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Preliminary communication

Identification of the methylation products of 3-acetamido-3,6-dideoxy-L-glucose by g.l.c.–m.s.

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Methylation analysis is an important method for the structural elucidation of complex polysaccharides[1]; it involves permethylation of the polysaccharide, followed by hydrolysis to a mixture of partially methylated sugars that is routinely analyzed[2,3] as alditol acetates by g.l.c.–m.s. in order to reveal structural information on the points of attachment of the interglycosidic linkages and on the carbohydrate composition.

This approach is also qualitatively effective in structural investigations of polysaccharides containing amino sugar residues. These are usually encountered as the *N*-acetyl derivatives of 2-amino-2-deoxy-D-hexoses[4]. Analysis and structural characterization of 2-acetamido-2-deoxy-D-hexoses by g.l.c.–m.s. has been the subject of several investigations[5–8], but few such studies are available on the partially methylated alditol acetates from other amino sugars.

We recently reported the occurrence of the rare amino sugar 3-acetamido-3,6-dideoxy-L-glucose as an integral part of the core oligosaccharide obtained from the lipopolysaccharides of the Gram-negative bacteria *Aeromonas hydrophila*[9] and *Vibrio anguillarum*[10]. The present communication describes the analysis of a range of possible methyl ethers of 3-acetamido-3,6-dideoxy-L-glucitol acetates in relation to their retention times in g.l.c. both on packed and open, tubular columns, and to their mass spectra and fragmentation patterns. The core oligosaccharides of *A. hydrophila* and *V. anguillarum* were methylated by the Hakomori method[11], hydrolyzed with 90% formic acid for 1 h at 100° and then 2M trifluoroacetic acid for 6 h at 100°, the sugars reduced with sodium borohydride, and the alditols acetylated with acetic anhydride–pyridine for 1 h at 100°. The g.l.c. retention-times and the mass spectra of the individual methylated alditol acetates were identified by comparison with synthetic standards. The synthetic methylated alditol acetates were obtained by complete (1 mmol of sugar, 3 mmol of methylsulfinyl anion), and incomplete (1 mmol of sugar, 1 mmol of methylsulfinyl anion) Hakomori methylation[11] of methyl 3-acetamido-3,6-dideoxy-α-L-glucoside, followed by hydrolysis with 2M trifluoroacetic acid for 10 h at 100°, reduction of the sugar with sodium borohydride, and acetylation of the alditol. The retention times (relative to that of hexa-*O*-acetyl-D-glucitol) in two different columns are given in Table I.

Unlike the partially methylated 2-deoxy-2-(*N*-methylacetamido)hexitol acetates, for which fragmentation of the carbon chain by fission between C-2 and C-3 yields the

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TABLE I

RETENTION TIMES [a] OF THE METHYLATION PRODUCTS OF 3-ACETAMIDO-3,6-DIDEOXY-L-GLUCITOL ACETATES

Methylated alditol acetate	*Retention time* [b]	
	Silar 7CP	*WCOT CP-Sil 5*
1,5-Di-*O*-acetyl-3,6-dideoxy-2,4-di-*O*-methyl-3-(*N*-methylacetamido)-L-glucitol	0.46	0.62
1,4,5-Tri-*O*-acetyl-3,6-dideoxy-2-*O*-methyl-3-(*N*-methylacetamido)-L-glucitol	0.74	0.70
1,2,5-Tri-*O*-acetyl-3,6-dideoxy-4-*O*-methyl-3-(*N*-methylacetamido)-L-glucitol	0.74	0.75
1,2,4,5-Tetra-*O*-acetyl-3,6-dideoxy-3-(*N*-methylacetamido)-L-glucitol	1.05	0.87

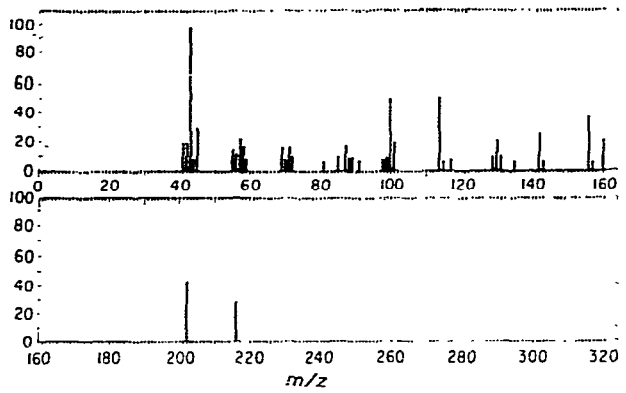
[a] Gas–liquid chromatography of the methylated alditol acetates was performed in packed columns (183 × 2 mm i.d.) of 1.5% of Silar 7CP on Gas Chrom Q (100–120 mesh) in a Perkin–Elmer Model 3920 gas chromatograph operated isothermally at 180°, with a helium flow of 40 mL/min. Gas chromatography was also performed in a column (25 m) of WCOT CP-Sil 5 (0.25 μm; Chrompak, The Netherlands) at 180° in the same instrument. [b] Retention time relative to that of D-glucitol hexaacetate as unity.

primary ion *m/z* 158 and its derived fragment *m/z* 116, the mass spectra of the methyl ethers of 3,6-dideoxy-3-(*N*-methylacetamido)-L-glucitol acetates were found to obey the same fragmentation rules as other partially methylated alditol acetates.

The fragmentation of 1,5-di-*O*-acetyl-3,6-dideoxy-2,4-di-*O*-methyl-3-(*N*-methylacetamido)-L-glucitol occurs by fission between C-2 and C-3 and between C-3 and C-4, giving the primary ions *m/z* 216 and 202, respectively. These primary ions give rise to secondary ions by elimination of acetic acid (60 m.u.), ketene (42 m.u.), and formaldehyde (30 m.u.), and there is no loss of methanol (32 m.u.) by β-elimination (see Fig. 1).

In the mass spectrum of 1,4,5-tri-*O*-acetyl-3,6-dideoxy-2-*O*-methyl-3-(*N*-methylacetamido)-L-glucitol, the order of precedence for the cleavage between adjacent carbon atoms is MeO-$\dot{C}$H-$\dot{C}$H-NMeAc > AcMeN-$\dot{C}$H-$\dot{C}$H-OAc, and hence the fission between C-2 and C-3, generating the major primary ion *m/z* 244, predominates over the fission between C-3 and C-4, which gives the minor primary ion *m/z* 202 (see Fig. 2). Similarly, the fragmentation pattern of 1,2,5-tri-*O*-acetyl-3,6-dideoxy-4-*O*-methyl-3-(*N*-methylacetamido)-L-glucitol gives a major primary ion *m/z* 230, generated by fission between C-3 and C-4, which predominates over the fission between C-2 and C-3 that gives the minor primary ion *m/z* 216 (see Fig. 3).

Finally, in the mass spectrum of 1,2,4,5-tetra-*O*-acetyl-3,6-dideoxy-3-(*N*-methylacetamido)-L-glucitol, the fragmentation pattern is governed equally by fission between



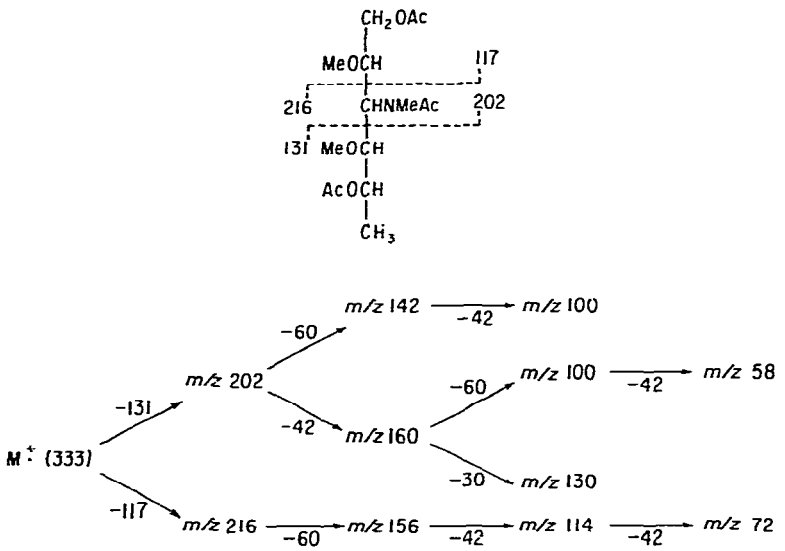
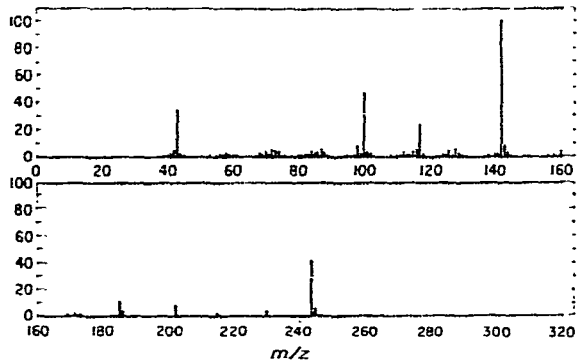


Fig. 1. Mass spectrum and fragmentation pattern of 1,5-di-*O*-acetyl-3,6-dideoxy-2,4-di-*O*-methyl-3-(*N*-methylacetamido)-L-glucitol. [Gas–liquid chromatography–mass spectrometry was performed in a Hewlett–Packard Model 5981A instrument controlled by a 5934A data system, with a capillary column (25 m) of WCOT CP-Sil 5 (0.25 μm), a source temperature of 200°, and an ionizing voltage of 70 eV.]



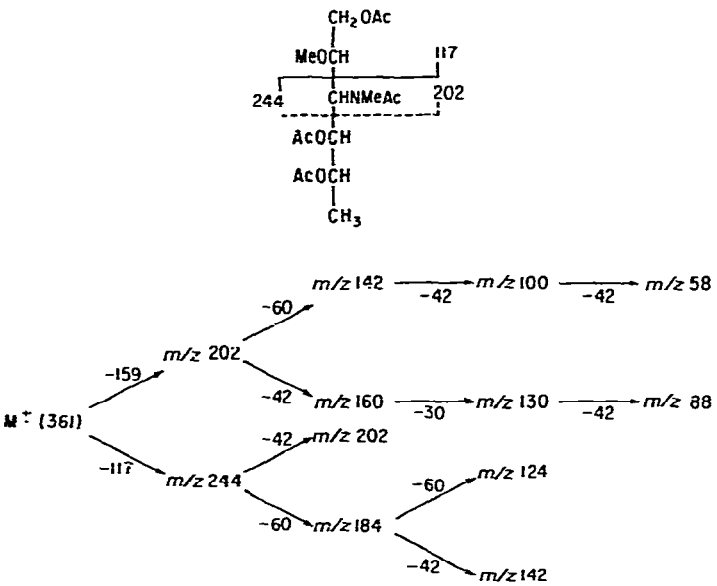
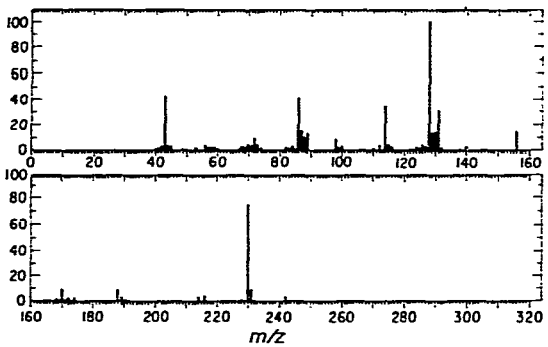


Fig. 2. Mass spectrum and fragmentation pattern of 1,4,5-tri-*O*-acetyl-3,6-dideoxy-2-*O*-methyl-3-(*N*-methylacetamido)-L-glucitol.

C-2 and C-3 and between C-3 and C-4. These primary ions yield series of secondary ions by losses of acetic acid and ketene (see Fig. 4).

In conclusion, the combined g.l.c.—m.s. technique provides clear identification of this series of partially methylated alditol acetates of 3-acetamido-3,6-dideoxy-L-glucose



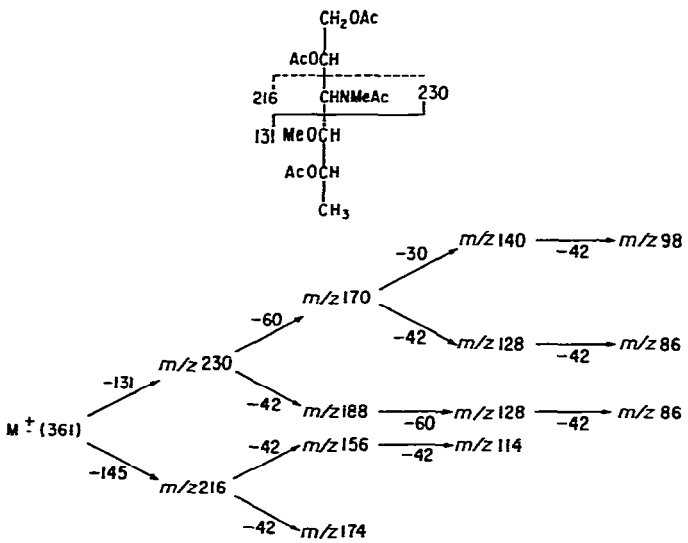


Fig. 3. Mass spectrum and fragmentation pattern of 1,2,5-tri-*O*-acetyl-3,6-dideoxy-4-*O*-methyl-3-(*N*-methylacetamido)-L-glucitol.

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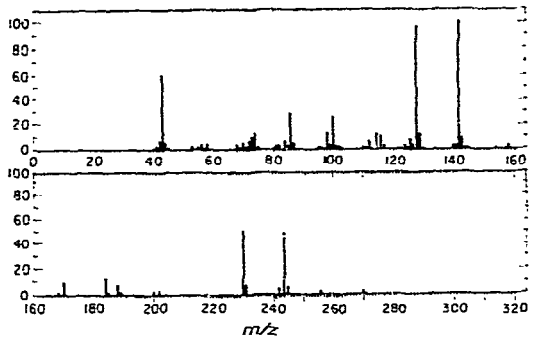

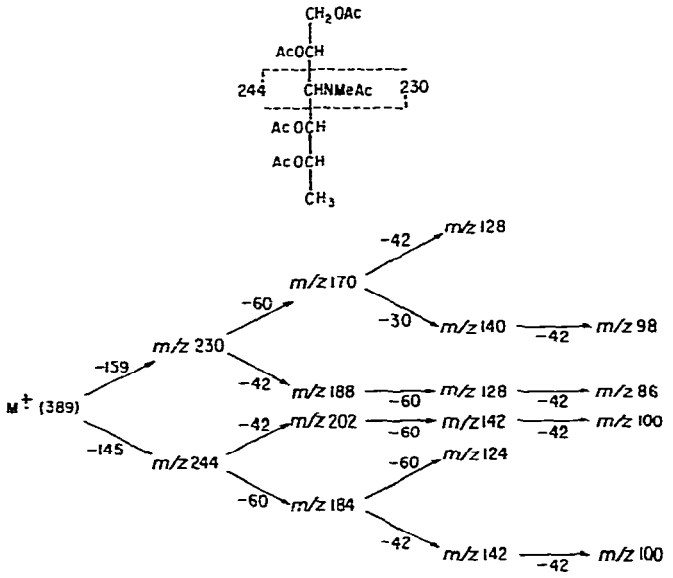

Fig. 4. Mass spectrum and fragmentation pattern of 1,2,4,5-tetra-*O*-acetyl-3,6-dideoxy-3-(*N*-methyl-acetamido)-L-glucitol.

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