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Note

Analysis of permethylated hexopyranosyl-2-acetamido-2-deoxyhexitols by g.l.c.-m.s.

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Methylated and trimethylsilylated di- and tri-saccharides, and their corresponding alditols, have been analysed by g.l.c.–m.s. to determine the position of the glycosidic linkages. These studies have been performed mainly with model substances containing simple hexoses[1–8]. Kamerling *et al.*[9] have studied trimethylsilylated 2-acetamido-2-deoxyhexosylhexoses, but relatively little is known about the fragmentation pattern of disaccharides containing amino sugars at the reducing end.

During the characterization[10] of 2-acetamido-2-deoxy-3-*O*-α-D-galactopyranosyl-D-galactitol, it was found that the fragmentation of the trimethylsilylated derivatives of disaccharide alditols was different from that of simple alditols, and, thus, the position of the glycosidic linkage can easily be erroneously assigned.

We now report on g.l.c.–m.s. of methylated hexopyranosyl-2-acetamido-2-deoxyhexitols.

The retention times in g.l.c. of the permethylated disaccharide alditols are shown in Table I; the methyl ethers were better resolved than the corresponding *O*-trimethylsilyl derivatives[10]. The partial mass spectra of these compounds are presented in Table II. Borohydride and borodeuteride were used for the reduction,

TABLE I

THE G.L.C. RETENTION TIMES (RELATIVE TO MALTOTRIITOL) OF THE PERMETHYLATED DISACCHARIDE ALDITOLS

Compound	*Stationary phase* *2.2% SE-30 (240°)*	*1% OV-225 (240°)*	*3% QF-1 (220°)*
1 α-D-Gal*p*-(1→3)-D-GalNAcol	0.28	0.37	0.66
2 β-D-Gal*p*-(1→3)-D-GalNAcol	0.29	0.45	0.77
3 β-D-Gal*p*-(1→3)-D-GlcNAcol	0.27	0.43	0.72
4 β-D-Gal*p*-(1→4)-D-GlcNAcol	0.29	0.47	0.87
5 α-D-Gal*p*-(1→6)-D-GlcNAcol	0.31	0.44	0.70
6 β-D-Gal*p*-(1→6)-D-GlcNAcol	0.34	0.57	0.83

TABLE II

PARTIAL MASS SPECTRA OF THE PERMETHYLATED HEXOPYRANOSYL-2-ACETAMIDO-2-DEOXYHEXITOLS

m/e	*(1→3)-link*			*(1→4)-link*	*(1→6)-link*	
	1	2	3	4	5	6
45	100	100	98	77^b	42^b	39^b
88	78^b	78^b	100^b	100^b	100^b	100^b
130	45^a	38^a	38^a	42^a	36^a	30^a
133	8.5	6.5	3.4	1.0	0.5	—
142	12	11	9.5	15^b	17^b	17^b
155	7.6	3.6	7.6	6.0	4.2	3.9
174	1.8	—	—	28^a	25^a	21^a
187	38	31	28	36	16	14
212	—	0.6^a	—	—	—	—
218	0.8^a	—	1.7^a	—	9.6^a	6.8^a
219	9.6	7.4	8.4	11	4.8	4.4
244	4.1^a	3.4^a	3.1^a	2.4^a	2.5^a	1.9^a
249	—	—	1.6	2.7	2.0	1.3
262	2.3	3.0	2.2	—	2.3^a	1.7^a
276	18^a	14^a	32^a	37^a	20^a	19^a
293	—	—	—	—	—	—
304	5.5	3.6	4.3	—	2.0	1.7
334	2.5	3.1	1.7	—	—	—
336	—	—	—	—	6.3^a	4.8^a
337	—	—	—	—	2.2^a	1.8^a
378	7.6^a	3.4^a	7.2^a	—	—	—
381	—	—	—	2.2	—	—
422	6.1^a	12^a	5.3^a	—	—	—
466	6.9	5.5	4.1	7.0	—	—

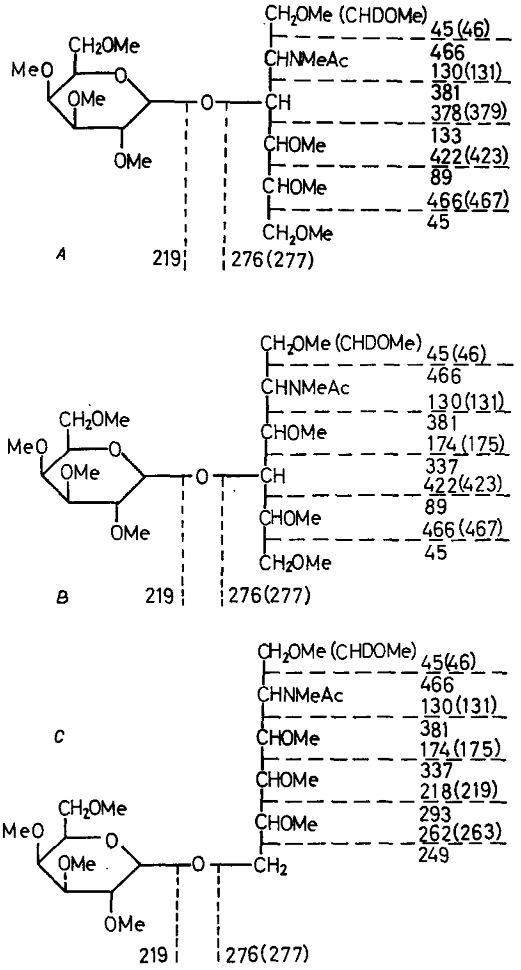
[a] In the deuterated derivative, the intensity is completely shifted to the next higher *m/e* value. [b] In the deuterated derivative, the intensity is partly shifted to the next higher *m/e* value.

to facilitate the interpretation of the mass spectra. However, the position of the glycosidic linkage can be determined without recourse to deuteration, because of the asymmetry of the alditol moiety, in contrast to simple disaccharides[7].

The molecular ion (*m/e* 511) was seen only in the mass spectra of the (1 → 6)-linked alditols. The (1 → 3)- and (1 → 4)-linked alditols showed an intense peak at *m/e* 466 (M − 45). The ions at *m/e* 219, 187 (219 − 32), and 155 (219 − 32 − 32) are formed by cleavage of the hexose unit from the molecule, followed by the elimination of methanol. These ions are unchanged in the spectra of alditols produced with borodeuteride. The corresponding ions from the alditol unit are found at *m/e* 276, 244, and 212, and are increased by one mass-unit after reduction with borodeuteride.

In many respects, the fragmentation of the disaccharide alditols in the low mass range is similar to that of partially methylated 2-acetamido-2-deoxyhexitols[11]. The intense ion at *m/e* 88 arises mainly *via* elimination of ketene from the common characteristic ion *m/e* 130, which arises by cleavage of the C-2–C-3 bond of the alditol moiety (Scheme 1).

The mass spectra of the (1 → 4)- and (1 → 6)-linked disaccharide alditols can



Scheme 1. Fragmentation of permethylated hexopyranosyl-2-acetamido-2-deoxyhexitols: *A*, (1→3)-link; *B*, (1→4)-link; *C*, (1→6)-link.

be distinguished from those of the (1 → 3)-linked derivatives on the basis of the intense ion at *m/e* 174, formed by cleavage of the C-3–C-4 bond of the alditol moiety. In the spectra of *O*-trimethylsilyl derivatives, the corresponding ion (*m/e* 276) is formed through a different pathway and is not useful for the differentiation of these linkages[10]. The (1 → 3)-linked alditols are also characterized by prominent ions at *m/e* 422 (M − 89) and 378. Although the latter ion could arise by primary cleavage between C-3 and C-4, it probably originates *via* a different mechanism[6,7]. The ion at *m/e* 133 can be formed by primary cleavage only from (1 → 3)-linked alditols, and it was almost absent from the spectra of the (1 → 4)- and (1 → 6)-linked derivatives.

The spectra of (1 → 4)-linked alditols show an intense peak at *m/e* 466 (see above). This fact and the absence of the ions at *m/e* 218 and 336 distinguish them from the (1 → 6)-linked derivatives. The prominent peaks at *m/e* 218 in the spectra of (1 → 6)-linked disaccharide alditols are formed by cleavage between C-4 and C-5 in the alditol moiety. The ion at *m/e* 336 was present only in the spectra of the (1 → 6)-linked alditols and was shifted by one mass-unit when deuterated. This ion could arise in a manner analogous to that of the ion at *m/e* 279 found in the mass spectra of the methyl glycosides of disaccharide methyl ethers[2,5], which contains a MeO = CH–O$^+$ group formed from the non-reducing hexosyl unit. However, for methyl glycosides, this kind of fragmentation of the hexosyl unit is typical of (1 → 3)- and (1 → 4)-linkages, but not of (1 → 6)-linkages.

The present study shows that the position of the glycosidic linkages of permethylated hexopyranosyl-2-acetamido-2-deoxyhexitols can be assigned on the basis of ions arising from the primary fragmentation of the alditol unit. For *O*-trimethylsilyl derivatives, other pathways of fragmentation complicate the interpretation of the spectra.

EXPERIMENTAL

The disaccharides 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-glucose, 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose, and 2-acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-D-glucose were kindly supplied by Dr. A. Gauhe, and 2-acetamido-2-deoxy-6-*O*-α-D-galactopyranosyl-D-glucose by Professor W. M. Watkins. 2-Acetamido-2-deoxy-3-*O*-α-D-galactopyranosyl-D-galactitol and 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactitol were purified from rat brain[12]. Maltotriose was a commercial sample. Disaccharide alditols and maltotriitol were prepared by reduction ($NaBH_4$ or $NaBD_4$) of the corresponding saccharides. The disaccharide alditols were methylated by the method of Hakomori[13]. The reaction mixture was partitioned between water and chloroform (1:1), the chloroform phase was washed with water (5×), and concentrated under nitrogen. G.l.c. was performed with a Perkin–Elmer 900 instrument using columns (2 mm × 2 m) of 2.2% of SE-30 (240°), 1% of OV-225 (240°), and 3% of QF-1 (220°). The mass spectra (70 eV) were recorded with a Varian MAT CH-7 instrument equipped with a Varian 1700 gas chromatograph and a SpectroSystem 100 MS data processing system.

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