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ANHYDROALDITOLS IN THE SUGAR ANALYSIS OF METHANOLYSATES OF ALDITOLS AND OLIGOSACCHARIDE-ALDITOLS*

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ABSTRACT

In the context of the methanolysis procedure for sugar analysis, several alditols were investigated for their capacity to form anhydro derivatives in M methanolic HCl (24 h, 85°). Xylitol, D-arabinitol, L-fucitol, D-glucitol, galactitol, 2-acetamido-2-deoxy-D-galactitol, and the alditols of *N*-acetylneuraminic acid were very prone to form anhydrides, whereas 2-amino-2-deoxy-D-galactitol, 2-amino-2-deoxy-D-glucitol, D-mannitol, and 2-acetamido-2-deoxy-D-glucitol formed little anhydride. Anhydride formation was observed for the relevant alditols when present in reduced oligosaccharides. This finding is of importance in the quantification of sugar residues based on methanolysis, *N*-(re)acetylation, trimethylsilylation, and subsequent capillary g.l.c.

INTRODUCTION

Various derivatives are employed for the determination of the sugar composition of complex carbohydrates by g.l.c. Neutral and aminodeoxy sugars, obtained by acid hydrolysis, are usually analysed as alditol acetates[1–3]. Methyl glycosides of neutral monosaccharides, aminodeoxy sugars, uronic acids (methyl esters), and sialic acids (methyl esters) obtained by methanolysis can be determined after *N*-acetylation–trimethylsilylation[4–6] or trifluoroacetylation[7]. Each monosaccharide gives rise to a characteristic group of methyl glycosides.

The methanolysis procedure has some complications. The analysis of *N*-glycosylically bound carbohydrate chains of glycoproteins (GlcNAc–Asn type) has shown that, under the usual conditions (M methanolic HCl, 24 h, 85°), the linkage between GlcNAc and Asn is split to only a very limited extent[8,9] and mainly the free monosaccharide is liberated. On methanolysis, several uronic acids form 6,3-lactones, gulose yields ~30% of the 1,6-anhydride[10,11], and *N*-acetylneuraminic acid (Neu5Ac) gives ~3% of the 2,7-anhydride[12].

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Recently, we observed another complication in studies of the structure of the carbohydrate chains of the mucin type of glycoproteins, which are linked to Ser or Thr through GalNAc. Sugar analysis of the oligosaccharide-alditols obtained after alkaline borohydride reduction[13] gave three unknown peaks in g.l.c., in addition to the normal pattern of trimethylsilylated monosaccharide methyl glycosides and trimethylsilylated 2-acetamido-2-deoxygalactitol. G.l.c.–m.s. data indicated the unknown peaks to be due to anhydrides of 2-acetamido-2-deoxygalactitol. In the light of this finding, the tendency of other alditols to form anhydrides in methanolic HCl has been studied and is now reported.

TABLE I

RETENTION TIMES (RELATIVE TO THAT OF TRIMETHYLSILYLATED D-MANNITOL) OF TRIMETHYLSILYLATED ALDITOLS AND ANHYDROALDITOLS ON CPsil5 (SEE EXPERIMENTAL)

Alditol	$T_{Mannitol}$	*Peak area ratio*[a] *(%)*	*Products*[a]	*Assignments of anhydro forms based on g.l.c.–m.s.*
D-Xylitol[b]	0.32	23	Anhydro-D-xylitol[b]	1,4 and 2,5
	0.60	77	D-Xylitol[b]	
D-Arabinitol	0.29	5	Anhydro-D-arabinitol	1,4
	0.62	95	D-Arabinitol	
L-Fucitol	0.36	8	Anhydro-L-fucitol	1,4
	0.40	2	Anhydro-L-fucitol	2,5[c]
	0.76	90	L-Fucitol	
2-Amino-2-deoxy--D-galactitol	0.96	100	2-Amino-2-deoxy--D-galactitol	
2-Amino-2-deoxy--D-glucitol	0.97	100	2-Amino-2-deoxy--D-glucitol	
D-Mannitol	1.00	100	D-Mannitol	
D-Glucitol	0.65	20	Anhydro-D-glucitol	1,4
	1.01	80	D-Glucitol	
D-Galactitol[d]	0.65	14	Anhydro-D-galactitol[d]	1,4 and 3,6
	1.02	86	D-Galactitol[d]	
2-Acetamido-2-deoxy--D-glucitol	1.23	100	2-Acetamido-2-deoxy--D-glucitol	
2-Acetamido-2-deoxy--D-galactitol	0.51	6	2-Acetamido-dianhydro--2-deoxy-D-galactitol	1,4:3,6
	0.90	17	2-Acetamido-anhydro--2-deoxy-D-galactitol	3,6
	0.96	12	2-Acetamido-anhydro--2-deoxy-D-galactitol	1,4
	1.24	65	2-Acetamido-2-deoxy--D-galactitol	
Epimeric alditols of *N*-acetyl-D-neuraminic acid methyl ester[e]	1.89	18 } 43	Anhydro forms of the epimeric alditols	2,7[c]
	1.91	25		
	1.97	13 } 22	Lactone forms of the epimeric alditols	1,4[c]
	1.99	9		
	2.09	16 } 35	Epimeric alditols	
	2.13	19		

[a]G.l.c. peaks ≤1% have not been included. [b]D-Xylitol-*1-d*. [c]Tentative assignments. [d]D-Galactitol-*1-d*. [e]Trimethylsilylation time, 2 h instead of 30 min.

RESULTS AND DISCUSSION

The products present in the methanolysates (M methanolic HCl, 24 h, 85°) of various alditols are noted in Table I, together with the g.l.c. retention-times of the corresponding trimethylsilylated derivatives on CPsil5. Capillary g.l.c.–m.s. was used for the identification of the various anhydroalditols and the lactones of the epimeric alditols of Neu5Ac. In order to facilitate interpretation of the mass spectra of the trimethylsilylated anhydroalditols, deuterium labels were introduced at specific positions. Sodium borodeuteride was used as a mass marker for C-1.

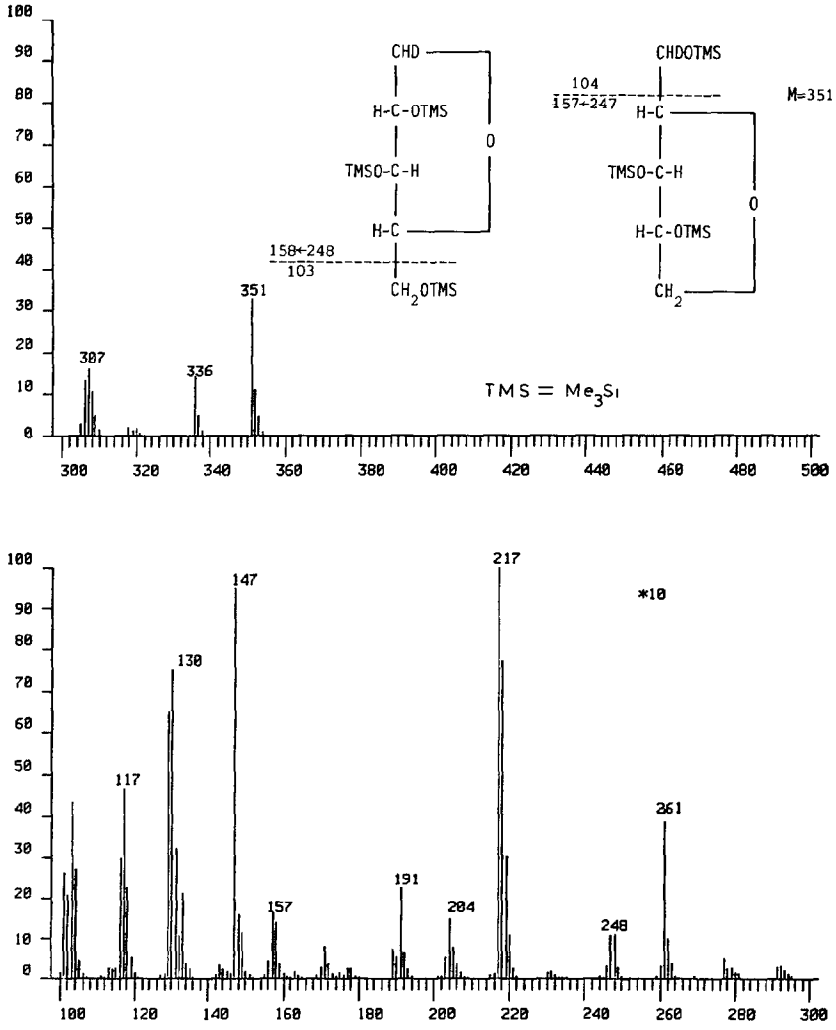

Fig. 1. E.i.-mass spectrum (70 eV) of the mixture of trimethylsilylated 1,4-anhydro-D-xylitol-*1-d* and 2,5-anhydro-D-xylitol-*1-d*.

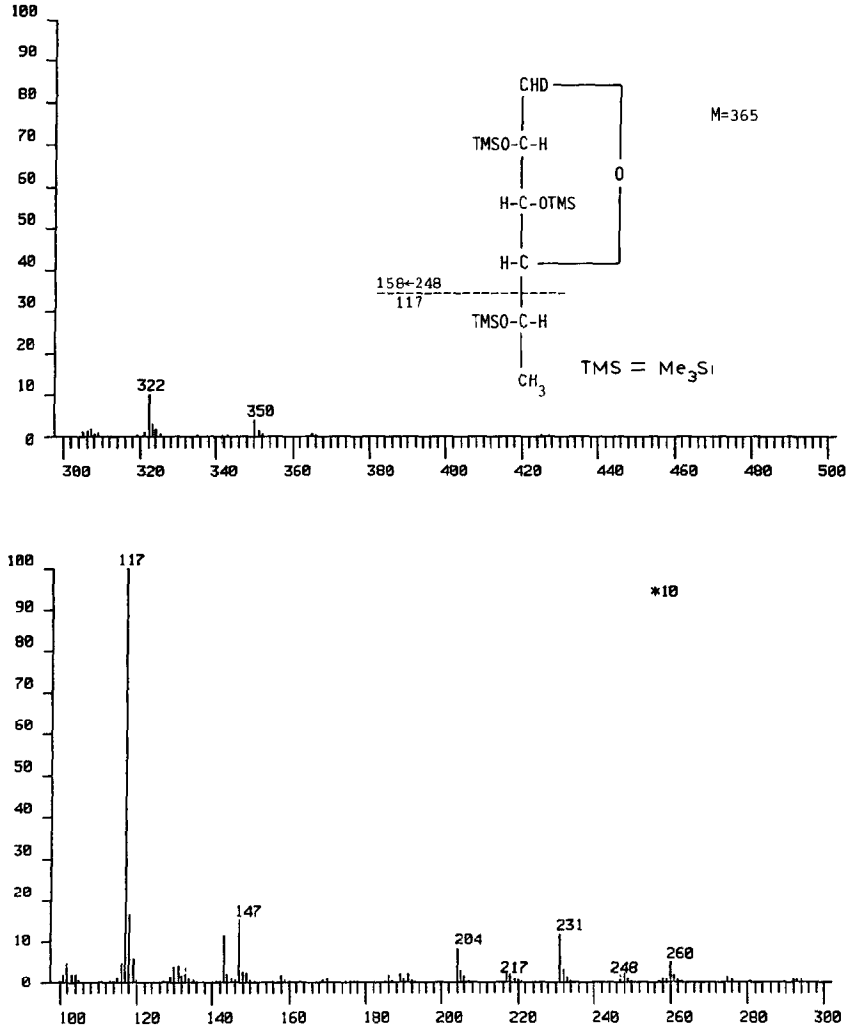

Fig. 2. Mass spectrum of trimethylsilylated 1,4-anhydro-L-fucitol-*1-d*.

Deuterated trimethylsilylation reagents and deuterated acetic anhydride were used to label hydroxyl and amino groups, respectively.

The data in Table I show that many alditols gave >1% of anhydride under the solvolysis conditions. The epimeric alditols of Neu5Ac also gave lactones. However, in the gas chromatogram of xylitol, D-arabinitol, and D-glucitol, small peaks ($\leq$1%) suggested the presence of other anhydrides. The methanolysate of D-mannitol contained <1% of a monoanhydride, the main product being probably 1,4-anhydro-D-mannitol. Mass spectra of some typical trimethylsilylated anhydro-alditols, labelled at C-1, are depicted in Figs. 1–5.

The partial conversion of alditols into anhydrides was also demonstrated for

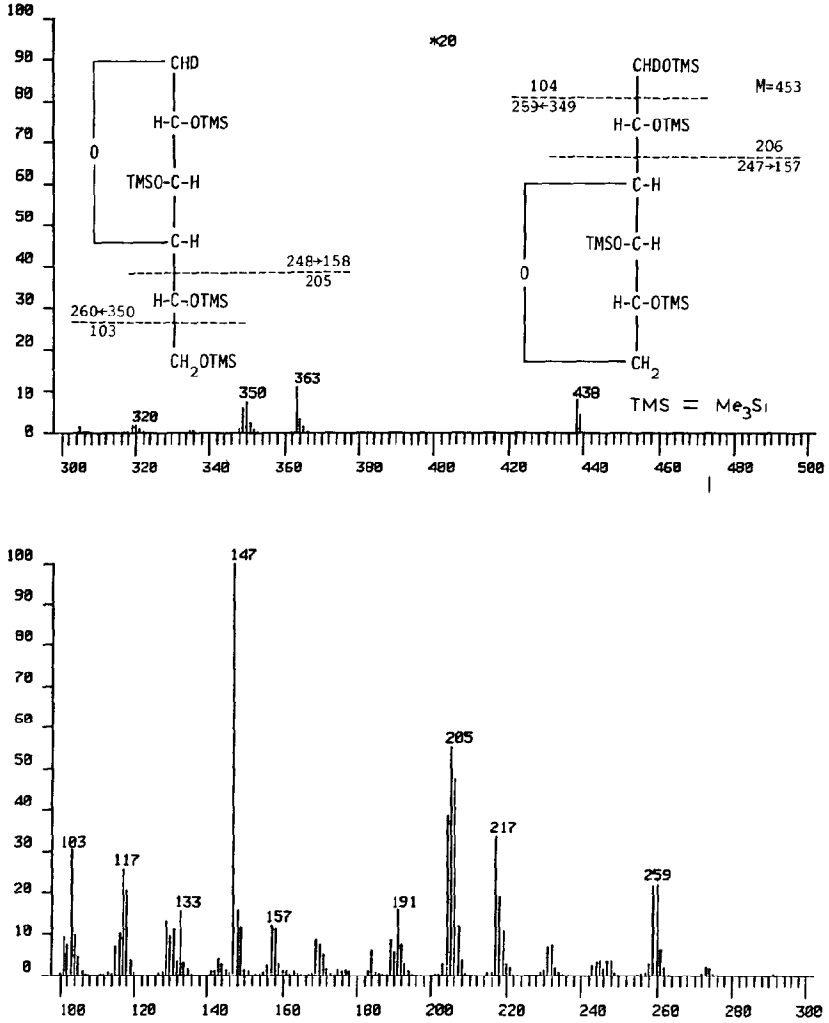

Fig. 3. Mass spectrum of the mixture of trimethylsilylated 1,4-anhydro-D-galactitol-*1-d* and 3,6-anhydro-D-galactitol-*1-d*.

the methanolysates of several oligosaccharide-alditols having xylitol, galactitol, glucitol, or 2-acetamido-2-deoxygalactitol as the alditol residue (Table II). Only for 2-acetamido-2-deoxygalactitol were the peak-area ratios of the anhydrides [6:17:12:65 (see Table I)] significantly different from those for the corresponding oligosaccharide-alditols (2:5:20:73). Oligosaccharide-alditols having mannitol or 2-acetamido-2-deoxyglucitol residues did not yield detectable amounts of anhydrides.

Thus, under the methanolysis conditions used in routine procedures for sugar analysis, numerous alditols and oligosaccharide-alditols will be converted, to some

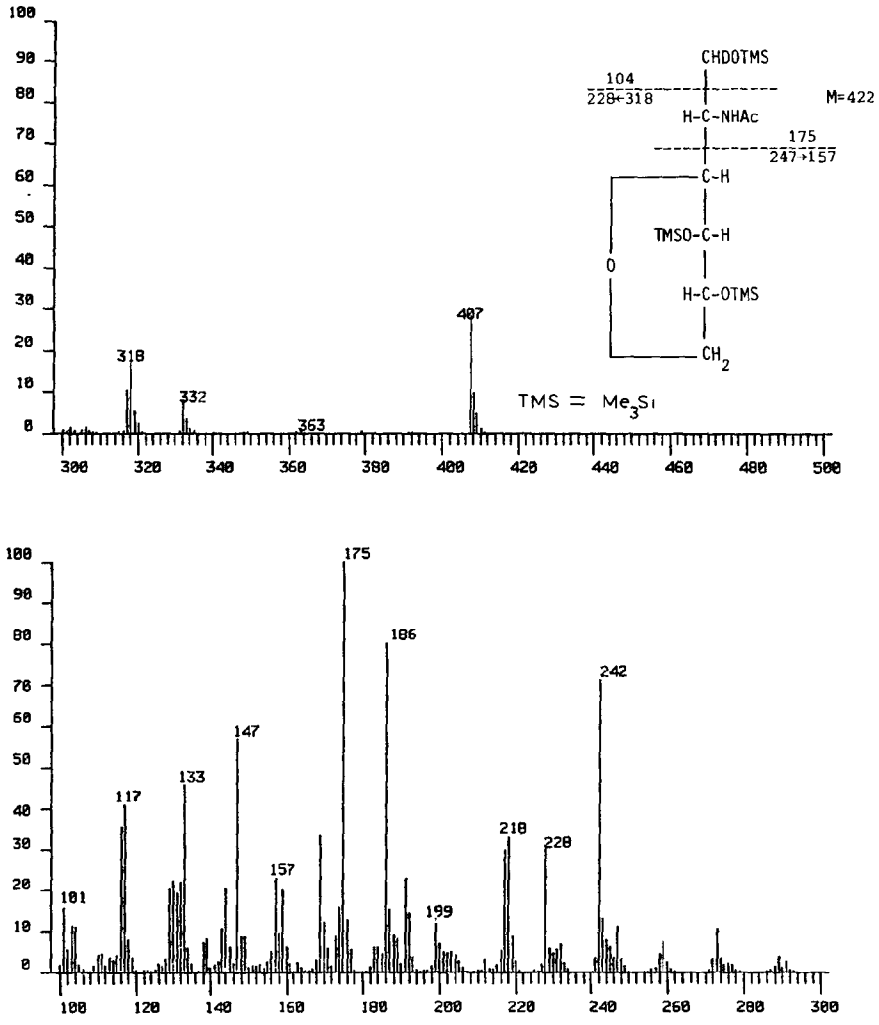

Fig. 4. Mass spectrum of trimethylsilylated 2-acetamido-3,6-anhydro-2-deoxy-D-galactitol-*1-d*.

extent, into anhydrides, and appropriate correction factors are required. The use of mannitol as internal standard is desirable, because it gives a negligible amount of anhydride. The formation of anhydrides by acid-catalysed dehydration of alditols is well known[14–20] and the formation of 1,4-anhydroglucitol from glucitol phosphate has been mentioned[21]. However, in relation to sugar analysis methods based on methanolysis, anhydride formation has not been reported hitherto.

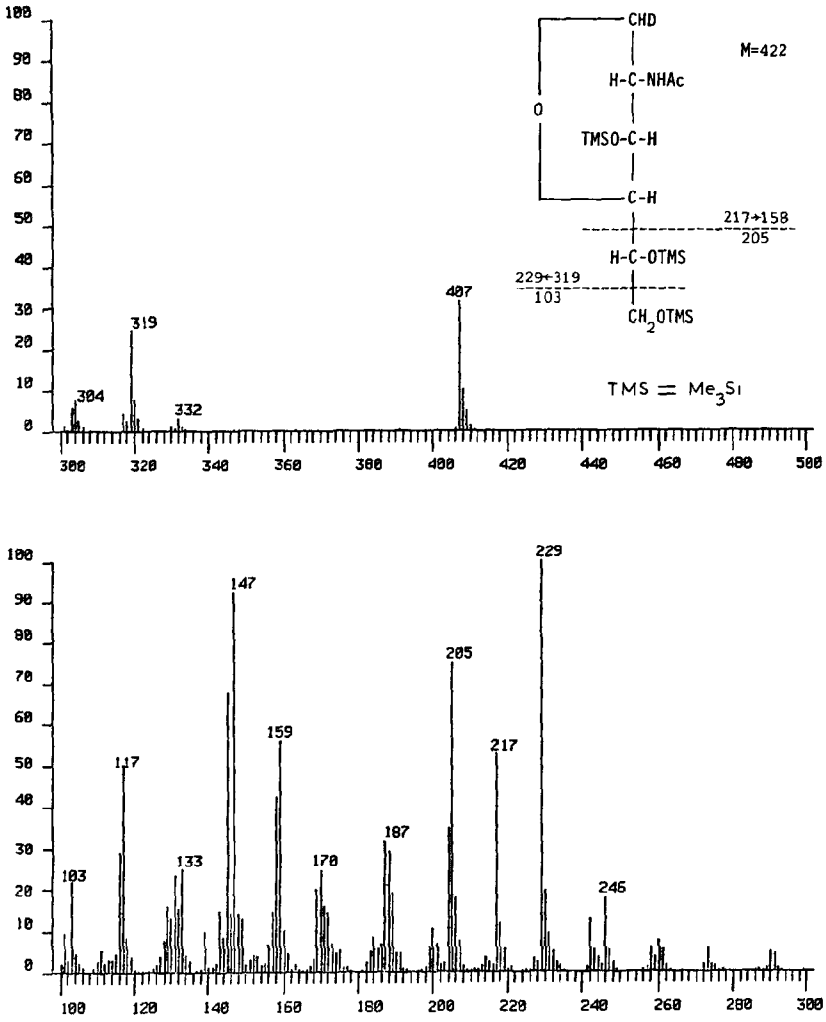

Fig. 5. Mass spectrum of trimethylsilylated 2-acetamido-1,4-anhydro-2-deoxy-D-galactitol-*1-d*.

TABLE II

LIST OF OLIGOSACCHARIDE-ALDITOLS INVESTIGATED FOR THEIR ANHYDROALDITOL FORMATION DURING METHANOLYSIS

β-Gal-(1→4)-Xyl-ol	β-Gal-(1→4)-Glc-ol
β-Neu5Ac-(2→6)-Gal-ol	β-GlcNAc-(1→4)-Man-ol
β-Gal-(1→6)-Gal-ol	β-GlcNAc-(1→2)-[β-GlcNAc-(1→4)]-Man-ol
β-Glc-(1→2)-Glc-ol	α-Man-(1→3)-β-Man-(1→4)-GlcNAc-ol
β-Glc-(1→3)-Glc-ol	α-Neu5Ac-(2→3)-β-Gal-(1→3)-GalNAc-ol
β-Glc-(1→4)-Glc-ol	α-Neu5Ac-(2→3)-β-Gal-(1→3)-[α-Neu5Ac-(2→6)]-GalNAc-ol
α-Gal-(1→6)-Glc-ol	

EXPERIMENTAL

Sugar analysis. — Ampoules each containing (oligosaccharide-)alditol (~0.5 mg) were dried over P_2O_5 in a vacuum desiccator. M Methanolic HCl (0.5 mL) was then added and, when necessary, D-mannitol (500 nmol) was added as the internal standard. Nitrogen was bubbled through each solution for 30 s, and each ampoule was then sealed, stored for 24 h at 85°, opened, and neutralised with solid silver carbonate. *N*-Acetylation was effected by the addition of acetic anhydride (10 μL). Each resulting mixture was kept at room temperature for 24 h in the dark, the precipitate was then triturated thoroughly, and, after centrifugation, the supernatant solution was collected. The residue was washed twice with dry methanol (2 × 0.5 mL). The combined supernatant solution and washings were concentrated under reduced pressure at 35°, and each final residue was dried for 12 h in a vacuum desiccator over P_2O_5. Before g.l.c., each sample was trimethylsilylated with pyridine–hexamethyldisilazane–chlorotrimethylsilane (5:1:1, 100 μL) for 30 min at room temperature. G.l.c. was carried out on a CPsil5 WCOT fused-silica capillary column (25 m × 0.32 mm i.d.) with flame-ionisation detection. The oven temperature of the Varian Aerograph 3700 gas chromatograph was programmed from 130 → 220° at 2°/min and kept isothermally at 220° for 1 min. The carrier gas was nitrogen at 1.5 mL/min with make-up nitrogen at 35 mL/min. The split ratio was 1:10. The injection-port temperature was 210°, and the detector temperature was 230°. In the labelling experiments, the *N*-acetylation was carried out with acetic anhydride-d_6 and the trimethylsilylation with pyridine–hexamethyldisilazane-d_{18}–chlorotrimethylsilane-d_9.

Samples. — (Oligosaccharide-)alditols were prepared by reduction (sodium borohydride or borodeuteride) of corresponding saccharides, or obtained from the alkaline borohydride reductive cleavage of *O*-glycosylically linked carbohydrate chains of glycoproteins.

G.l.c.–m.s. — Combined g.l.c.–m.s. was performed on a Carlo Erba GC/Kratos MS80/Kratos DS55 system: electron energy, 70 eV; accelerating voltage, 2.7 kV; ionising current, 100 μA; ion-source temperature, 225°; CPsil5 WCOT fused-silica capillary column (25 m × 0.32 mm i.d.); oven-temperature programme, 130 → 220° at 2°/min.

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