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Note

Two-dimensional n.m.r. spectra of *O*-methylated cyclomalto-oligosaccharides: assignment of ^{1}H and ^{13}C resonances and conformational analysis

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Cyclomalto-oligosaccharides (cyclodextrins, CDs) are cyclic oligosaccharides consisting of six or more (1→4)-linked α-D-glucopyranosyl residues[1], which can form inclusion complexes with various substances[1-3]. The CD molecule has been modified chemically in attempts to improve solubility and complexation ability. Methylation of CD affords 2,6-di- and 2,3,6-tri-*O*-methyl derivatives, which not only have higher solubilities in water[4] but also appear to have significantly enhanced complexation[5] and improved chiral recognition[6] abilities. There are many data on the crystal structures of *O*-methylated CDs and their inclusion complexes[7-11]. Harata *et al.*[6] recently reported an induced-fit type of inclusion complexation between 2,3,6-tri-*O*-methyl-α-CD (α-TMCD) and (*S*)- and (*R*)-mandelic acids, and it is of interest to see whether this phenomenon occurs in solution.

N.m.r. spectroscopy has been used quite successfully to investigate the structure of CDs and their inclusion complexes[12-18], and we now report on a 2D-n.m.r. study of α-TMCD and 2,6-di-*O*-methyl- and 2,3,6-tri-*O*-methyl-β-CDs (β-DMCD and β-TMCD, respectively).

The ^{1}H–^{1}H COSY spectrum of β-DMCD in D_2O is illustrated in Fig. 1 together with the 500-MHz ^{1}H-n.m.r. spectrum; the connectivities, which are related *via* scalar-coupling, are shown with the successive vertical and horizontal lines. Beginning with the resonance for H-1 at 5.18 p.p.m., all the ring proton resonances can be assigned. Based on the assignment of the ^{1}H resonances, the ring ^{13}C resonances can be assigned by ^{1}H–^{13}C COSY. The ^{1}H–^{13}C COSY spectrum of β-DMCD in D_2O, together with the ^{1}H and ^{13}C spectra, is shown in Fig. 2. The appearance of eight resonances in the ^{1}H–^{13}C 2D-spectrum clearly establishes the eight different ^{1}H–^{13}C connectivities in β-DMCD, and therefore the assignment of the ^{13}C resonances can be made unambiguously. Similar experiments were carried out on α-TMCD, β-TMCD, and γ-CD in D_2O and $(CD_3)_2SO$, and the results are summarised in Table I. The $J_{1,2}$ values were in the range 3.1–3.7 Hz (results not shown), indicating that there was no significant distortion of the original *C1* conformation of the constituent glucopyranosyl residues.

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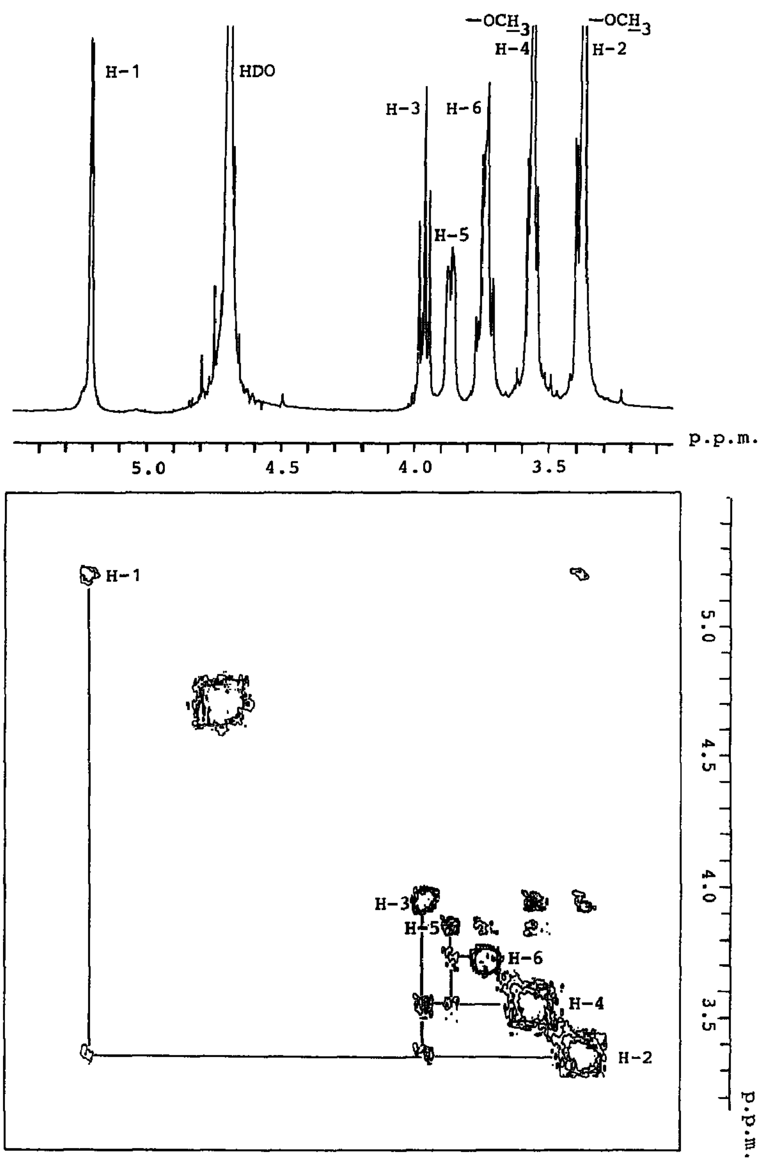

Fig. 1. ^1H–^1H COSY spectrum of β-DMCD in D_2O at 35°. The connectivities between the protons are indicated. The 500-MHz ^1H-n.m.r. spectrum is shown along the F_2 axis.

The H-6a,6b resonances become inequivalent in TMCDs, whereas they are nearly degenerate in β-DMCD as well as in CDs. The non-equivalence of H-6a,6b in TMCDs may be attributed to a restricted rotation about the C-5–C-6 bond (see below). The hydrogen bonding proposed between HO-3 and O-2 of the adjacent glucopyranosyl residue in β-DMCD[4] has been confirmed by the temperature-

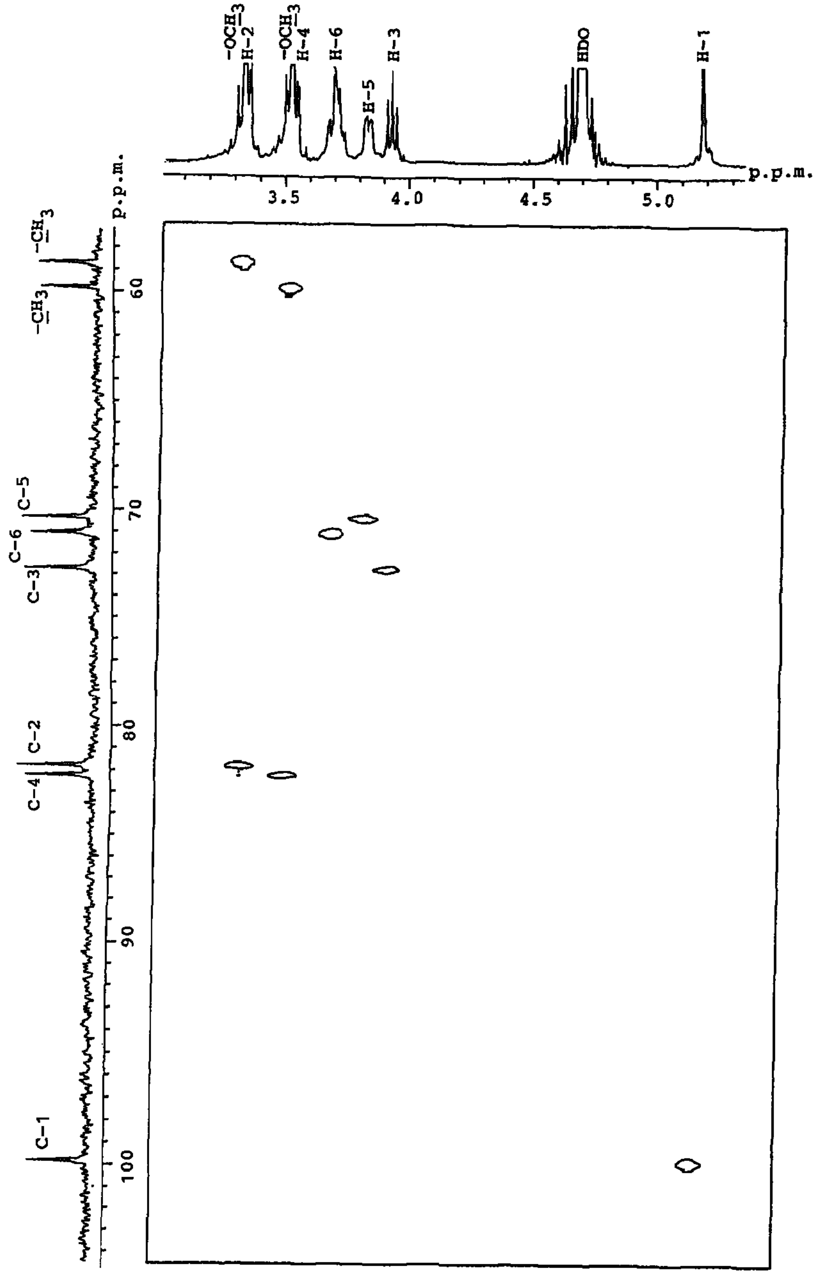

Fig. 2. ^{1}H–^{13}C COSY spectrum of β-DMCD in D_2O at 35°. The ^{1}H- and ^{13}C-n.m.r. spectra are shown along the F_1 and F_2 axes, respectively.

TABLE I

^{1}H- AND ^{13}C-N.M.R. CHEMICAL SHIFTS[a] FOR CDs AND *O*-METHYLATED CDs IN D_2O AND $(CD_3)_2SO$

	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*2-OH*	*3-OH*	*6-OH*	*-OCH$_3$*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*-OCH$_3$*
D_2O[b]																	
α-CD[c]	5.05	3.62	3.99	3.57	3.83	3.89					102.23	72.44	74.06	82.05	72.80	61.13	
β-CD[c]	5.09	3.64	3.88	3.59	3.78	3.90					102.81	72.85	74.05	81.95	72.85	61.01	
β-DMCD	5.18	3.37	3.94	3.55	3.85	3.72				3.36	99.86	81.65	72.71	82.16	70.30	70.97	58.58
										3.55							59.73
α-TMCD[d]	5.15	3.24	3.63	3.62	3.76	3.61				3.33	98.12	80.43	81.21	79.65	71.00	71.43	58.11
						3.76				3.44							58.74
										3.55							60.39
β-TMCD[d]	5.22	3.29	3.63	3.69	3.78	3.59				3.32	97.73	80.73	81.60	77.84	71.00	71.40	58.68
						3.78				3.45							58.96
										3.54							60.40
$(CD_3)_2SO$[e]																	
α-CD	4.60	3.08	3.57	3.19	3.39	3.44	5.32	5.26	4.28		102.8	73.0	74.1	83.0	73.0	60.9	
β-CD	4.68	3.16	3.51	3.21	3.41	3.47	5.52	5.48	4.26		102.2	73.3	73.9	81.8	73.0	60.8	
β-CD	4.74	3.19	3.47	3.22	3.39	3.51	5.53	5.57	4.32		102.5	73.4	73.7	81.8	73.0	60.8	
β-DMCD	4.77	2.99	3.51	3.14	3.50	3.37		4.70		3.05	100.5	82.2	73.2	83.2	70.3	71.2	58.4
										3.31							60.6
α-TMCD	4.32	2.74	3.10	3.02	3.41	3.67				2.93	99.4	82.4	82.3	81.8	71.0	71.8	58.1
						3.21				3.05							58.7
										3.17							61.8
β-TMCD	4.85	2.88	3.13	3.13	3.53	3.52				3.05	98.4	82.2	81.9	80.1	71.0	71.8	58.7
						3.25				3.20							59.0
										3.30							61.5

[a] In p.p.m. from the signal for Me_4Si. [b] At 30°. [c] At 22°, from ref. 17. [d] ^{1}H assignment made in ref. 25 is revised. [e] At 35°.

dependence study of hydroxyl-proton resonance[19], and therefore the conformation of the macrocyclic ring should be similar to that of the parent CD.

The changes in chemical shift of the signals for the ring carbons of CDs induced by methylation at positions 2 and 6 parallel those reported for α-D-glucopyranose[20]. From a comparison of the ^{13}C shifts observed for β-CD, β-DMCD, and β-TMCD, the effect of methylation at position 3 can be deduced (see Table II) and shown to induce large shifts in the signals for C-1 and C-4 of CD, although typical σ-type substituent effects are observed for α-D-glucopyranose. A simple substituent effect cannot account for such large chemical shift changes. Dependency of the chemical shifts of the signals for C-1 and C-4 of α-CD on the dihedral angles ϕ and ψ has been established[21–24]. Semi-qualitatively, the observed changes in the chemical shifts of the signals for C-1 and C-4 of CD arising from methylation at position 3 account for a change of $>10°$ in both ϕ and ψ. Therefore, due to the lack of hydrogen bonding between glucopyranosyl residues in TMCDs, conformations similar to those of the corresponding parent CDs cannot be retained. The wider rim of the torus appears to become larger and more flattened. Molecular models indicate that there is restricted rotation around the C-5–C-6 bond of the glucopyranosyl residues because of steric hindrance by MeO-6. X-Ray structural analyses of TMCD inclusion-complexes have revealed similar flattened shapes for TMCD[8] and it is now shown that this structure is retained in solution.

EXPERIMENTAL

α-, β-, and γ-CD (Nakarai Chemicals) and β-TMCD (Toshin Chemical Co.) were used without further purification. α-TMCD was synthesised[4] from α-CD. β-DMCD (Toshin Chemical Co.) was purified by using the procedures reported by Koizumi *et al.*[26]. The ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra were recorded for solutions in D_2O and $(CD_3)_2SO$ with a JEOL GX-500 spectrometer, using the standard pulse sequences and procedures. For a typical ^{1}H–^{1}H COSY spectrum, 250 free-induction decays were acquired with 1k data points and a sweep-width of 1250 Hz. The time-domain data matrix was expanded to 512 × 1k by zero filling and a sine bell function was applied to apodise the spectrum in both dimensions. The initial data matrix for

TABLE II

CHANGES IN CHEMICAL SHIFTS[a] OF THE RING ^{13}C RESONANCES INDUCED BY METHYLATION AT POSITION 3

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
CD[b]						
in D_2O	−2.13	−0.92	8.89	−4.32	0.70	0.43
in $(CD_3)_2SO$	−2.1	0.0	8.7	−3.1	0.7	0.6
α-D-Glucopyranose[c]	0.1	−0.5	9.7	−0.6	−0.1	0.1

[a] In p.p.m. [b] Shift changes are calculated from the equation $(\delta_{\beta\text{-TMCD}} - \delta_{\beta\text{-CD}}) - (\delta_{\beta\text{-DMCD}} - \delta_{\beta\text{-CD}})$, for the corresponding resonances. [c] In aqueous solution: from ref. 18.

the ^1H–^{13}C COSY spectrum was 2k (^{13}C–6500 Hz) × 128 (^1H–1250 Hz) in the ω_2 and ω_1 dimensions, respectively, and was expanded to the final matrix size 2k × 512 by zero filling. The data matrix was apodised with a sine bell function in both dimensions and the spectrum is presented in the absolute value mode. Chemical shifts are given relative to that of Me_4Si.

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