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Note

Assignment of ^{13}C NMR signals for reduced nigerooligosaccharides prepared by partial acid hydrolysis of (1 → 3)-α-D-glucan

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Nigerooligosaccharides are nigerose and its homologous oligomers, and can be obtained by partial degradation of (1 → 3)-α-D-glucan. Major ^{13}C NMR signals[1,2] of the glucan have been assigned, as well as numerous oligo- and poly-saccharides having other linkages. However, except for nigerose[3], the α-(1 → 3)-linked oligomers have not yet been studied by NMR spectroscopy.

The glucan is soluble in alkaline but not in neutral solution[4]. Most saccharides are also soluble in alkaline solution, and their alditols are alkali-stable. Therefore, ^{13}C NMR spectra for the alkaline solutions of the reduced saccharides should be of wide use. As for glucitol derivatives, a few spectra of glucitol[5–7], maltitol[5,8], and isomaltitol[5] have been reported. Recently, the conformations of glucitol and maltitol were analyzed by ^{1}H NMR spectroscopy[9], and the ^{13}C signals of reduced isomaltooligosaccharides in 0.5 M NaOH were assigned[10]. We now report ^{13}C NMR spectra of the reduced nigerooligosaccharides in neutral and alkaline solutions, and complete assignment of the signals.

EXPERIMENTAL

Preparation of oligosaccharide.—(1 → 3)-α-D-Glucan was synthesized from sucrose by the D-glucosyltransferase from *Escherichia coli* MAF10 harboring a recombinant plasmid[11] containing a *gtfI* gene of *Streptococcus downei* MFe28, as previously reported[12]. The glucan (3.6 g) was hydrolyzed in 100 mL of 0.1 M H_2SO_4 with stirring under reflux for 2 h at 100°. The mixture was centrifuged, the glucan precipitated was further hydrolyzed four more times, and the supernatants were pooled. About 64% of the original glucan was hydrolyzed. The pooled hydrolyzate was neutralized with $BaCO_3$, and insoluble material was removed by

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centrifugation. After concentration under diminished pressure, the hydrolyzate (10 mL) was applied to a column (3.6 × 68 cm) of Bio-Gel P2 (−400 mesh) and eluted with distilled water. The fractions of dp 2–6 each were pooled and concentrated. The oligosaccharides were then reduced with $NaBH_4$ in distilled water, as previously reported[4].

NMR spectroscopy.—One-dimensional ^{13}C NMR spectra for 5% samples in neutral and alkaline aqueous solutions were recorded, as previously reported[10]. Chemical shifts are expressed as ppm relative to an internal standard of sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS).

Two-dimensional (2D) incredible natural abundance double quantum transfer experiment (INADEQUATE) spectra[13,14] of nigeritol and nigerotriitol (40% each) in neutral solution containing 20% D_2O were also measured, as previously reported[10]. The carrier frequency was 72.8 ppm for both spectra. The spectral widths for F1 and F2 were 3000 and 1500 Hz for nigeritol, and 3200 and 1600 Hz for nigerotriitol.

RESULTS

First of all ^{13}C signals for the reduced nigerooligosaccharides in neutral solutions were assigned (Fig. 1, Table I). In the spectrum of nigeritol (Fig. 1A), assignment of signals A′, H, and Y were based on the published ^{13}C NMR data for (1 → 3)-α-D-glucan[1,2] and the nonreducing terminal Glc*p* residue[15]. Nigeritol reduced with $NaBD_4$ instead of $NaBH_4$ gave the same spectrum except for the signal U which was shifted upfield by ~0.5 ppm (data not shown), allowing the assignment of the signals T and U to C-6 and C-1, respectively, of the glucitol residue (G_{OH}). The other signals were assigned by the 2D INADEQUATE spectrum (Fig. 2). All of the signals arose from G_{OH} and the nonreducing terminal Glc*p* residue ($G_{T\text{-}GOH}$) linked to G_{OH}.

The ^{13}C signals for nigerotriitol (Fig. 1B) were also assigned by the 2D spectrum (not shown). The signals arose from the nonreducing terminal Glc*p* residue (G_T) linked to the Glc*p* residue and the internal Glc*p* residue ($G_{I\text{-}GOH}$) linked to G_{OH}, and G_{OH}. These signals were observed clearly for the higher oligomers (Fig. 1C–E), where only the three signals of C-4 to C-6 of G_T were shifted; the C-4 signal (S′) was shifted downfield by ~0.3 ppm and overlapped the signal S, and the C-5 (N) and C-6 (Y) signals were shifted upfield by ~0.2 ppm.

For nigerotetraitol (Fig. 1C), six new signals appeared in addition to the signals for the nigerotriitol component. The six signals, four new (B, F, R, and V) and two overlapped (L and P) signals, arose from the internal Glc*p* residue ($G_{I\text{-}GT}$) adjacent to G_T and also to the Glc*p* residue on the other side. The assignment of these six signals was based on that for the more internal residue, as described next.

Nigeropentaitol (Fig. 1D) gave six additional signals arising from the more internal Glc*p* residue (G_I), which was adjacent to other internal residues on both sides. Four signals, C, E, M, and W, were new, and the other two overlapped

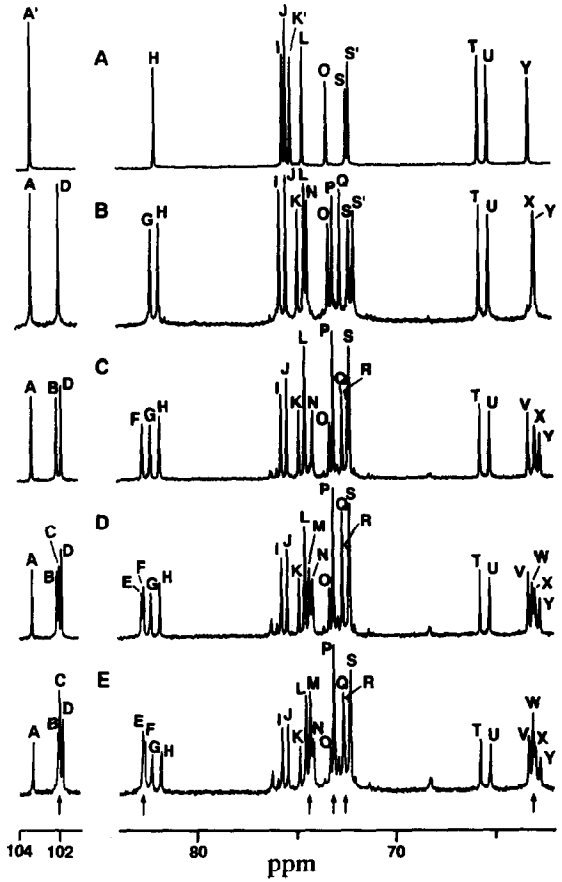

Fig. 1. ^{13}C NMR spectra of reduced nigerooligosaccharides of dp 2–6 (A–E) in H_2O. Signals are designated as A to Y from low to high field. Primed signals A′ and K′ are different in assignment from unprimed signals A and K. Signal S′ entirely overlapped signal S in the higher oligomers. Arrows represent the six signals, which increased in intensity with increasing G_I.

signals P and Q. These six signals became more intense with increasing G_I in nigerohexaitol (Fig. 1E) and become major signals in higher oligomers and polymers, as assigned in (1 → 3)-α-D-glucan[1,2]. The signals of the internal residue, $G_{I\text{-}GT}$, in the dp 4–6 oligomers (Fig. 1C–E) were always located closely to the signals of G_I, which allowed similar assignment for $G_{I\text{-}GT}$. Thus, all of the spectra in neutral solutions were assigned (Table I). The ^{13}C-chemical shifts of the higher oligomers, which consist of G_T, $G_{I\text{-}GT}$, G_I, $G_{I\text{-}GOH}$, and G_{OH} are fundamentally the same as those for nigerotetraitol.

The spectra of nigeritol (Fig. 3) and nigerotriitol (not shown) in 0–0.5 M NaOH were recorded to correlate the signals in neutral and alkaline solutions. Only the signals of C-1 and C-2 of G_{OH} shifted upfield with increasing NaOH concentration, while the other signals shifted downfield similarly. Based on these observa-

TABLE I

Assignments of ^{13}C signals of reduced nigerooligosaccharides in H_2O

Dp	Residue [a]		Chemical shift [b] (ppm)					
			C-1	C-2	C-3	C-4	C-5	C-6
2	α-D-Glc*p*-(1 → 3)-	($G_{T\text{-}GOH}$)	103.29 (A')	74.51 (L)	75.50 (I)	72.19 (S')	75.11 (K')	63.15 (Y)
	-glucitol	(G_{OH})	65.21 (U)	75.34 (J)	82.00 (H)	72.32 (S)	73.31 (O)	65.71 (T)
3	α-D-Glc*p*-(1 → 3)-	(G_T)	101.94 (D)	74.44 (L)	75.70 (I)	71.93 (S')	74.31 (N)	62.88 (Y)
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GOH}$)	103.31 (A)	73.04 (P)	82.22 (G)	72.67 (Q)	74.79 (K)	62.93 (X)
	-glucitol	(G_{OH})	65.19 (U)	75.37 (J)	81.81 (H)	72.23 (S)	73.26 (O)	65.69 (T)
4	α-D-Glc*p*-(1 → 3)-	(G_T)	101.85 (D)	74.49 (L)	75.68 (I)	72.26 (S)	74.10 (N)	62.67 (Y)
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GT}$)	102.09 (B)	73.06 (P)	82.68 (F)	72.36 (R)	74.49 (L)	63.27 (V)
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GOH}$)	103.32 (A)	73.06 (P)	82.28 (G)	72.62 (Q)	74.79 (K)	62.94 (X)
	-glucitol	(G_{OH})	65.20 (U)	75.38 (J)	81.82 (H)	72.26 (S)	73.26 (O)	65.70 (T)
5	α-D-Glc*p*-(1 → 3)-	(G_T)	101.86 (D)	74.49 (L)	75.68 (I)	72.25 (S)	74.11 (N)	62.69 (Y)
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GT}$)	102.09 (B)	73.08 (P)	82.65 (F)	72.31 (R)	74.49 (L)	63.26 (V)
	-α-D-Glc*p*-(1 → 3)-	(G_I)	102.00 (C)	73.08 (P)	82.75 (E)	72.63 (Q)	74.28 (M)	63.06 (W)
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GOH}$)	103.32 (A)	73.08 (P)	82.28 (G)	72.63 (Q)	74.80 (K)	62.94 (X)
	-glucitol	(G_{OH})	65.20 (U)	75.38 (J)	81.83 (H)	72.25 (S)	73.27 (O)	65.70 (T)

[a] Abbreviation of residue in parentheses. [b] Designation of signal in parentheses.

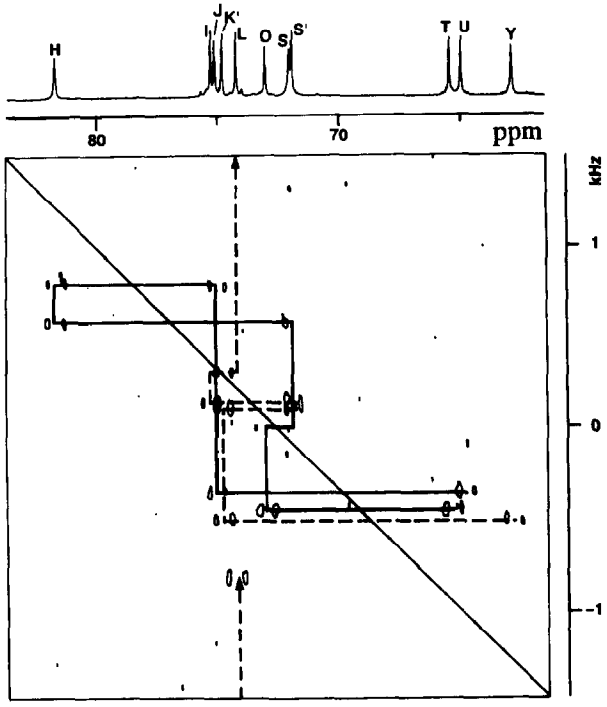

Fig. 2. 2D INADEQUATE spectrum of nigeritol from 61–84 ppm. Continuous and dashed lines indicate connectivities of ^{13}C satellite signals arising from G_{OH} and $G_{T\text{-}GOH}$, respectively. The satellite signals at −0.8 kHz for F1 and 74.5 ppm for F2 are the folding signals coupled with glucosidically linked C-1.

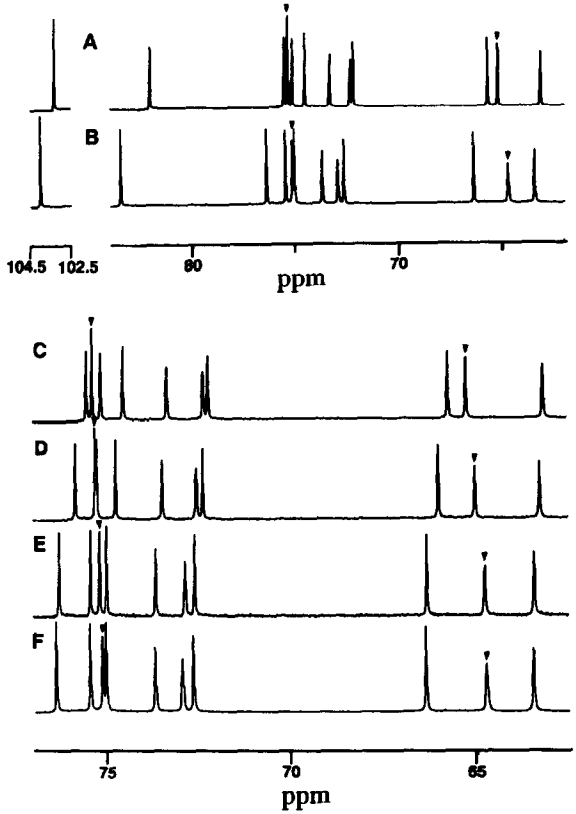

Fig. 3. ^{13}C NMR spectra of nigeritol in 0 M (A and C), 0.1 M (D), 0.3 M (E), and 0.5 M (B and F) NaOH. Arrow heads represent the signals shifted upfield in the alkaline solutions.

tions and the comparison of signals arising from nigero-tetraitol to -hexaitol (not shown), the ^{13}C-signals of all the oligomers in 0.5 M NaOH were also assigned (Table II).

DISCUSSION

We have prepared the reduced nigerooligosaccharides of dp 2–6 and have assigned the ^{13}C NMR signals in neutral and alkaline solution (Figs. 1 and 2, Tables I and II). The assignments in alkaline solution are more useful than those in D_2O alone, since most polysaccharides, including (1 → 3)-α-D-glucan, are alkali-soluble and the data can be readily compared. In this study, the signals of each residue were well resolved for the neutral solution and somewhat overlapped for the alkaline solution. Therefore, the signals were assigned first for the neutral solutions and then for the alkaline solutions.

The signals of the internal residues, $G_{I\text{-}GT}$, G_I, and $G_{I\text{-}GOH}$, were distinguished from each other, particularly in the neutral solutions. The signals of the nonreduc-

TABLE II

Assignments of ^{13}C signals of reduced nigerooligosaccharides in 0.5 M NaOH

Dp	Residue [a]		Chemical shift (ppm)					
			C-1	C-2	C-3	C-4	C-5	C-6
2	α-D-Glc*p*-(1 → 3)-	($G_{T\text{-}GOH}$)	103.98	75.03	76.38	72.65	75.46	63.46
	-glucitol	(G_{OH})	64.74	75.13	83.47	72.95	73.70	66.36
3	α-D-Glc*p*-(1 → 3)-	(G_T)	103.61	75.42	76.95	72.65	75.15	63.50
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GOH}$)	103.98	73.40	85.81	73.08	75.81	63.60
	-glucitol	(G_{OH})	64.69	75.15	83.51	72.99	73.71	66.48
4	α-D-Glc*p*-(1 → 3)-	(G_T)	103.32	75.42	76.86	72.84	75.01	63.50
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GT}$)	103.48	73.75	85.26	72.97	75.42	63.74
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GOH}$)	103.84	73.44	85.54	73.04	78.80	63.58
	-glucitol	(G_{OH})	64.69	75.22	83.23	72.97	73.65	66.43
5	α-D-Glc*p*-(1 → 3)-	(G_T)	103.39	75.45	76.91	72.89	75.05	63.56
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GT}$)	103.39	73.81	85.43	72.97	75.45	63.75
	-α-D-Glc*p*-(1 → 3)-	(G_I)	103.39	73.81	85.21	73.38	75.45	63.83
	-α-D-Glc*p*-(1 → 3)-	($G_{I\text{-}GOH}$)	103.83	73.45	85.76	73.05	75.81	63.56
	-glucitol	(G_{OH})	64.68	75.20	83.29	72.97	73.67	66.46

[a] Abbreviation of residue in parentheses.

ing terminal residues, $G_{T\text{-}GOH}$ and G_T, were also distinguishable. In dp 5 or higher oligomers, the signals arose from five types of residues, G_T, $G_{I\text{-}GT}$, G_I, $G_{I\text{-}GOH}$, and G_{OH}. Thus, the signals of these residues were definitely affected by their adjacent residues. In addition, the signals of G_T in α-(1 → 3) and α-(1 → 6)[10] linkages in 0.5 M NaOH were also unambiguously distinguishable, except for the C-6 signals.

Conversion of a reducing terminal residue to the corresponding alditol residue has distinct advantages in NMR spectroscopy. First, the alditol is stable even in alkaline solution. Second the ^{13}C signals of the alditol increase in intensity as compared to those of its reducing forms in their α- and β-configurations, since the signals of the reducing forms are divided into two groups and each intensity is diminished. Furthermore, quantitative analysis of a terminal residue as an alditol may be possible in polysaccharides of low molecular weight, as observed in dextran T10 (data not shown).

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