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Note

Separation by gas–liquid chromatography, and identification by mass spectrometry, of the methyl ethers of 2-deoxy-2-(*N*-methylacetamido)-D-glucose*

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The combination of gas–liquid chromatography with mass spectrometry (g.l.c.–m.s.) is an important tool for the separation and identification of methylated sugars obtained in the determination of carbohydrate structures by methylation procedures[1–8]; only a small amount of material is required, and the information given by the mass-spectral pattern and the retention times is sufficient for unambiguous identification, without any need for standard compounds for comparison. Björndal *et al.*[9] have applied this method to the alditol acetates from partially methylated, neutral sugars derived from permethylated oligo- and poly-saccharides, and this report describes the alditol acetates derived from fully or partially methylated 2-acetamido-2-deoxy-D-glucose.

After this study had been completed and reported[1,2], a similar procedure was published by Stellner *et al.*[10] on some of the compounds reported in this paper, but the 4-methyl ether was not investigated. The main difference between the two studies resides in the reduction step, which, in the later work[10], was performed with sodium borohydride, whereas sodium borodeuteride was used in the investigation. The derivatives prepared[10] with the borohydride give a main diagnostic ion at *m/e* 158, but "bleeding" of the lipid phase of the g.l.c. medium also gives an ion, of varying intensity, at *m/e* 158, which vitiates the quantitative determinations based on this main peak. In addition, in the later work[10], numerous peaks, helpful for the identification of the methyl ethers, were deleted from the artist's interpretation of the original

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spectra, perhaps because the poor response of the instrument required multiplication of the intensity of peaks above *m/e* 117 by a factor of 10.

Previous reports described the gas–liquid chromatographic separation of various peracetylated methyl ethers of 2-deoxy-2-(methylamino)-D-glucitol and -D-galactitol[11], and the g.l.c. separation, and identification by m.s., of the 3,4,6-tri-*O*-methyl and 6-*O*-acetyl-3,4-di-*O*-methyl derivatives of both methyl 2-deoxy-2-(*N*-methylacetamido)-α-D-galactopyranoside and methyl 2-deoxy-2-(*N*-methylacetamido) α-D-glucopyranoside[12].

The partially methylated alditol acetates that were obtained from various 2-acetamido-2-deoxy-D-glucose derivatives by *O*- and *N*-methylation, followed by hydrolysis, borodeuteride reduction, and acetylation, were satisfactorily separated by g.l.c. on an ECNSS-M column, a single peak being observed for each derivative. The results were similar to those reported by Stellner *et al.*[10], except that the replacement of their stationary phase by glass beads (GLC 110) coated with only 0.05% of ECNSS-M gave an improved separation of the 3,4- and 4,6-dimethyl ethers (see Table I). The fragmentation pattern was, in general, (*a*) similar to that of the alditol

TABLE I

RETENTION TIMES IN GAS–LIQUID CHROMATOGRAPHY OF THE ACETATES OF THE METHYL ETHERS OF 2-DEOXY-2-(*N*-METHYLACETAMIDO)-D-GLUCITOL[a]

Compounds	*Substituents*		R_T	*Relative*[b]
	O-*Acetyl*	O-*Methyl*	(*min*)	R_T
1	1,5	3,4,6	4.72	1.00
2	1,3,5	4,6	10.7	2.27
3	1,4,5	3,6	8.00	1.69
4	1,5,6	3,4	11.2	2.35
5	1,3,4,5	6	13.8	2.91
6	1,3,5,6	4	27.6	5.85
7	1,4,5,6	3	17.2	3.64

[a]On a column of 0.05% of (cyanoethyl)silicone–1,2-ethanediol succinate on GLC 110 at 210°; see details in Experimental part. [b]Relative to 1,5-di-*O*-acetyl-2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol.

acetates derived from neutral sugars[9,13], and (*b*) easier to interpret than that of methyl or trimethylsilyl glycosides (see Figs. 1–7). The most diagnostic ion for 2-deoxy-2-(*N*-methylacetamido)alditol acetate derivatives is that at *m/e* 158, which is formed by fission between C-2 and C-3; however, this ion is also obtained by "bleeding" of the ECNSS-M liquid phase, still the most effective medium discovered for the g.l.c. separation of these compounds. The reduction with borodeuteride presents the advantage of shifting ion *m/e* 158 to 159, thus improving the interpretation of the spectra. The primary ions expected from the fragmentation are shown in Table II. Ion A tends to release ketene readily, to give ion A′ (*m/e* 117). Ions at *m/e* 43, 103, and 145

correspond to CH_3C^+O, CH_3–CO–$(\overset{+}{O}H)$–CO–CH_3, and CH_3–CO–$\overset{+}{O}$=$(COCH_3)_2$, respectively, and are characteristic for many acetyl derivatives.

TABLE II

PRIMARY FRAGMENTS (*m/e*) EXPECTED FROM THE FISSION OF THE CARBON ATOM CHAIN OF ALDITOL ACETATES FROM VARIOUS METHYL ETHERS OF 2-DEOXY-2-(*N*-METHYLACETAMIDO)-D-GLUCOSE-*1-d*

Ions	*Fragmentation between C atoms*	*C atoms in chain*	*Methoxyl groups*[a] *at C-*						
			3,4,6 (1)	4,6 (2)	3,6 (3)	3,4 (4)	6 (5)	4 (6)	3 (7)
A	2,3	1,2	159	159	159	159	159	159	159
B	3,4	1–3	203		203	203			203
C	4,5	1–4	247	275		247		275	
R	2,3	3–6	205		233	233			261
S	3,4	4–6	161	161		189		189	
T	5,6	6	45	45	45		45		

[a]Number assigned to compound is given in parentheses.

In addition to the primary fragments, the following ions were observed: for the 3,4,6-trimethyl ether (**1**), an ion at *m/e* 145, which derives from the ion R by loss of acetic acid (see Fig. 1); for the 4,6-dimethyl ether (**2**), an ion ($M^+ + 1$) at *m/e* 393 (see Fig. 2); for the 3,6-dimethyl ether (**3**), ions of small intensity ($M^+ - 60$) and ($M^+ - 59$) at *m/e* 332 and 333, representing loss of acetic acid and acetamide, respectively, from the molecular ion (see Fig. 3); for the 6-methyl ether (**5**), an ion at *m/e* 315

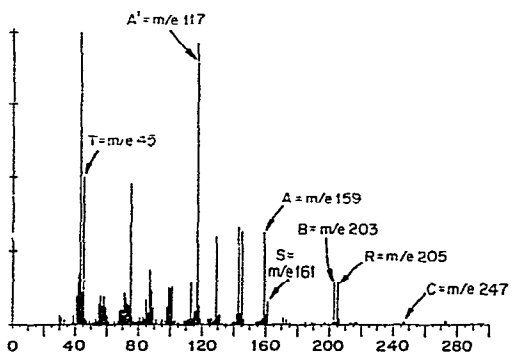

Fig. 1. Mass spectrum of 1,5-di-*O*-acetyl-2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol-*1-d* (**1**) recorded on a Perkin–Elmer model 900 gas–liquid chromatograph, equipped with a stainless-steel column (0.3 × 152 cm) containing 0.05% of ECNSS-M on GLC 110, and combined with a Hitachi–Perkin–Elmer model RMU-6L mass spectrometer, by means of a fritted-glass–helium separator. Ionizing-electron energy, 70 eV. Temperature of column, 210°; of molecular separator, 250°; and of ion source, 250°. The spectrum was recorded, as bar graphs, with an on-line IBM 1800 data-aquisition and -processing system.

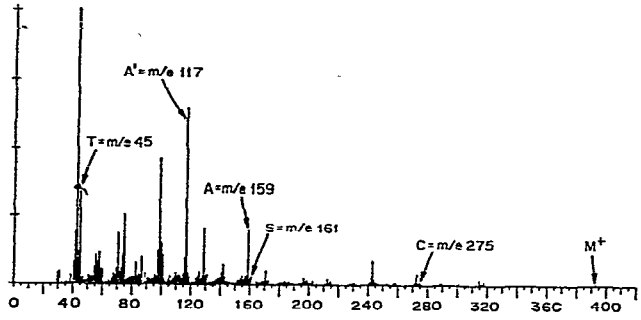

Fig. 2. Mass spectrum of 1,3,5-tri-*O*-acetyl-2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol-*1-d* (2). Instruments and conditions are described in the legend to Fig. 1.

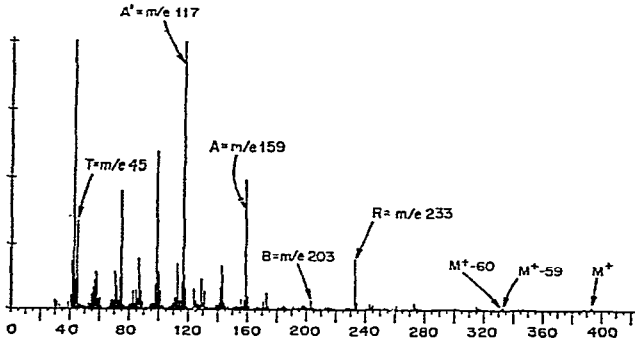

Fig. 3. Mass spectrum of 1,4,5-tri-*O*-acetyl-2-deoxy-3,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol-*1-d* (3). Instruments and conditions are described in the legend to Fig. 1.

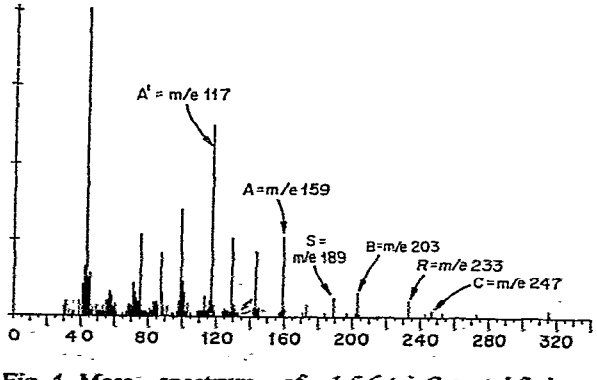

Fig. 4. Mass spectrum of 1,5,6-tri-*O*-acetyl-2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol-*1-d* (4). Instruments and conditions are described in the legend to Fig. 1.

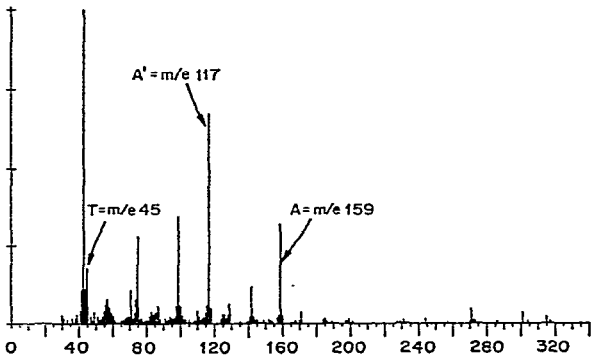

Fig. 5. Mass spectrum of 1,3,4,5-tetra-*O*-acetyl-2-deoxy-6-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol-*1-d* (5). Instruments and conditions are described in the legend to Fig. 1.

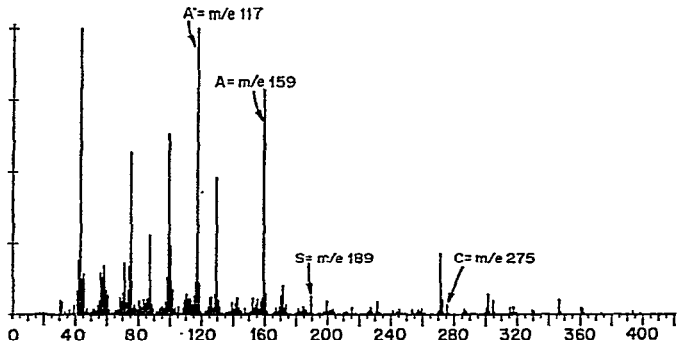

Fig. 6. Mass spectrum of 1,3,5,6-tetra-*O*-acetyl-2-deoxy-4-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol-*1-d* (6). Instruments and conditions are described in the legend to Fig. 1.

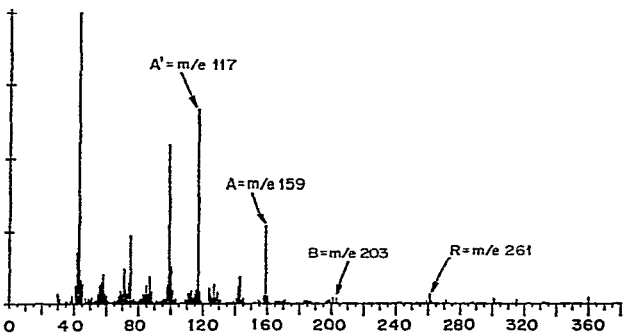

Fig. 7. Mass spectrum of 1,4,5,6-tetra-*O*-acetyl-2-deoxy-3-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol-*1-d* (7). Instruments and conditions are described in the legend to Fig. 1.

that might derive by ionization of the fragment containing C-1 to C-5 of the chain, after loss of acetic acid, and ions at *m/e* 301 and 271 that cannot as yet be explained (see Fig. 5); for the 4-methyl ether (**6**) ions at *m/e* 360 and 361 that are derived from the molecular ion by loss of acetic acid and acetamide, respectively (see Fig. 6); and for the 3-methyl ether (**7**), an ion at *m/e* 360, having a relatively high intensity, that is derived from the molecular ion by loss of acetic acid (see Fig. 7). For each methylated derivative, a unique mass spectrum is obtained, and thus, definite location of the *O*-methyl groups can be assigned.

EXPERIMENTAL

Methyl ethers of 2-deoxy-2-(methylamino)-D-glucose. — These ethers were prepared from starting materials that were resistant to the strongly alkaline conditions of the methylation[14,15] and that were available in our laboratory: methyl 2-acetamido-2-deoxy-α-D-glucopyranoside[16] for the 3,4,6-trimethyl ether, methyl 2-acetamido-2-deoxy-6-*O*-trityl-α-D-glucopyranoside[17] for the 3,4-dimethyl ether, benzyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranoside[18] for the 4,6-dimethyl, ether, 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine[19] for the 3,6-dimethyl ether, and methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[20] for the 3-methyl ether.

Each derivative (~10 μmoles) was dissolved in dry dimethyl sulfoxide (1.0 ml) in a screw-capped (Teflon-lined) reaction-vial, and a solution (0.1 ml) of M sodium (methylsulfinyl)methylene in dimethyl sulfoxide was added. The mixture was stirred under a stream of dry nitrogen for 1 h at room temperature. Then, methyl iodide (four 10-μl portions) was added stepwise, with vigorous stirring under nitrogen, during 2 h, and the solution was kept overnight. Water (3 ml) was added, and the solution was transferred to a column (0.9 × 5 cm) of 2:1 Norit A–Celite 535 (prewashed successively with 6M hydrochloric acid, water, ethanol, chloroform, ethanol, and water) suspended in water. The column was then successively washed with water (65 ml), 1:1 (v/v) methanol–water (1 ml), and methanol (1 ml) to remove the dimethyl sulfoxide and salts. The permethylated sugar derivatives were then eluted with 2:1 (v/v) chloroform–methanol (80 ml). The eluate was evaporated, the oily residue was hydrolyzed with 3M trifluoroacetic acid (1.0 ml) for 4 h at 105° under nitrogen, and the solution was evaporated by being kept *in vacuo* in the presence of sodium hydroxide pellets.

The 4-methyl ether was prepared by hydrolysis of benzyl 2-deoxy-4-*O*-methyl-2-(*N*-methylacetamido)-6-*O*-trityl-α-D-glucopyranoside[21], with 3M trifluoroacetic acid in 25% acetic acid under the conditions just described.

Samples of 2-deoxy-6-*O*-methyl-2-(methylamino-)-D-glucopyranose and 2-deoxy-3-*O*-methyl-2-(methylamino)-D-glucopyranose were provided by Dr. P. A. J. Gorin, and samples of 2-deoxy-3,4,6-tri-*O*-methyl-2-(methylamino)-D-glucopyranose hydrochloride and 2-deoxy-3,4-di-*O*-methyl-2-(methylamino)-D-glucopyranose hydrochloride by Dr. P. J. Stoffyn.

The results obtained with the 3,4,6-trimethyl, 3,4-dimethyl, and 3- and 6-methyl ethers prepared in our laboratory were identical with those obtained with the products provided by Drs. Gorin and Stoffyn.

Methyl ethers of peracetylated 2-deoxy-2-(N-methylacetamido)-D-glucitol-1-d derivatives. — Reduction of the 2-deoxy-2-(methylamino)-D-glucose (2 μmoles) was performed overnight at room temperature with M sodium borodeuteride (0.5 ml). The excess of the borodeuteride was decomposed by careful addition of acetic acid to pH 5.0, and the solution was passed through a column (1.2 × 1.8 cm) of AG 50W X-8 (H^+) cation-exchange resin (200–400 mesh) to remove the sodium ions. Boric acid and acetic acid were eluted with water (10 ml), and the 2-deoxy-2-(methylamino)-D-glucitol derivative with M aqueous ammonia until the pH of the eluted mixture exceeded 9. The sugar-containing fraction was evaporated *in vacuo*, and the residue acetylated with an excess of 1:1 (v/v) acetic anhydride–pyridine (0.5 ml) for 3 h at 105°, under nitrogen. The excess of acetic anhydride–pyridine was removed by keeping the solution *in vacuo* in the presence of sodium hydroxide pellets and concentrated sulfuric acid.

Gas–liquid chromatography and mass spectrometry. — The alditol acetates from the methylated sugars were dissolved in chloroform (0.1–0.2 ml), and aliquots were analyzed by g.l.c. with a Perkin–Elmer model 900 gas chromatograph equipped with a stainless-steel column (0.3 × 152 cm) containing 0.05% of (cyanoethyl)silicone–1,2-ethanediol succinate (ECNSS-M) on GLC 110 (120–140 mesh) (Supelco Inc., Bellefonte, Pa. 16823, U.S.A.) at 210°, with nitrogen as the carrier gas at a flow rate of 40 ml/min.

Combined g.l.c.–m.s. was performed, with helium as the carrier gas, with a Perkin–Elmer model 900 gas chromatograph, connected to a Hitachi–Perkin–Elmer model RMU-6L mass spectrometer by means of a fritted-glass–helium separator. The ion source and the molecular separator were maintained at 250°, and the ionizing-electron energy was 70 eV. The spectra were recorded, as bar graphs, with an on-line IBM 1800 data-acquisition and -processing system. The g.l.c. conditions were identical with those just described.

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