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Note

Assignment of ^{13}C-n.m.r. signals for reduced isomaltooligosaccharides

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Numerous ^{13}C-n.m.r. studies have been devoted to mono-, oligo-, and polysaccharides. Among the oligomers of D-glucose, disaccharides[1–7] of the α- and β-configurations have been extensively examined, and among the higher oligomers, series of α-(1→4)-linked[3,5], β-(1→4)-linked[3,5], α-(1→6)-linked[3], and β-(1→6)-linked[4] oligosaccharides have been principally studied.

Spectra of saccharides in alkaline solution would be more useful for direct comparison than those in neutral solution, since most of the saccharides are soluble[8,9] in alkaline solution. Although dimethyl sulfoxide is also used as a solvent, the spectra of carbohydrates in this solvent are often low in resolution because of viscosity[10]. One problem under alkaline conditions is the degradation of saccharides possessing a reducing-terminal residue. However, they may be effectively protected against alkaline degradation by $NaBH_4$ reduction, which affords the alkali-stable alditols[11]. Thus far, few alditols of D-glucose and related oligomers have been studied by n.m.r.; those reported include spectra of glucitol[2,12,13], maltitol[2], and isomaltitol[2]. Recently, ^{13}C signals of maltitol were assigned in detail by the two-dimensional (2D) Incredible Natural Abundance Double Quantum Transfer Experiment (INADEQUATE) without need for reference to model compounds[14]. In addition, the X-ray structures of isomaltitol and mannitol have been analyzed[15]. However, the ^{13}C spectra of higher oligomers have not yet been reported.

Herein, we show the one-dimensional (1D) ^{13}C-n.m.r. spectra of reduced isomaltooligosaccharides having d.p. 2–6 and reduced dextran T10 in 0.5M NaOH, and demonstrate how the signals arise from the terminal 6-*O*-linked glucitol residue (G_{OH}), the internal Glc*p* residue ($G_{I\text{-}GOH}$) directly linked to the glucitol residue, all the other internal Glc*p* residues (G_I), and the nonreducing terminal Glc*p* residue (G_T), which constitute the oligomers. The signals were then assigned by comparing the spectra and by further measuring 2D-INADEQUATE spectra of isomaltitol and isomaltotriitol, which provided the direct assignments of pairs of coupled ^{13}C–^{13}C signals.

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In the 1D-spectrum of isomaltitol (d.p. 2, Fig. 1A), signals A, P, R, and S were assigned (Table I), based on the unambiguous assignments[2] in D_2O. To assign all the other signals, the 2D-INADEQUATE spectrum was measured (Fig. 2). For G_T, starting from the unambiguous signals A of C-1 and S of C-6, the connectivities of the C-1–C-6 signals (A-H-E-N-G-S) were determined. Although the signal N was overlapped, the connectivities of the C-1–C-5 signals (R-F-N-K-O) of G_{OH} were also determined. As the C-6 signal (P) of G_{OH} was assigned in the 1D-spectrum, all of the signals of isomaltitol were thus assigned (Table I).

The signals A, F, K, P, and R appeared in all of the oligomers (Fig. 1A to 1E) and dextran T10 (Fig. 1F), but disappeared in the higher polysaccharide, dextran T70 (ref. 11). These five signals would be closely related to G_{OH}, as the C-6 signal (S) of the other terminal, G_T, appeared in both of the dextrans. The signals F, K, P, and R were certainly attributable to G_{OH}, as already described. The signal A was the C-1 signal of G_T linked to G_{OH} in isomaltitol. However, in the higher oligomers and polymers, signal A would be

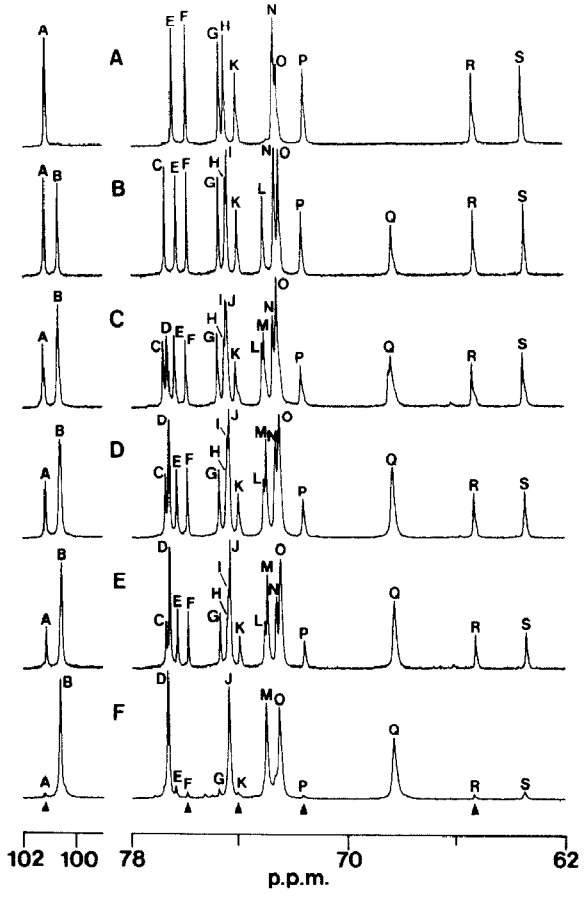

Fig. 1. ^{13}C-N.m.r. spectra of reduced isomaltooligosaccharides of d.p. 2 (A), d.p. 3 (B), d.p. 4 (C), d.p. 5 (D), and d.p. 6 (E) and of reduced dextran T10 (F). Signals are designated as "A to S" from downfield to upfield. Arrow heads represent the signals which disappeared[11] in reduced dextran T70.

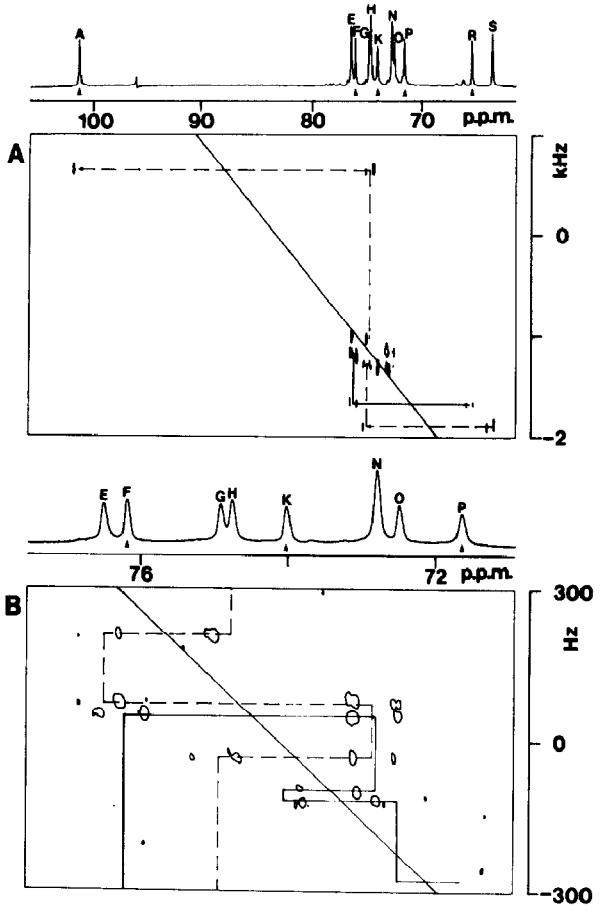

Fig. 2. 2D-INADEQUATE spectra of isomaltitol ranging from 61 to 105 p.p.m. (A) and from 70 to 78 p.p.m. (B). The spectra were measured independently as described in the experimental section. Continuous and dashed lines indicate connectivities of ^{13}C-satellite signals for the carbon atoms of G_{OH} and G_T, respectively. Arrow heads show the signals that disappeared[11] in reduced dextran T70.

the C-1 signal of $G_{I\text{-}GOH}$, and signal B would be the C-1 signal of G_T and G_I. This speculation was consistent with the observation that the C-1 atom linked to a Glc*p* residue resonated at higher field when compared with C-1 linked to a glucitol residue, as previously reported by Colson *et al.*[12] for maltose and maltitol, and isomaltose and isomaltitol, dissolved in neutral D_2O. They found that the C-1 atoms were more shielded by the pyranose rings than by the aliphatic chains.

In the spectrum of isomaltotriitol (d.p. 3, Fig. 1B, Table I), the six signals related to $G_{I\text{-}GOH}$ appeared in addition to the spectrum of isomaltitol. Five of the six new signals, B, C, I, L, and Q were observed, and the other one was apparently superimposed on signal O. These signals arose from $G_{I\text{-}GOH}$, except for the signal B of the C-1 atom of G_T. The 2D-INADEQUATE spectrum of this oligomer was also measured (data not shown) and all of the signals arising from G_T, $G_{I\text{-}GOH}$, and G_{OH} were assigned (Table I).

The spectrum of isomaltotetraitol (d.p. 4, Fig. 1C, Table I) was compared with

TABLE I

Assignments of ^{13}C-signals[a] of reduced isomaltooligosaccharides

D.p.	*Residue*	*Chemical shift (p.p.m.)*[b]					
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
2	α-D-Glc*p*-(1→6)-	101.18 (A)	74.57 (H)	76.49 (E)	72.73 (N)	74.75 (G)	63.55 (S)
	glucitol	65.39 (R)	75.97 (F)	72.73 (N)	74.12 (K)	72.62 (O)	71.64 (P)
3	α-D-Glc*p*-(1→6)-	100.71 (B)	74.52 (H)	76.35 (E)	72.72 (N)	74.78 (G)	63.49 (S)
	α-D-Glc*p*-(1→6)-	101.21 (A)	74.45 (I)	76.76 (C)	72.56 (O)	73.15 (L)	68.40 (Q)
	glucitol	65.38 (R)	75.94 (F)	72.72 (N)	74.09 (K)	72.56 (O)	71.74 (P)
4	α-D-Glc*p*-(1→6)-	100.62 (B)	74.50 (H)	76.35 (E)	72.71 (N)	74.76 (G)	63.49 (S)
	α-D-Glc*p*-(1→6)-	100.62 (B)	74.40 (J)	76.61 (D)	72.58 (O)	73.06 (M)	68.38 (Q)
	α-D-Glc*p*-(1→6)-	101.18 (A)	74.45 (I)	76.75 (C)	72.58 (O)	73.12 (L)	68.38 (Q)
	glucitol	65.38 (R)	75.93 (F)	72.71 (N)	74.09 (K)	72.58 (O)	71.71 (P)

[a] Letter in parenthesis indicates the signal designated. [b] Two decimal points used is reliable in relative comparisons among signals of a spectrum, but might have some uncertainty in comparisons among spectra.

that of isomaltotriitol to ascertain how the six signals of an additional G_I would appear. Three new signals, D, J, and M, were observed, and the other three signals were superimposed on signals B, O, and Q. These six signals became more intense with increasing G_I in higher oligomers (d.p. 5 and 6, Fig. 1D and 1E), and were the major signals in dextran T10 (Fig. 1F), as already assigned[16,17]. As isomaltotetraitol and the higher oligomers were composed of G_T, G_I, $G_{I\text{-}GOH}$, and G_{OH}, all of the ^{13}C-chemical shifts of the higher oligomers were consistent with those of isomaltotetraitol, as presented in Table I.

Signals of some saccharides in 0.5M NaOH shifted slightly downfield when compared with those in neutral water. Shifts of +0.3 to +0.6 p.p.m. were observed for dextran T10 (data not shown), although the spectral features under alkaline and neutral conditions were essentially similar. Downfield shifts of 0–0.5 p.p.m. were also observed for maltitol in 0.5M NaOH (not shown), as compared with the published data[14] obtained in H_2O and D_2O. Therefore, the signals of isomaltooligosaccharides would also be similarly shifted downfield under alkaline conditions.

In ^{13}C spectra of saccharides in ionic solution, the signal-to-noise ratio (S/N) was drastically decreased when a 10-mm sample tube was used[18], which was attributable to a heterogeneous heating of the samples by the broad-band decoupling power. In the present study using a 5-mm tube, no significant decrease of S/N was observed, as had been reported[18], and the spectra of the oligomers could be measured almost quantitatively. This improved resolution might arise because of the lower decoupling power of the multinuclear probe used. Nevertheless, the slightly unsymmetrical line-shape (Fig. 1A to 1C) may be dependent in part on temperature effects caused by heterogeneous heating.

EXPERIMENTAL

Compounds. — Isomaltooligosaccharides of d.p. 2 (Nakarai Chem., Kyoto) and of d.p. 3–6 (Seikagaku Kogyo, Tokyo) and dextran T10 (Pharmacia, Uppsala, Sweden) were reduced for several days at room temperature with $NaBH_4$ in distilled water, mixed with Bio-Rad AG 50W-X8 resin, filtered, and dried with MeOH, as previously reported[11].

Spectra. — One-dimensional ^{13}C-n.m.r. spectra were obtained with a Jeol JNM-GX-270 spectrometer equipped with a 5-mm multinuclear probe and operated[11] at 67.9 MHz in the Fourier-transform (F.t.) mode with complete proton decoupling at 18–20°. All samples were dissolved in 0.5M NaOH containing 10% D_2O and 1% sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). Final concentration of the samples was 5% (w/v). 1D-Spectra were measured under the following conditions: sweep width, 8 kHz; pulse width, 90° (7.2 μs); acquisition time, 1.0 s; data points, 65k. Chemical shifts are expressed as p.p.m. relative to the internal standard of DSS.

The 2D-INADEQUATE spectra[19,20] of isomaltitol and isomaltotriitol dissolved in 0.5M NaOH in final concentration of 40% (w/v) were measured using a standard Jeol pulse program. A data matrix ranging from 61–105 p.p.m. consisted of 64 (F1 dimen-

sion) × 2k (F2 dimension) points; the spectral width for F1 was 6 kHz and for F2 it was 3 kHz; the carrier frequency was positioned at 83.58 p.p.m. The matrix was zero-filled to 256 × 8k prior to F.t. For isomaltitol, a matrix ranging from 70–78 p.p.m. was also obtained: data points, 64 × 2k; the spectral widths, 1100 (for F1) and 550 (for F2) Hz; the carrier frequency, 74.00 p.p.m. The matrix was zero-filled to 256 × 8k prior to F.t. For isomaltotriitol, a matrix ranging from 67.8–80.7 p.p.m. was also obtained: data points, 64 × 2k; the spectral widths, 1760 (F1) and 880 (F2) Hz; the carrier frequency, 74.26 p.p.m. The matrix was zero-filled to 256 × 8k prior to F.t.

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