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Note

Determination of position of substitution on 2-acetamido-2-deoxy-D-glucosyl residues in glycolipids

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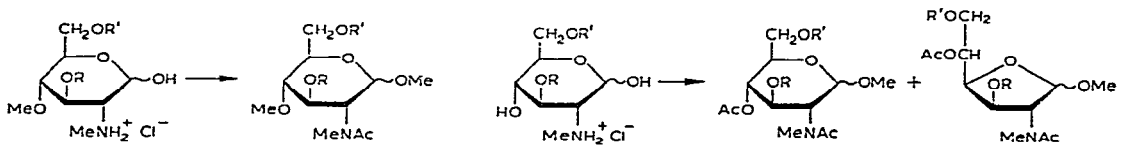
In an accompanying publication[1], we describe a procedure for determining the location of substituents on 2-acetamido-2-deoxy-D-galactosyl residues in glycolipids. Methanolysis of the permethylated glycolipid gave methyl glycosides which, following acetylation, were identified by g.l.c. on columns of OV-1 and OV-225. We now report a parallel study of analogous derivatives of 2-acetamido-2-deoxy-D-glucose and illustrate their use with a glycolipid of known structure.

The recently synthesized methyl ethers of 2-deoxy-2-(*N*-methylamino)-D-glucose[2] were converted into the methyl glycoside standards according to the procedure previously described[1]. As reported for the derivatives of 2-amino-2-deoxy-D-galactose[1], some of the compounds (**1–4**) form pyranosyl glycosides only, whereas others (**9–12**) form furanosyl glycosides as well.



1 R = R′ = Me
2 R = Me, R′ = H
3 R = H, R′ = Me
4 R = R′ = H

5 R = R′ = Me
6 R = Me, R′ = Ac
7 R = Ac, R′ = Me
8 R = R′ = Ac

9 R = R′ = Me
10 R = Me, R′ = H
11 R = H, R′ = Me
12 R = R′ = H

13 R = R′ = Me
14 R = Me, R′ = Ac
15 R = Ac, R′ = Me
16 R = R′ = Ac

17 R = R′ = Me
18 R = Me, R′ = Ac
19 R = Ac, R′ = Me
20 R = R′ = Ac

The chromatograms (Fig. 1) obtained after subjecting the three di-*O*-methyl ethers (**2**, **3**, and **9**) and the single tri-*O*-methyl ether (**1**) to the reaction sequence, followed by g.l.c. on a OV-225 column are all characterized by two peaks, a major

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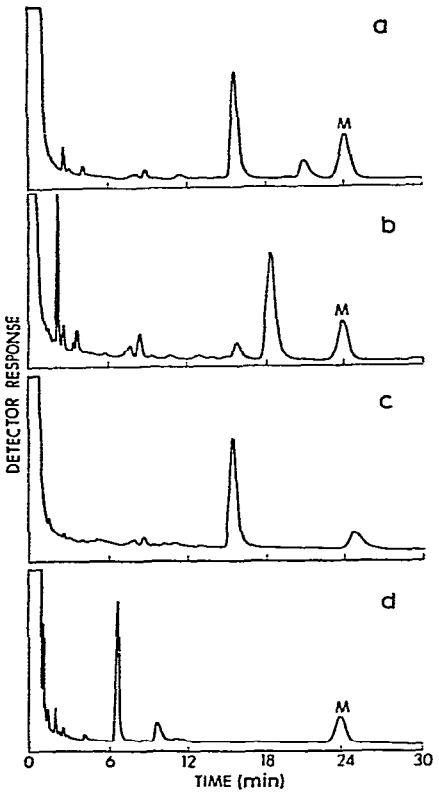
and a minor one, even though the 3,6-dimethyl ether (**9**) can potentially yield as many as four glycosides (a corresponding treatment of 2-amino-2-deoxy-D-galactose gave three products[1]). Fig. 2 shows the g.l.c. patterns of the same methyl glycosides (**5–7, 13, 17**) on an OV-1 column.



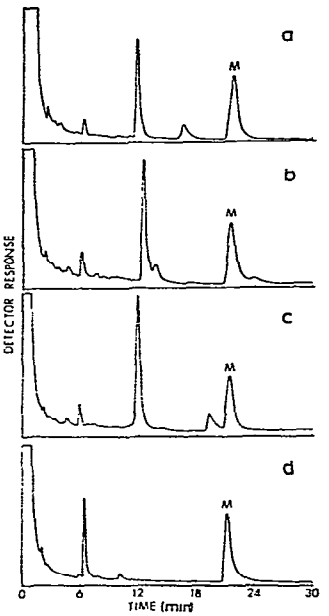


Fig. 1. Gas–liquid chromatograms of methyl glycosides of 2-deoxy-2-(*N*-methylacetamido)-D-glucose with various *O*-acetyl and *O*-methyl substituents; the products in this series contain either two or three *O*-methyl groups. The OV-225 column was operated isothermally at 190°. M, D-mannitol hexaacetate used as standard (omitted from c because of overlap with smaller peak); a, methyl 4(or 5)-*O*-acetyl-2-deoxy-3,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucosides (**13** or **17**); b, methyl 3-*O*-acetyl-2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucopyranosides (**7**); c, methyl 6-*O*-acetyl-2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucopyranosides (**6**); and d, methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucopyranosides (**5**).

Fig. 2. Gas–liquid chromatograms of the same methyl glycosides depicted in Fig. 1, chromatographed on an OV-1 column operated isothermally at 160°. Chromatograms are arranged in same sequence as in Fig. 1.

More overlapping of the g.l.c. peaks was observed with these derivatives than with the corresponding 2-amino-2-deoxy-D-galactose compounds. Thus, the minor peak of the 6-*O*-acetyl-3,4-di-*O*-methyl-D-glucoside mixture (**6**) overlapped with D-mannitol hexaacetate on elution from the OV-225 column (Fig. 1c), and the inter-

nal standard was omitted in this chromatography. The major peaks of the 6-*O*-acetyl-3,4-di-*O*-methyl (**6**) (Fig. 1c) and the 4-*O*-acetyl-3,6-di-*O*-methyl (**13** or **17**) (Fig. 1a) derivatives overlapped on chromatograms obtained by g.l.c. on the OV-225 column, but the two minor peaks have appreciably different elution times and can be used to differentiate the two isomers. On elution from the OV-1 column, the two major peaks of the same derivatives had retention times (relative to D-mannitol hexaacetate) of 0.56 and 0.53, respectively (Table I), which is a difference sufficient for resolution and characterization; the minor peaks again showed an even greater difference.

TABLE I

G.L.C. CHARACTERISTICS OF METHYL GLYCOSIDES OF *O*-ACETYL-*O*-METHYL-SUBSTITUTED 2-DEOXY-2-(*N*-METHYLACETAMIDO)-D-GLUCOSE

Positions of substituents			*Starting compound*	*Retention times[a] of peaks on columns of*			
Methyl	*Acetyl*			*OV-225*		*OV-1*	
				Major	*Minor*	*Major*	*Minor*
3,4,6		(5)	**1**	0.28 (84, β)	0.41 (16, α)	0.30 (92, β)	0.47 (8, α)
3,4	6	(6)	**2**	0.67 (82, β)	1.05 (18, α)	0.56 (85, β)	0.91 (15, α)
4,6	3	(7)	**3**	0.79 (89, β)	0.69 (11, α)	0.58 (88, β)	0.64 (12, α)
3,6	4	(13, 17)	**9**	0.67 (85)	0.88 (15)	0.53 (82)	0.76 (18)
3	4,6	(14, 18)	**10**	1.35 (73)	1.86 (27)	1.0 (75)	1.43 (25)
4	3,6	(8)	**4**	1.78 (79)	1.62 (21)	1.07 (77)	1.21 (23)
6	3,4	(15, 19)	**11**	1.14 (71)	1.07 (20) 1.35 (5)	0.81 (70)	0.92 (17) 1.36 (6) 1.50 (7)
	3,4,6	(16, 20)	**12**	2.21 (88)	1.74 (12)	1.31 (69)	1.08 (5) 1.56 (22) 1.84

[a]Retention times are relative to D-mannitol hexaacetate. Percentages in parentheses are relative peak areas of glycosides resulting from methanolysis in 0.1M methanolic hydrogen chloride for 18 h at 80°. G.l.c. was performed isothermally at 190° and 210° with the OV-225 column, and 160° and 170° with OV-1.

The chromatograms (Figs. 3 and 4) of the remaining glycoside mixtures include the methyl glycosides obtained from 2-deoxy-2-(*N*-methylacetamido)-D-glucose and its three mono-*O*-methyl derivatives. The compounds resulting from the 3-*O*-methyl (**10**) and 4-*O*-methyl (**4**) starting compounds each gave essentially two peaks when chromatographed on OV-225 (Fig. 3) and OV-1 (Fig. 4) columns, whereas the 6-*O*-methyl derivative (**11**) and the compound without *O*-methyl groups (**12**) each gave rise to multiple peak patterns. Table I summarizes the relative retention times of these products on elution from the two columns employed. The compounds (**15** and **19**) obtained from the 6-*O*-methyl derivative gave, on elution from an OV-225 column, a mixture somewhat more complex than indicated in the Table, owing to the presence of partially unresolved shoulders.

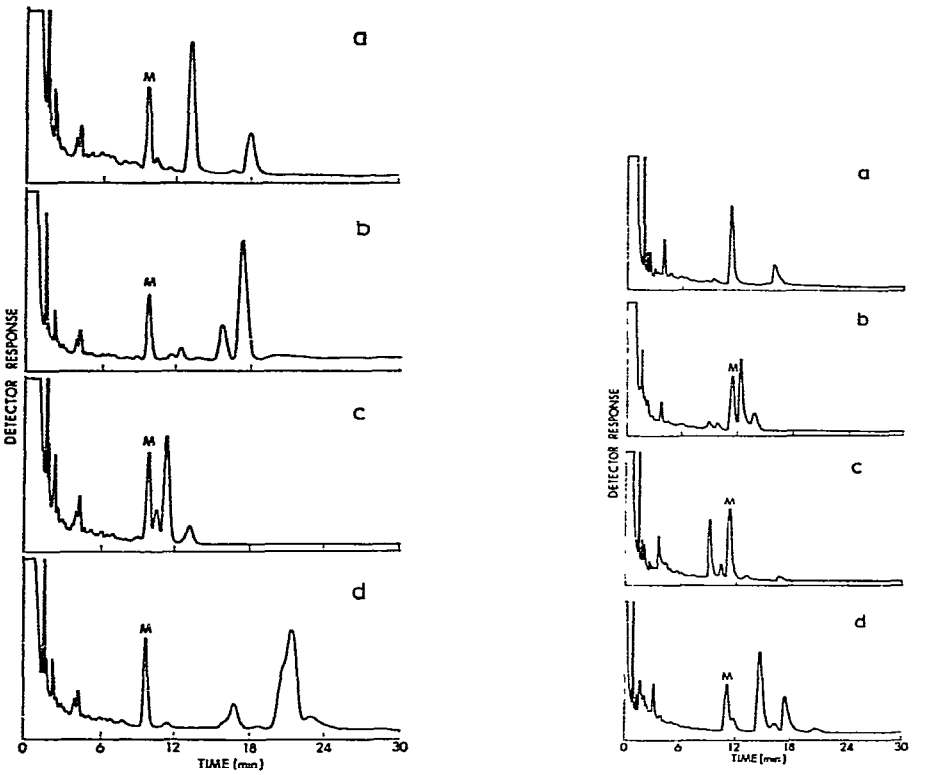


Fig. 3. Gas–liquid chromatograms of methyl glycosides of 2-deoxy-2-(*N*-methylacetamido)-D-glucose with various *O*-acetyl and *O*-methyl substituents; the products in this series contain either one or zero *O*-methyl groups. The OV-225 column was operated isothermally at 210°. M, D-mannitol hexaacetate used as standard; a, methyl 4,6(and 5,6)-di-*O*-acetyl-2-deoxy-3-*O*-methyl-2-(*N*-methylacetamido)-D-glucosides (**14** and **18**); b, methyl 3,6-di-*O*-acetyl-2-deoxy-4-*O*-methyl-2-(*N*-methylacetamido)-D-glucopyranosides (**8**); c, methyl 3,4(and 3,5)-di-*O*-acetyl-2-deoxy-6-*O*-methyl-2-(*N*-methylacetamido)-D-glucosides (**15** and **19**); and d, methyl 3,4,6(and 3,5,6)-tri-*O*-acetyl-2-deoxy-2-(*N*-methylacetamido)-D-glucosides (**16** and **20**).

Fig. 4. Gas–liquid chromatograms of the same methyl glycosides depicted in Fig. 3, chromatographed on an OV-1 column operated isothermally at 170°. Chromatograms are arranged in the same sequence as in Fig. 3. D-Mannitol hexaacetate standard was omitted from a, because of overlap with major peaks.

The procedure was tested with "paragloboside", a glycolipid of known structure[3]: β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-β-D-Glc-(1→1)-ceramide. After methanolysis and acetylation of the permethylated glycolipid, the chromatograms obtained on elution from both OV-225 and OV-1 columns (Figs. 5 and 6) were clearly those of 4-*O*-acetyl-3,6-di-*O*-methylglycosides (**13** or **17**), indicating substitution on the 4-hydroxyl group of the 2-amino-2-deoxy-D-glucose residue, as previously shown by Hakomori[3]. It may be noted that methyl glycosides of the *O*-methylated neutral sugars that form simultaneously during the reaction

sequence are eluted from the columns soon after the solvent front and, hence, do not interfere with the hexosamine peaks.

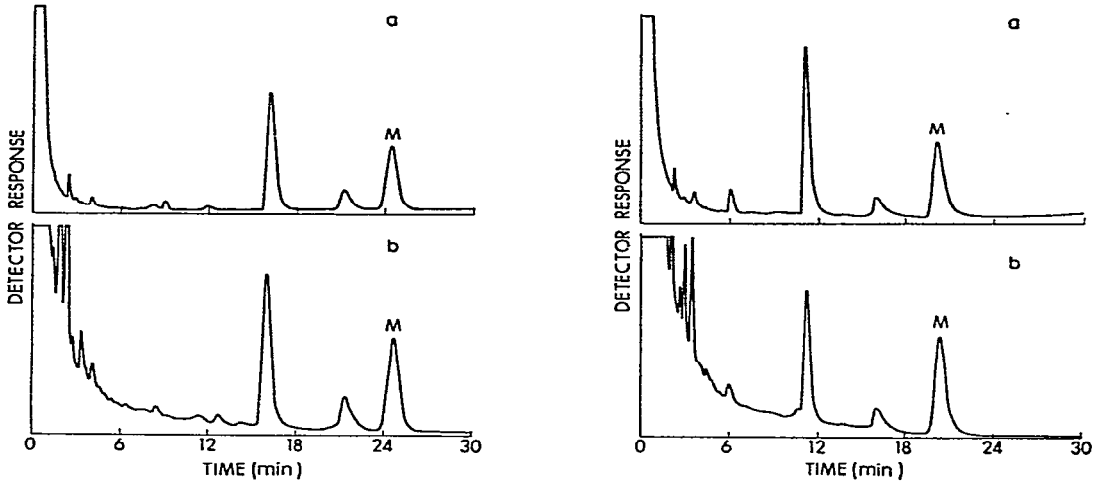


Fig. 5. Comparison of the methyl glycosides obtained from permethylated paragloboside with standards: a, methyl 4(or 5)-*O*-acetyl-2-deoxy-3,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucosides (13 or 17); b, corresponding methyl glycosides obtained by methanolysis of permethylated paragloboside; M, D-mannitol hexaacetate used as standard. G.l.c. was performed with an OV-225 column operated isothermally at 190°.

Fig. 6. Comparison of the methyl glycosides obtained from permethylated paragloboside with standards: the same samples were employed as in Fig. 5, but chromatographed on a OV-1 column operated isothermally at 160°.

The majority of naturally-occurring glycolipids that contain hexosamine units have such units in either a terminal position or in a nonterminal, unbranched location. These structures would give rise to the relatively simple chromatograms depicted in Figs. 1 and 2. The fact that most glycolipids appear to contain a single hexosamine unit would also tend to favor the simpler patterns. Attempts to apply the present method to 2-amino-2-deoxy-D-hexose-containing oligosaccharides did not give clearly delineated chromatograms, possibly because of the presence of a sensitive reducing group, which rendered the molecule susceptible to degradation by the strongly basic permethylation reagent. Preliminary experiments with the Forssman oligosaccharide suggest that the structure of such compounds may be successfully determined when permethylation is preceded by borohydride reduction of the free aldehyde group.

As with 2-amino-2-deoxy-D-galactose derivatives[1], we have found that the area ratios as well as the retention times of the patterns of multiple g.l.c. peaks are quite reproducible, provided the experimental conditions are standardized. The resulting "fingerprints" thus provide reliable criteria for identification of the substitution positions on 2-amino-2-deoxy-D-glucose units located within an oligosaccharide

chain. The method has been successfully applied to 20 μg of glycolipid, and probably could be scaled down.

EXPERIMENTAL

Virtually all procedures followed the description given in the accompanying report on 2-amino-2-deoxy-D-galactose derivatives[1]. Starting materials for preparation of the 2-amino-2-deoxy-D-glucose derivatives as standards for g.l.c. were 2-deoxy-2-(*N*-methylamino)-D-glucose, and all the mono-, di- and tri-*O*-methyl derivatives that were recently synthesized[2]. The glycolipid "paragloboside" was a kind gift of Dr. S. Hakomori. The methyl glycosides of the glucosamine derivatives were separated on both OV-1 and OV-225 columns in the presence of D-mannitol hexaacetate as standard for relative retention times. Although the separation on the OV-225 column alone would have provided a reasonably definitive identification of the 4-*O*-acetyl-3,6-di-*O*-methyl glycosides resulting from paragloboside, the separation on both columns made the assignment virtually unequivocal. In preparing the OV-1 column, it was important to employ the high-performance Chromosorb W-HP as solid support, in order to obtain the desired resolution. Configurational assignments (Table I) were made on the basis of analogy with the corresponding derivatives of 2-acetamido-2-deoxy-D-galactose[1].

ACKNOWLEDGMENTS

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