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Note

One- and two-dimensional high-resolution ^{1}H-n.m.r. spectra of permethylated cyclomaltohexaose and cyclomaltoheptaose: spectral assignments and conformational analysis

YOSHIO INOUE*, YŪJI TAKAHASHI, AND RIICHIRŌ CHŪJŌ

Department of Polymer Chemistry, Tokyo Institute of Technology, O-okayama 2-chome, Meguro-ku, Tokyo 152 (Japan)

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The cyclomalto-oligosaccharides [cyclodextrins (CDs); cyclic (1→4)-linked α-D-glucosaccharides] can form inclusion complexes with a wide variety of organic molecules. There have been numerous investigations of this phenomenon and of the use of these oligosaccharides in catalysis, as enzyme models, and as carriers of drugs[1–5], which depends on the size of the cavity. The complexation and catalytic properties of chemically modified CDs have been extensively investigated[4,5]. Partially and fully methylated derivatives can also form inclusion complexes in aqueous solution[6], some of which are more stable than those of the parent CD.

High-resolution n.m.r. spectroscopy has been used for the analysis of the structure and molecular dynamics of CDs and their inclusion complexes in solution[7], and high-resolution cross-polarisation magic-angle spinning ^{13}C-n.m.r. spectroscopy has been applied to cyclomaltohexaose and cyclomaltoheptaose inclusion-complexes in the solid state[8].

The ^{1}H-n.m.r. (60 and 100 MHz) data for hexakis- and heptakis-(2,3,6-tri-*O*-methyl)cyclomalto-hexaose and -heptaose (α- and β-TMCD, respectively) in chloroform[9,10] are difficult to analyse, since the resonances, except that of H-1, are severely masked by extraordinarily large methoxyl-proton resonances[11]. We now report on the 500-MHz ^{1}H-n.m.r. spectra of aqueous solutions of α- and β-TMCD. The assignments were made with the aid of two-dimensional ^{1}H chemical-shift correlation spectroscopy (COSY) experiments[12]. Data on the inclusion complexes will be published elsewhere.

Figs. 1 and 2 show the 500-MHz ^{1}H-n.m.r. spectra of α- and β-TMCD, respectively, in aqueous solution; the resonances of the methoxyl and other protons are well resolved. The resonances of H-1 and the methoxyl groups have been assigned[9]. The resonances of the ring protons of β-TMCD are better resolved than those of α-TMCD. For β-TMCD, the scalar connectivities of the ring-proton

*To whom inquiries should be addressed.

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resonances were established by homonuclear spin-decoupling experiments based on the pre-assigned H-1 resonance. This lengthy process was not effective for α-TMCD, since some of the resonances were too severely contiguous. Therefore, the two-dimensional technique, *i.e.*, the ^{1}H COSY method[12], was used to assign the ring-proton resonances. The results are illustrated in Figs. 3 and 4. Beginning on the diagonal with the resonance for H-1, the resonances of H-2 may be located by tracing of its cross peaks with H-1, and so on for the other ring protons and H-6,6; the assignments made by these procedures are shown in Fig. 1. The accurate centers of the H-3,4,5 resonances were confirmed by extrapolating the plots of chemical shift displacements, induced for these resonances by the aromatic guest compound, *versus* [Host CD]/[Guest] molar ratio, details of which will be published elsewhere.

The 500-MHz ^{1}H-n.m.r. spectra of cyclomaltohexaose (α-CD) and cyclomaltoheptaose (β-CD) and the assignments[13] are shown in Figs. 5 and 6, respectively, and the coupling constants are listed in Table I.

The line-shape analyses of the H-3,4,5,6 resonances of α-TMCD and of H-6 of β-TMCD are difficult even at 500 MHz. On the ^{1}H-n.m.r. time-scale, all six 2,3,6-tri-*O*-methyl-α-D-glucopyranosyl residues of α-TMCD have the same conformation, and the macrocyclic ring has hexagonal symmetry as do α-CD and the β-CD derivative. The magnitudes of $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ for α- and β-TMCD are in agreement with the corresponding values for α- and β-CD, and are reasonably consistent with the $^4C_1$ chair form as indicated by partial analysis of 60-MHz

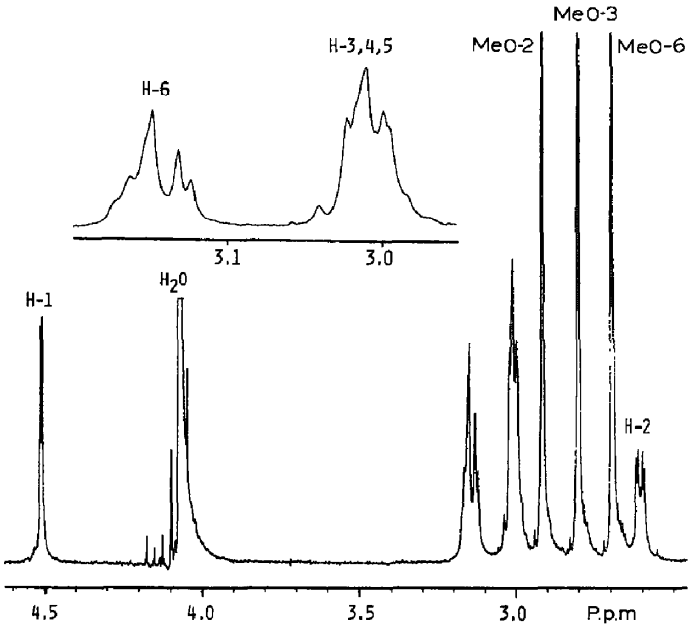

Fig. 1. 500-MHz ^{1}H-N.m.r. spectrum of 0.01M hexakis(2,3,6-tri-*O*-methyl)cyclomaltohexaose in ^2H_2O at p^{2}H 3.

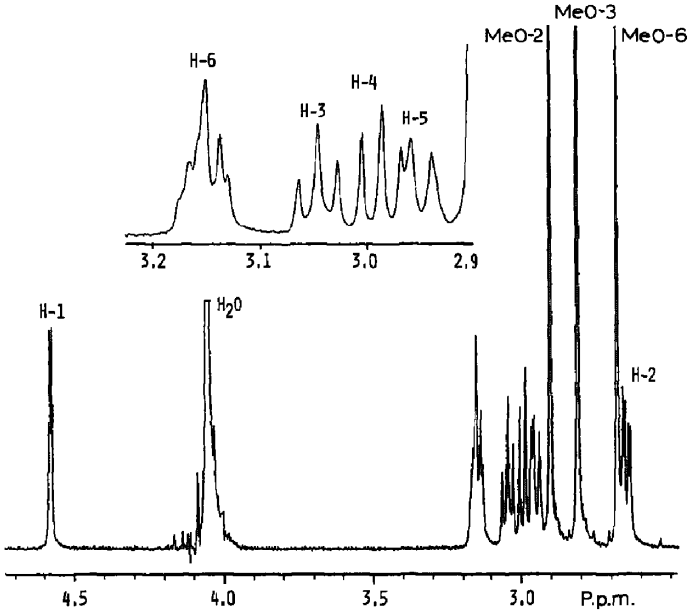

Fig. 2. 500-MHz ^{1}H-N.m.r. spectrum of 0.01M heptakis(2,3,6-tri-*O*-methyl)cyclomaltoheptaose in ^2H_2O at p^{2}H 10

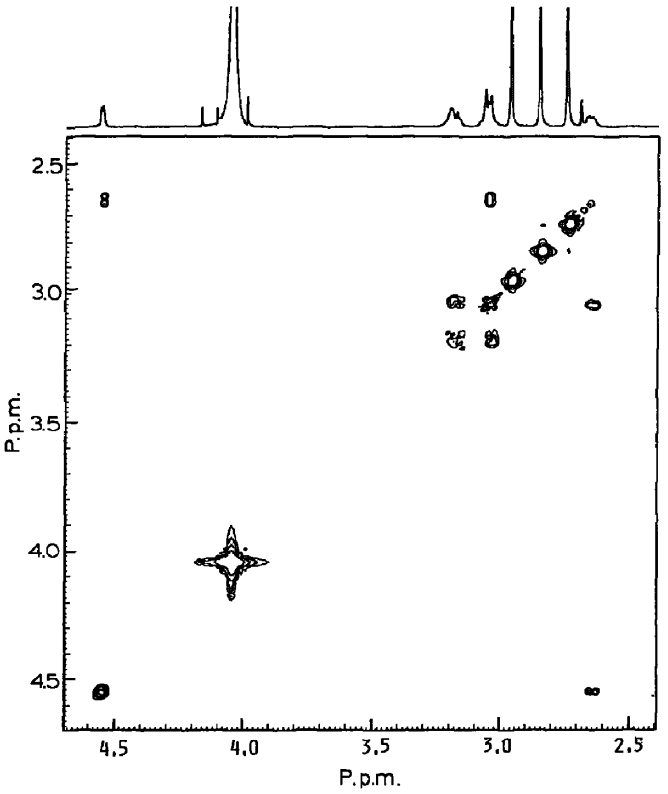

Fig. 3. 400-MHz ^{1}H-COSY spectrum of 0.01M hexakis(2,3,6-tri-*O*-methyl)cyclomaltohexaose in ^2H_2O.

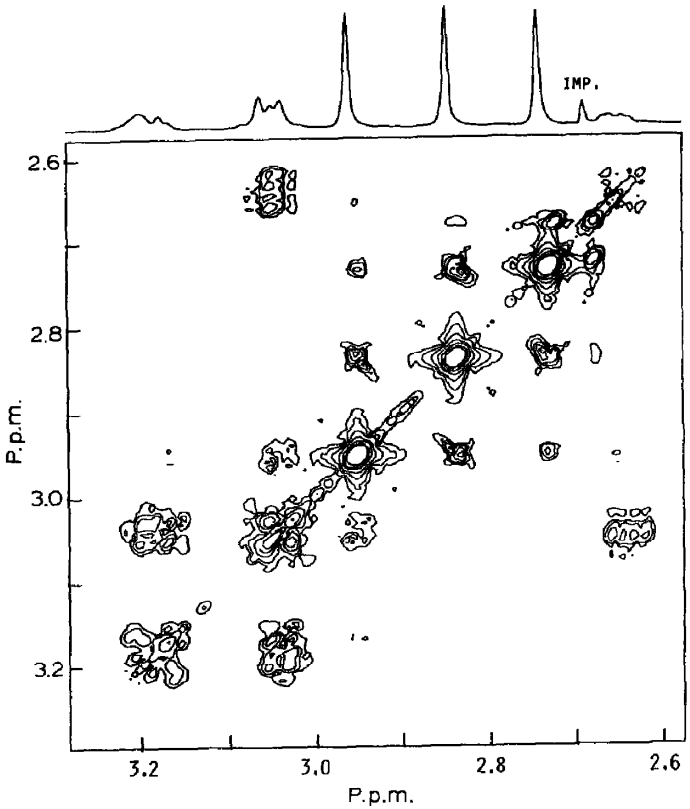

Fig. 4. The same as that shown in Fig. 3, but only the 2.3–3.4 p.p.m. region was observed. IMP is due to an impurity.

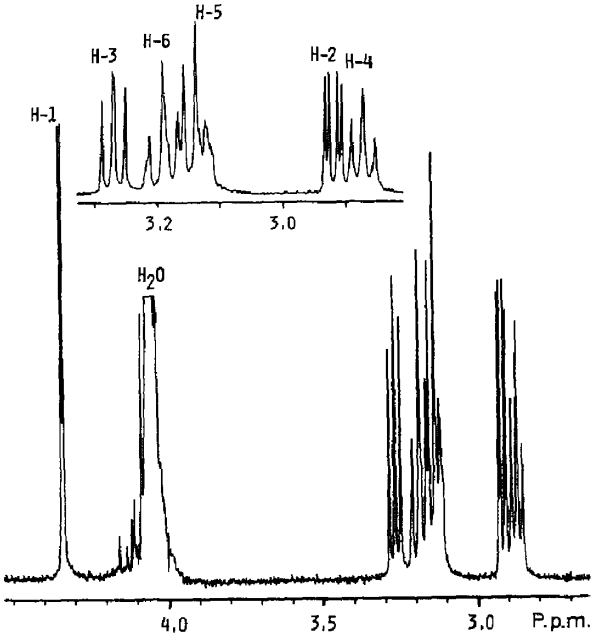

Fig. 5. 500-MHz ^{1}H-N.m.r. spectrum of 0.01M cyclomaltohexaose in ^2H_2O at p^{2}H 10.

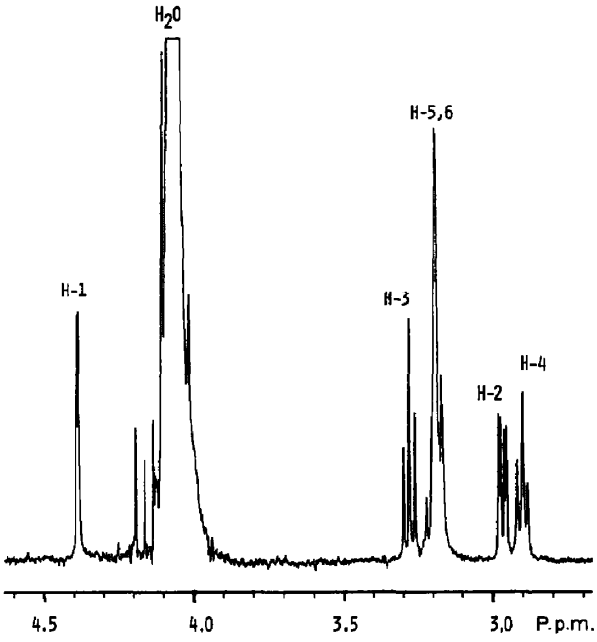

Fig. 6. 500-MHz ^{1}H-N.m.r. spectrum of 0.01M cyclomaltoheptaose in ^2H_2O at p^{2}H 3.

spectra[9]. Therefore, permethylation does not cause significant distortions of the conformation. Furthermore, the conformations of the α-D-glucopyranosyl rings in α- and β-TMCD, as well as in α- and β-CD, are not affected by the p^{2}H changes in the range 3–10.

There are no crystallographic data for α- and β-TMCD, but the data for their inclusion complexes with *p*-iodoaniline, benzaldehyde, and *p*-nitrophenol indicate that the $^4C_1$ conformations are maintained in agreement with that in aqueous solution[14]. The X-ray data further show that the macrocyclic rings of α- and β-TMCD in the above inclusion complexes are distorted because of steric hindrance involving the methyl groups and the inability to form intramolecular hydrogen-bonds, which

TABLE I

VICINAL COUPLING CONSTANTS (Hz) FOR AQUEOUS SOLUTIONS OF α- AND β-TMCD AND α- AND β-CD

	α-TMCD		*β-TMCD*		*α-CD*		*β-CD*	
	p²H 3	*p²H 10*	*p²H 3*	*p²H 10*	*p²H 3*	*p²H 10*	*p²H 3*	*p²H 10*
$J_{1,2}$	3.7	3.7	3.7	3.7	3.1	3.7	3.1	3.7
$J_{2,3}$	9.8	9.8	9.8	9.5	10.1	10.1	9.8	10.4
$J_{3,4}$	[a]	[a]	8.9	8.9	9.2	9.0	9.5	9.5
$J_{4,5}$	[a]	[a]	9.2	9.2	9.2	9.2	8.9	8.8

[a]Not determined.

stabilise the macrocyclic conformation of α- and β-CD. The averaged macrocyclic conformations of α- and β-TMCD in solution are expected to be different from those of α- and β-CD, respectively, but these differences are not reflected in the ^1H coupling constants as observed here. The differences in macrocyclic conformation and/or the diversity of the conformations about the glycosidic linkage may be reflected[8] in the chemical shifts of C-1 and C-4.

EXPERIMENTAL

Materials. — α- and β-CD and β-TMCD were commercial samples. α-TMCD was synthesised[9] from α-CD, and recrystallised several times from hot water.

Methods. — ^1H-N.m.r. spectra (500 Hz) were recorded at 27°, using a JEOL JNM GX-500 spectrometer with digital resolution of 0.0012 p.p.m. (0.61 Hz). Two-dimensional ^1H COSY spectra (400 MHz) were recorded with a Bruker AM400 spectrometer. Chemical shifts were measured in p.p.m. downfield from external Me_4Si.

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