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Note

Two-dimensional n.m.r. spectra of 6-*O*-α-D-glucopyranosylcyclomaltohexaose; ^1H and ^{13}C resonance assignments and primary structure determination

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6-*O*-α-D-Glucopyranosylcyclomaltohexaose (**1**), which can be isolated[1,2] after the action of *Bacillus macerans* cycloamylose glucanotransferase on starch, is more water-soluble and more resistant to enzymic degradation than cyclomaltohexaose (α-CD), but forms inclusion complexes[3] in a similar manner.

Various techniques have been applied to characterise the physicochemical properties of 6-*O*-α-D-glucopyranosylcyclomalto-oligosaccharides[3], but their structures in solution and inclusion phenomena have not been studied in detail. We now report on a 2D-n.m.r. study of 6-*O*-α-D-glucopyranosylcyclomaltohexaose (**1**).

2D-N.m.r. spectroscopy has been applied to various oligosaccharides[4–8] and we have employed phase-sensitive double-quantum-filtered COSY (DQF-COSY) spectra to assign the ^1H resonances of **1**. The DQF-COSY contour plot and the ^1H-n.m.r. spectrum are illustrated in Fig. 1. There are three sets of independent spin net-work systems arising from three different types of glucopyranosyl residues. Based on the analysis of the intensities of the resonances for the anomeric protons at 4.9–5.1 p.p.m. (see the inset of the ^1H-n.m.r. spectrum in Fig. 1), the doublets at 4.93 and 5.05 p.p.m. and the central doublet at 5.04 p.p.m. corresponded to single and five protons, respectively. The resonance at 5.05 p.p.m. is connected to the down-field resonance of H-6 at 4.02 p.p.m. in the DQF-COSY plot, indicating that this residue is 6-linked. The doublet at 4.93 p.p.m. is involved in the spin net-work containing the resonance for H-4 at 3.43 p.p.m., suggesting that this residue is not 4-linked. Consequently, the three resonances for anomeric protons can be assigned to residues B, C, and A, respectively, in **1**. The $J_{1,2}$ value of 3.6 Hz for residue A indicates that it is α-(1→6)-linked to residue B and confirms the primary structure of **1** assigned by Kobayashi *et al.*[1].

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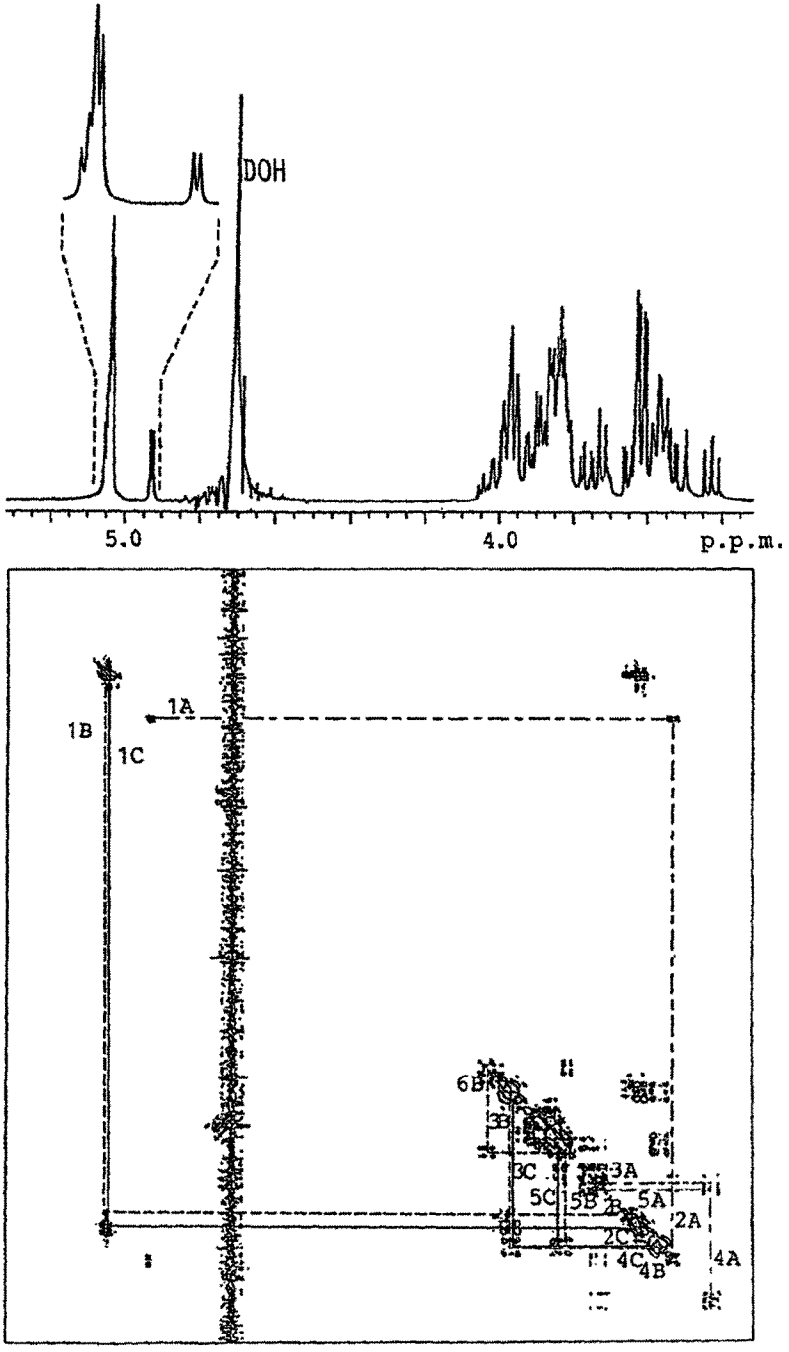
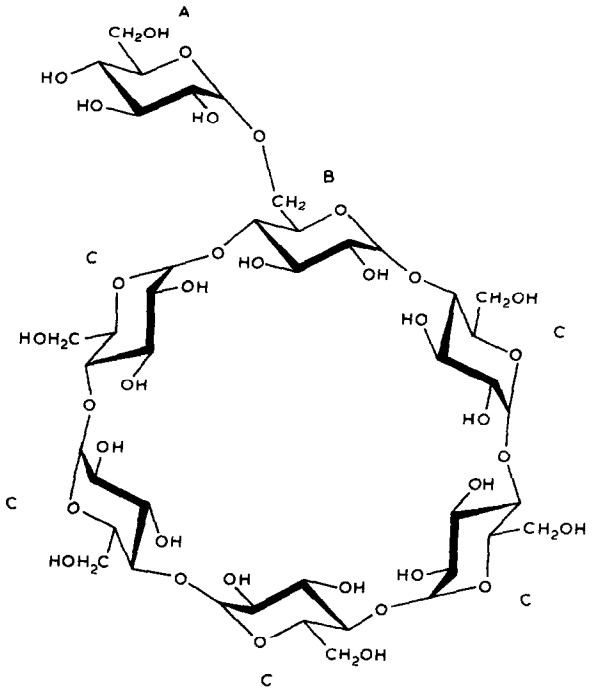


Fig. 1. DQF-COSY spectrum of **1** in D_2O at 30°. Both positive and negative contours are shown. The connectivities between the protons are indicated. The 500-MHz ^{1}H-n.m.r. spectrum is shown along the F_2 axis.



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From the assignment of the ^1H resonances, the ^{13}C resonances can be assigned using ^1H–^{13}C COSY spectra. The ^1H–^{13}C COSY contour plot of **1** together with ^1H- and ^{13}C-n.m.r. spectra are shown in Fig. 2. The connectivities in ^1H–^{13}C COSY spectra are established by the ^1H and ^{13}C nuclei, the Larmor frequencies of which are related through scalar-coupling. The assignments of ^1H and ^{13}C resonances of **1** together with those of the parent compound are summarised in Tables I and II, respectively. The observed ^1H and ^{13}C shifts of the signals of residue C in **1** are similar to those of the corresponding resonances of α-CD, suggesting that the substitution of residue A perturbs the electronic structure only of residue B.

TABLE I

^1H-N.M.R. DATA FOR **1**

Glucopyranosyl residue	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*[b]
(1)						
A	4.93[a]	3.57	3.74	3.43	3.72	3.75
B	5.05	3.65	3.98	3.57	3.83	4.02
C	5.04	3.62	3.97	3.57	3.84	3.88
α-CD[c]	5.05	3.62	3.99	3.57	3.83	3.89

[a]P.p.m. from the signal for Me_4Si. [b]The value indicates the average shift of H-6,6. [c]At 22°; data from ref. 4.

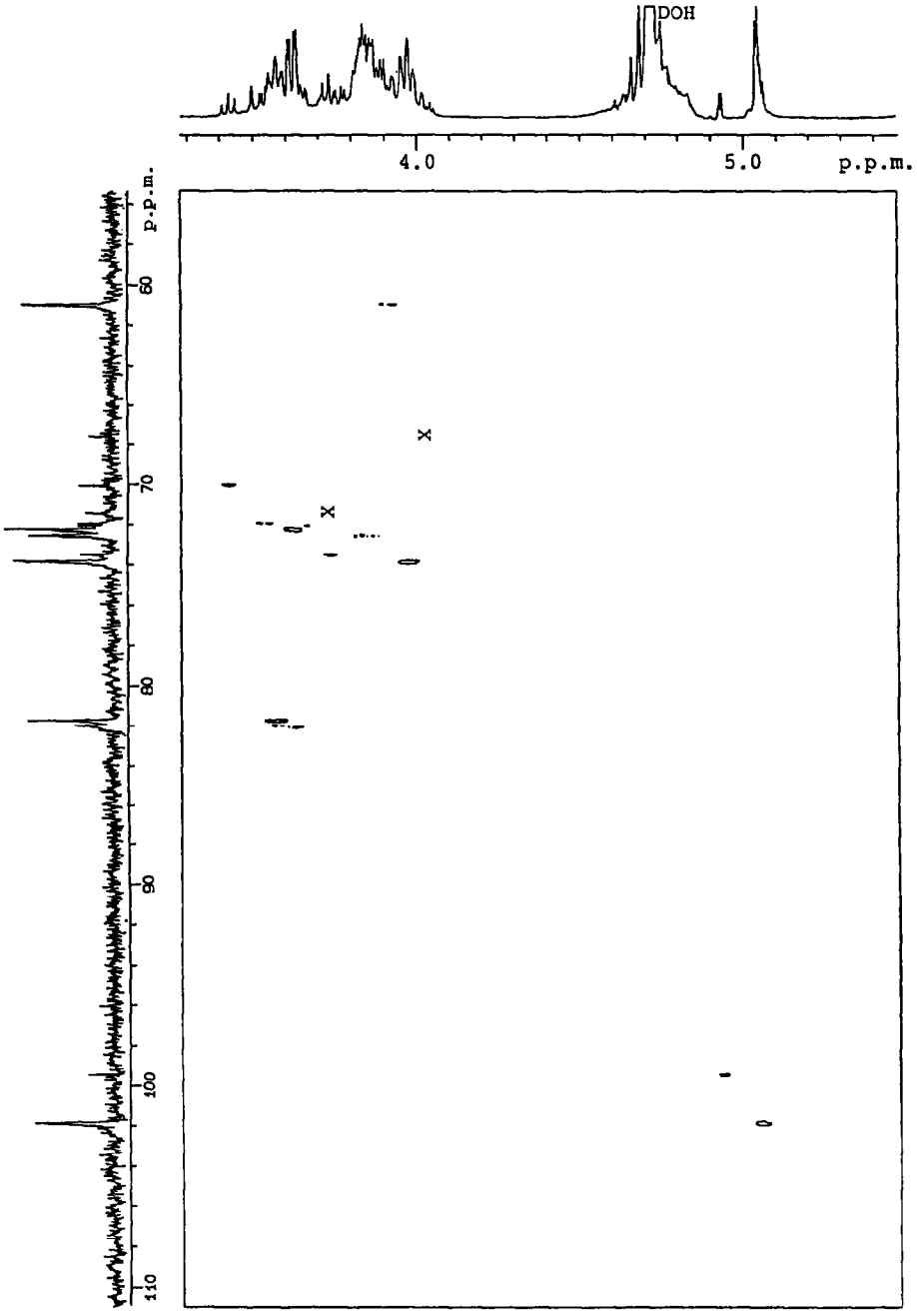

Fig. 2. ^{1}H–^{13}C COSY spectrum of **1** in D_2O at 30°. "×" designates cross-peaks which can be observed at lower contour levels. The ^{1}H- and ^{13}C-n.m.r. spectra are attached along F_1 and F_2 axes, respectively.

TABLE II

^{13}C-N.M.R. DATA FOR **1**

Glucopyranosyl residue	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
(1)						
A	99.4[a]	72.2	73.7	70.3	71.6	61.2
B	102.1	72.3	74.0	82.2	72.7	67.8
C	102.1	72.4	74.0	81.9	72.7	61.2
α-CD[b]	102.23	72.44	74.06	82.05	72.80	61.13

[a]P.p.m. from the signal for Me_4Si. [b]At 22°; data from ref. 4.

EXPERIMENTAL

6-*O*-α-D-Glucopyranosylcyclomaltohexaose (**1**) has been described elsewhere[1]. The n.m.r. spectra were obtained on a solution of **1** (12 mg) in D_2O (0.45 mL). All of the n.m.r. spectra were recorded at 30° with a JEOL GX-500 F.t.-n.m.r. instrument operating in the quadrature mode at 500 and 125 MHz for ^{1}H and ^{13}C nuclei, respectively.

The phase-sensitive DQF-COSY spectrum was obtained by using the pulse sequence (PD–90°–t_1–90°–D–90°–t_2)[9–10]; D was 10 μs for a clear shift in the phase of radiofrequency. For DQF-COSY, 256 free induction decays were acquired with 1K data points, a sweepwidth of 1000 Hz, and the phase cycling of 90°-pulse and receiver described by States *et al.*[11]. The solvent resonance was suppressed by a selective irradiation except during the t_1 and t_2 periods. The time-domain data matrix was expanded by zero filling and then processed according to the standard procedure.

The ^{1}H–^{13}C COSY spectrum was recorded by using the pulse sequence [PD–90° (^{1}H)–t_1/2–180° (^{13}C)–t_1/2–D_1–90° (^{1}H, ^{13}C)–D_2–t_2 (with ^{1}H decoupling)][12]. The delay times D_1 and D_2 were set to 3.4 and 1.7 ms, respectively. A total of 128 transients was accumulated per t_1 value with a pulse delay of 1 s. The initial data matrix was 2K(^{13}C–6500 Hz) × 128(^{1}H–1000 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 apodized with a sine-bell function and the absolute value mode spectrum is presented.

Chemical shifts are given in parts per million (p.p.m.) downfield from that of Me_4Si.

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