSY and the INAPT measurements are shown in Figs. 2 and 3, respectively. In the INAPT measurement, a soft pulse was performed to irradiate the specific proton signals and cause selective long-range

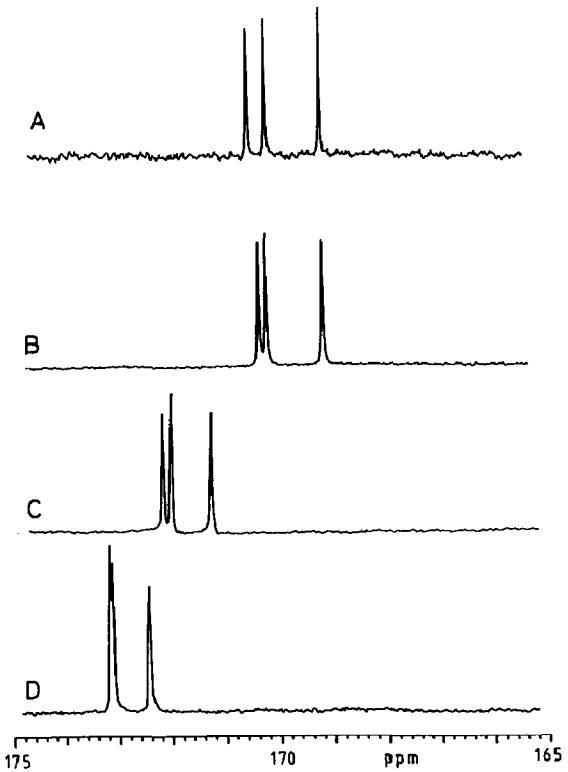

Fig. 1. 100-MHz ^{13}C NMR carbonyl region spectra of peracetylated β-CD in $CDCl_3$ at 40°C [A], C_6D_6 at 40°C [B], acetone-d_6 at 40°C [C], and Me_2SO-d_6 at 100°C [D].

polarization-transfer to the carbonyl carbon of the acetyl groups. Both methods gave identical assignments of carbonyl peaks from the acetyl groups, namely, the 3 (169.4), 6 (170.4), and 2 (170.6 ppm) positions on the glucose residue, respectively.

Based on the assignment of acetyl carbonyl carbon peaks in peracetylated β-CD, a methylated β-CD of the total ds 1.72 was peracetylated and subjected to ^{13}C NMR measurement. IR inspection, in addition to ^{1}H and ^{13}C NMR analysis, confirmed that acetylation of hydroxyl groups was quantitative. A full-range ^{13}C NMR spectra of the starting methylated β-CD (in Me_2SO-d_6) and its acetylated derivative (in $CDCl_3$) are compared in Fig. 4. It should be stressed that, although the methoxy methyl carbon signal in permethylated β-CD is resolved into a triplet and is indicative of the location of the substitution position [15], those in both partly methylated β-CD itself and its acetylated derivative gave two major peaks with additional minor peak splitting that is apparently not indicative of the location of the methyl groups on the glucose residue.

The carbonyl region of the spectrum of acetylated methylated β-CD obtained by a quantitative-mode ^{13}C NMR measurement is listed in Fig. 5, together with that of the peracetylated β-CD as a reference. The acetyl carbonyl carbon signal in

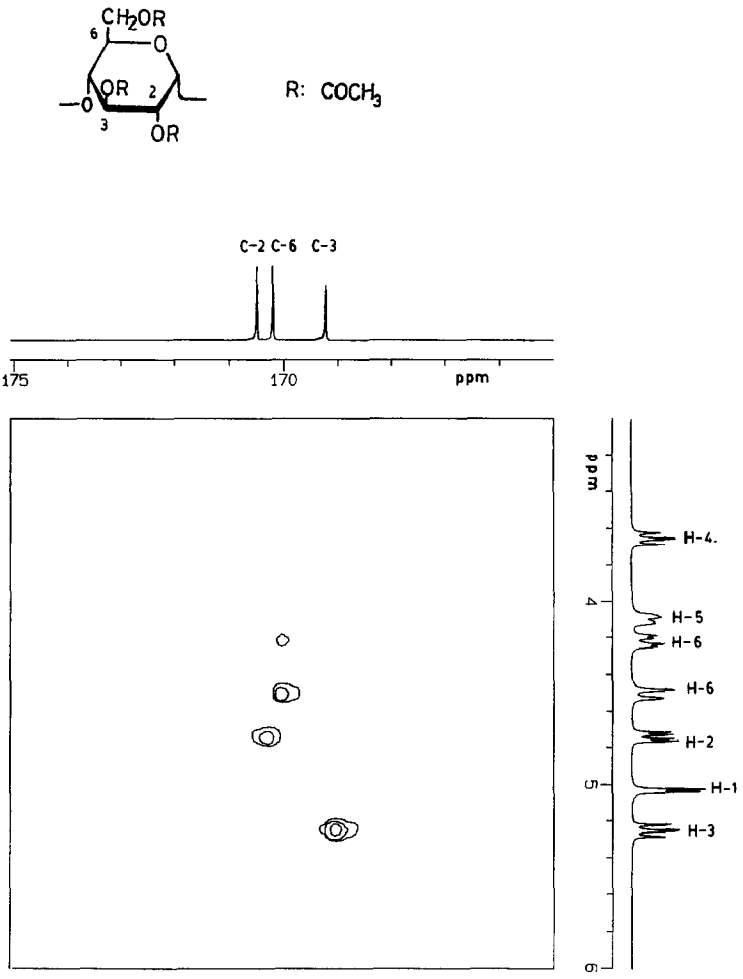

Fig. 2. Long-range C–H COSY carbonyl region spectrum of peracetylated β-CD (270 MHz, $CDCl_3$, 40°C).

the acetylated methylated β-CD sample showed a notable peak broadening, with further fine structure. Nevertheless, the carbonyl signal could be separated into three regions, and these could be referenced to three narrow peaks of peracetylated β-CD. The peak-broadening of the carbonyl carbon signals is presumably caused by the presence of numerous conformationally different forms in $CDCl_3$ produced through the interaction between methyl and acetyl substituents at the different substitution positions in the glucose residue. A relevant result was previously observed with acetylated *O*-methylcellulose in $CDCl_3$ [4]. This peak-broadening was even more extensive for an acetylated *O*-(2-hydroxypropyl) β-CD sample of ds of 0.70. The inclusion of a hydroxypropyl groups into the cavity of β-CD has been postulated [16,17], and this could cause a complex interaction

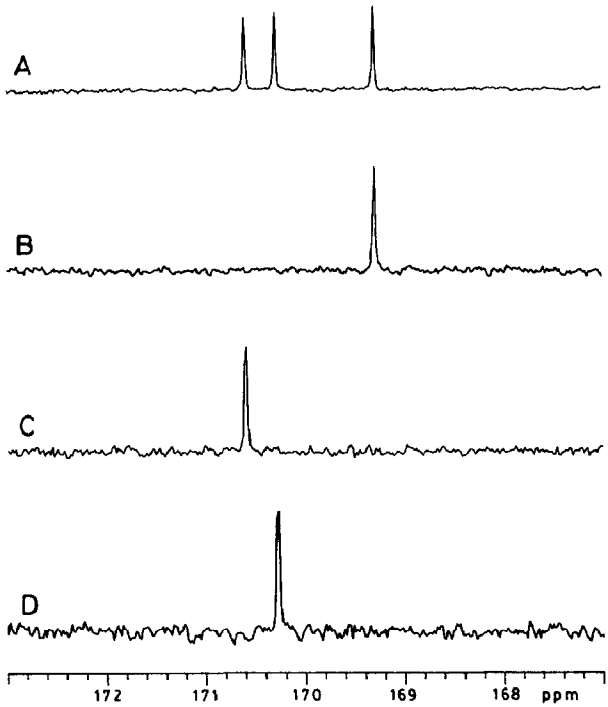

Fig. 3. Normal proton-decoupled ^{13}C NMR carbonyl region spectrum of peracetylated β-CD [A], and the INAPT spectra with irradiation of H-3 [B], H-2 [C], and H-6 of the lower magnetic field [D] (100 MHz, $CDCl_3$, 40°C).

between two substituents on the different substitution positions in the glucose residue. On the other hand, for the acetylated *O*-methylcellulose, such intramolecular interaction could be minimized in Me_2SO-d_6 at 100°C, and a resolved triplet was maintained up to high degrees of substitution [4]. In the present β-CD system, however, the separation of three carbonyl peaks in peracetylated β-CD itself was not sufficient (Fig. 1) in Me_2SO-d_6 at 100°C so as to apply this solvent system for the determination of the distribution of substituents.

As the acetyl carbonyl signal in acetylated methylated β-CD could be divided into three regions, and they could be referenced to three peaks of peracetylated β-CD, the relative content of three carbonyl regions was estimated by a simple peak-separation procedure. The absolute content of the acetyl groups at the 2, 3, and 6 positions was subsequently determined by comparing the intensity of each signal with that of the C-1 signal observed at ~ 100 ppm. Finally, subtraction of the acetyl-group distribution in each substitution position provides the methyl group distribution in the starting methylated β-CD sample. The results are summarized in Table 1, where the ds values obtained by GLC analysis [18] are also listed. The individual and the total ds values obtained by the two techniques showed reasonably good agreement with each other despite the peak-broadening. The apparent order of reactivity of hydroxyl groups at the 2, 3, and 6 positions in

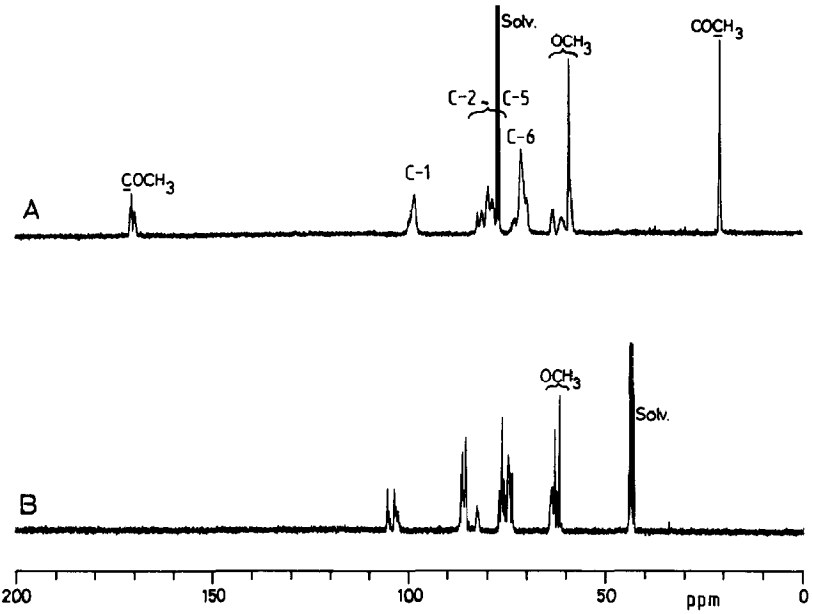

Fig. 4. ^{13}C NMR full-range spectra of methylated β-CD [B] (100 MHz, Me_2SO-d_6, 100°C), and its acetylated derivative (100 MHz, $CDCl_3$, 40°C).

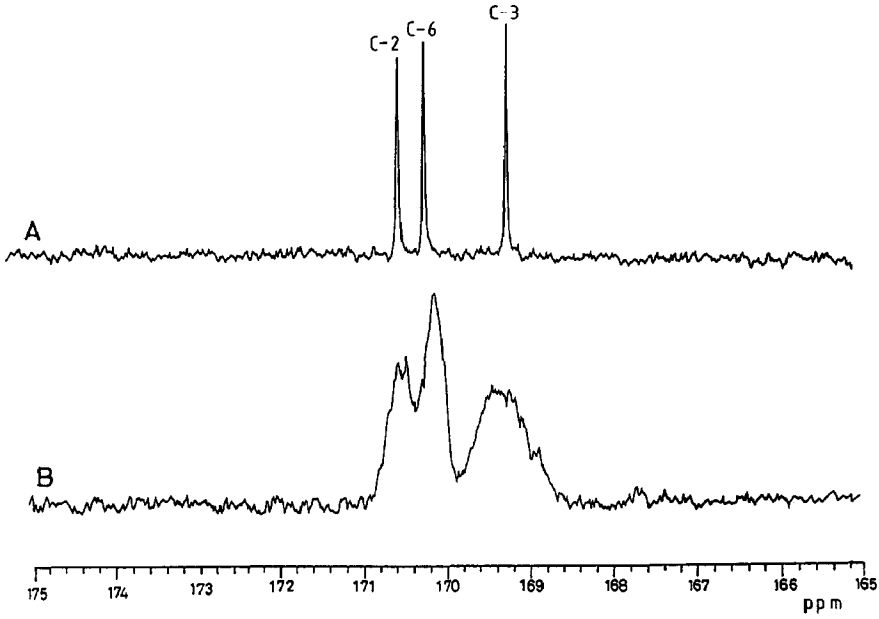

Fig. 5. ^{13}C NMR carbonyl region spectra of peracetylated β-CD [A] and acetylated methylated β-CD [B] (100 MHz, $CDCl_3$, 40°C).

Table 1
Distribution of methyl groups in methylated β-cyclodextrin

Method	Individual position			Total ds
	2	3	6	
NMR	0.71	0.49	0.60	1.80
GLC [a]	0.63	0.40	0.69	1.72

[a] Data supplied by Dr. Pitha [18].

the glucose residue was observed to be 2 > 6 > 3 in the methylation of β-CD, and this coincided with that observed in the methylation of cellulose [4].

In conclusion, the distribution of methyl groups in methylated β-CD may be determined by means of a ^{13}C NMR technique, with the resonance of the acetyl carbonyl carbon constituting a sensitive NMR probe.

3. Experimental

Samples.—A sample of peracetylated β-CD was prepared by the treatment of β-CD (Nacalai Tesque) with Ac_2O–pyridine for 10 h at 120°C. The product was isolated and purified by reprecipitation from the Me_2CO–cold water system and finally freeze-drying from benzene solution. A sample of methylated β-CD was supplied by Dr. J. Pitha (BW/1/7/92, total ds of 1.72) and 0.28 g of it was treated with Ac_2O (2.5 mL) and pyridine (5.0 mL) for 2 h at 120°C. The solution was evaporated and the product was isolated by freeze-drying from benzene; yield, 0.24 g (92%).

NMR measurements.—^1H and ^{13}C NMR measurements were performed with either a Jeol EX-400 or ESX-270 (W) spectrometers at 400 MHz or at 270 MHz for ^1H, and at 100 MHz or at 67.8 MHz for ^{13}C, respectively, with a 5-mm C–H dual probe in either $CDCl_3$ at 40°C, Me_2SO-d_6 at 100°C, acetone-d_6 at 40°C, or C_6D_6 at 40°C. Chemical-shift values were referenced from the signal of each solvent ($CDCl_3$; 7.30 for ^1H and 77.0 for ^{13}C, Me_2SO-d_6 43.5, acetone-d_6 (carbonyl); 206.0, and C_6D_6; and 128.0 for ^{13}C, respectively). Quantitative-mode ^{13}C NMR measurements were performed by a non-NOE gated-decoupling technique with a pulse-repetition time of 30 s and with 2000 transients.

H–H COSY measurements were performed by means of an ESX-270 (W) apparatus with a 1024 × 256 data matrix, and 16 transients were acquired for each t_1 value. A spectral width of 2500 Hz was used in both dimensions and a pulse delay of 1.0 s was used between scans. The spectrum was processed by using a sine-bell filtering function in both dimensions after zero-filling to a 1024 × 512 matrix.

Long-range C–H COSY measurements were performed with an ESX-270 (W) apparatus with a 2048 × 256 data matrix, and 256 transients were acquired for each t_1 value. A long-range J_{C-H} value of 10 Hz was applied and spectral widths of 14006 and 2500 Hz were used in carbon and proton spectral dimensions,

respectively, and a pulse delay of 1.0 s was used between scans. The spectrum was processed by using a sine-bell filtering function in both dimensions after zero-filling to a 2048×512 matrix.

INAPT (selective INEPT) measurements were performed by use of the EX-400 instrument with the delay time $\Delta_{1/2}$ and $\Delta_{2/2}$ being set to 68 and 73 ms, respectively, and the pulse width of the selective soft 90° pulse was set to 10 ms. Calibration of this 90° pulse was performed according to the pulse sequence of Bax [19] with incrementation of the attenuation using 90% ethylbenzene in acetone-d_6. The optimum value of the present system was found for an attenuation value of 390. Broad-band decoupling was applied during data acquisition and a pulse delay of 1.5 s was used between scans. Each of the INAPT spectra was obtained with up to 500 transients.

Other measurements.—IR spectra of the samples were recorded on a Shimadzu Model FT-IR 8100 infrared spectrophotometer by casting the sample on an NaCl plate.

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