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NITROUS ACID DEAMINATION OF METHYLATED AMINO-OLIGOSACCHARIDE GLYCOSIDES*†

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ABSTRACT

G.l.c.–mass spectrometry has been used to characterize the products of *N*-deacetylation–nitrous acid deamination of per-*O*-methylated derivatives (**8–11**) of methyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (**1**), methyl (**2**) and benzyl (**3**) 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosides, and methyl 2-acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (**4**). 2,5-Anhydrohexoses have been converted into alditol trideuteriomethyl ethers, alditol acetates, and aldononitriles. The importance of side reactions that lead to the formation of 2-deoxy-2-*C*-formylpentofuranosides is discussed.

INTRODUCTION

Nitrous acid deamination of 2-amino-2-deoxyhexopyranosides having equatorial amino groups is a highly selective reaction for the cleavage of glycosidic linkages under mild, acid conditions with the formation of 2,5-anhydrohexoses and with exposure of aglyconic hydroxyl groups[1]. Provided that both liberated sugar units and exposed hydroxyl groups can be unambiguously characterized, it is possible to obtain more structural information from the selective fragmentation of methylated carbohydrate polymers than from the parent oligo- or poly-saccharides[2,3]. In order to explore the potential value of the deamination reaction for the controlled depolymerization of per-*O*-methylated oligo- or poly-saccharides, such as those derived from glycoproteins, we have applied this reaction to some aminodisaccharide glycosides and their methylated derivatives. Particular attention has been given to the conversion of products into derivatives suitable for characterization by g.l.c.–mass spectrometry (m.s.).

*Dedicated to Dr. Elizabeth Percival.
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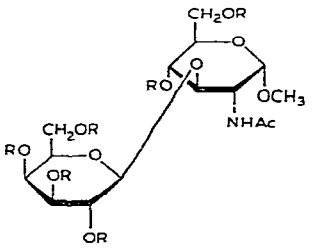
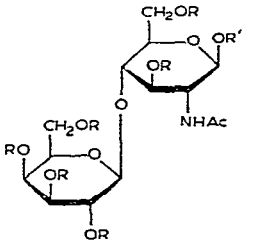
RESULTS AND DISCUSSION

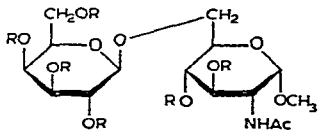
Methyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (**1**) was prepared as described by Matta and Barlow[4]. Methyl (**2**) and benzyl (**3**) 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosides were prepared by *O*-deacetylation of their hexa-*O*-acetyl derivatives (**5** and **6**) obtained from *N*-acetyl-β-lactosamine hepta-acetate[5] by the oxazoline method[6]. Methyl 2-acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (**4**) was prepared by the Bredereck condensation[7] of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide and methyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-trityl-α-D-glucopyranoside[8] to give the hexa-*O*-acetyl derivative (**7**), from which **4** was formed on *O*-deacetylation.



1 R = H 8 R = CH_3



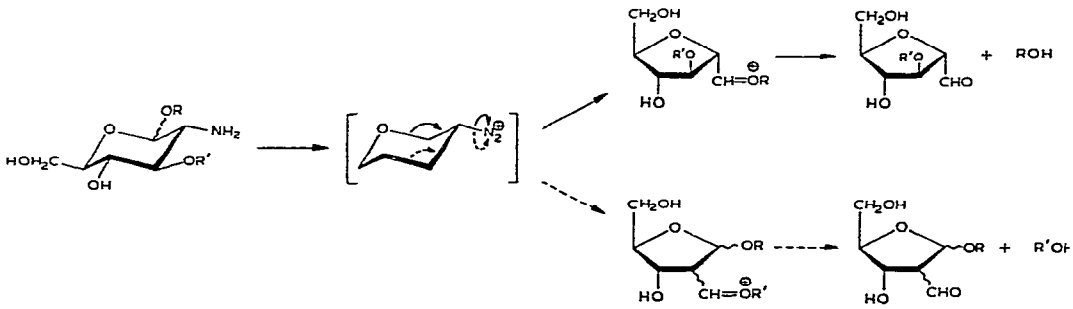
2 R = H, R′ = CH_3 6 R = Ac, R′ = CH_2Ph
3 R = H, R′ = CH_2Ph 9 R = CH_3, R′ = CH_3
5 R = Ac, R′ = CH_3 10 R = CH_3, R′ = CH_2Ph



4 R = H 11 R = CH_3
7 R = Ac

Erbing *et al.*[9] have shown that the dominant reaction in the nitrous acid deamination of methyl 2-amino-2-deoxy-α(or -β)-D-glucopyranoside, namely, the formation of 2,5-anhydro-D-mannose, is accompanied by an alternative ring-contraction, leading to the formation of methyl 2-deoxy-2-*C*-formylpentofuranosides. These authors have further suggested that this competing reaction would account for the observation of Dmitriev *et al.*[10] that deamination of benzyl 2-amino-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranoside, followed by reduction with sodium borohydride, led to the formation of galactitol and unidentified minor products in addition to the major product, 2,5-anhydro-3-*O*-β-D-galactopyranosyl-D-mannitol. The correctness of this suggestion has now been confirmed in the following reaction of methyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (**1**). The disaccharide glycoside **1** was *N*-deacetylated by hydrazinolysis[11], the product was treated with nitrous acid, and, in order to minimize the possibility of galactitol's being formed inadvertently by base-catalyzed β-elimination followed by reduction on treatment of the intermediate 2,5-anhydro-3-*O*-β-D-galactopyranosyl-D-mannose with sodium borohydride, the deamination products were reduced with sodium cyanoborohydride at pH 3.5. The major product was 2,5-anhydro-3-*O*-β-D-galacto-pyranosyl-D-mannitol, characterized as the hepta-acetate. The formation, as by-products, of galactitol and, in approximately equimolar proportions, a mixture of

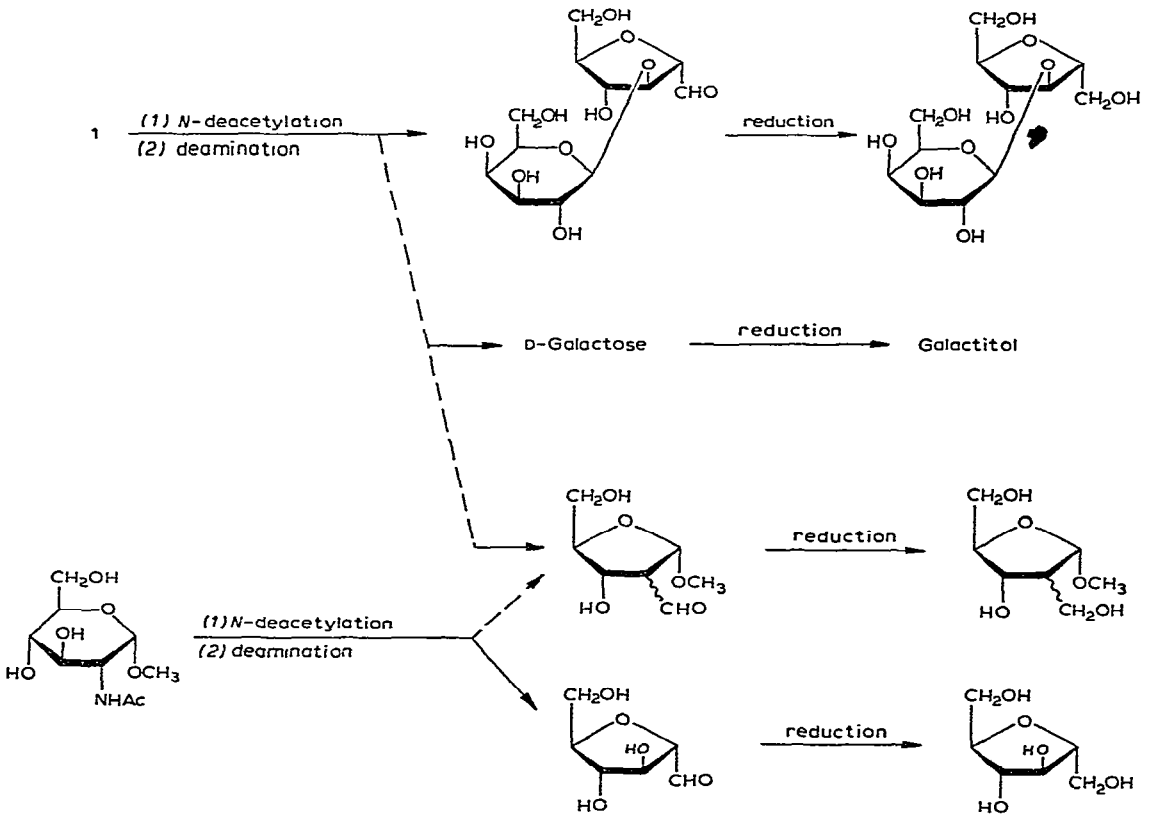
methyl 2-deoxy-2-*C*-hydroxymethyl-α-D-*ribo*-(and D-*arabino*-)pentofuranosides was shown by g.l.c. of their peracetates. Additionally, the mass spectra of the branched-chain glycoside acetates confirmed their identity with the compounds similarly derived from methyl 2-amino-2-deoxy-α-D-glucopyranoside[9].



Scheme 1 Alternative ring contractions during the nitrous acid deamination of 2-amino-2-deoxyglycosides having equatorial amino groups

The alternative ring-contraction which may accompany 2,5-anhydrohexose formation is outlined in Scheme 1. 3-*O*-Substituted 2,5-anhydrohexose units are indeed susceptible to base-catalyzed β-elimination, and this reaction has been used in the characterization of oligosaccharides liberated during the deamination of aminopolysaccharides[12]. It is now clear, however, that formation of alditols (*e.g.*, of galactitol above) during the deamination–reduction sequence of reactions need not arise inadvertently during the reduction step, as suggested by Dmitriev *et al.*[10], but is a direct consequence of the alternative ring-contraction of 3-*O*-substituted 2-amino-2-deoxyglycosides (Scheme 2), because alditol formation is accompanied by the formation of 2-deoxy-2-*C*-hydroxymethylpentofuranosides. In our experience, the use of unbuffered sodium borohydride reduction is not accompanied by significant base-catalyzed β-elimination. The alternative ring-contraction, which accounts for ~20% of the products from nitrous acid deamination, is likely to prove valuable in linkage and sequence analysis of amino-oligosaccharides, in being diagnostic for 3-*O*-substituted 2-amino-2-deoxyglycosidic linkages.

Per-*O*-methylated aminodisaccharide glycosides (**8–11**) were prepared (and isolated as crystalline compounds), without significant *N*-methylation, by treatment of the parent disaccharide glycosides (**1–4**) with methyl sulfate and sodium hydroxide. Small samples of the three methyl glycosides (**1**, **2**, and **4**) were also methylated by the Hakomori procedure[13]. In all cases, the mass spectra of the methylated disaccharides showed fragment ions confirming the nature of the reducing and non-reducing units. For the per-*O*-methylated (but not *N*-methylated) methyl (**9**) and benzyl (**10**) disaccharide glycosides, fragment ions at *m/e* 332 and 408 (baB_1) and 188 (bB_2) corresponded to those found at *m/e* 305 and 161 for permethylated hexopyranosyl-(1→4)-hexoses[14]. The methylated disaccharide glycoside **11** showed the fragment ion

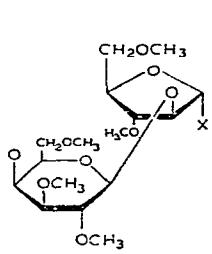


Scheme 2 *N*-Deacetylation and deamination followed by reduction, of methyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (1)

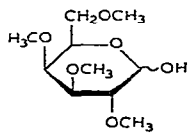
at *m/e* 353 (baD_1) which is characteristic of methylated hexopyranosyl-(1→6)-hexopyranoses[14]. The features most readily indicative of the (1→3)-linkage in the methylated methyl disaccharide-glycoside **8** are the relative proportions of fragment ions in the A series. In the further fragmentation of the baA_1 ion, the next preferred ion in the series is that resulting from β-elimination of the 3-*O*-substituent[14]. Thus, for the disaccharide derivatives **9** and **11**, the M−31 (baA_1 ion at *m/e* 450) and M−(31+32) (baA_2 ion at *m/e* 418) peaks are of approximately equal intensity, whereas for the (1→3)-linked compound **8**, the peak at *m/e* 418 is relatively weak and that at *m/e* 214 (bA_2) is relatively intense. Examination of the mass spectra reported by Hallgren and Lundblad[15] for permethylated lacto-*N*-tetraitol and methylated neolacto-*N*-tetraitol suggests that the relative intensities of these peaks in the A series are generally diagnostic for the presence of (1→3)-linkages.

Several groups of workers[16] have observed that 3-*O*-substituted 2-acetamido-2-deoxyglycosides are very resistant to basic *N*-deacetylation, and hydrazinolysis of the per-*O*-methylated acetamidodisaccharide glycosides proceeded extremely

sluggishly (up to 120 h in boiling hydrazine). The variously linked, methyl disaccharide glycosides were treated with nitrous acid, and the products were submitted to three different procedures for the formation of derivatives suitable for analysis by g.l.c.–m.s.: (*a*) reduction with sodium borodeuteride followed by *O*-trideuteriomethylation; (*b*) reduction with sodium borodeuteride followed by acetylation; and (*c*) treatment with hydroxylamine, followed by acetic anhydride in pyridine*. In the case of the (1→3)-linked disaccharide derivative **8**, monosaccharide products from the alternative ring-contraction were first characterized. Each procedure led to readily identified derivatives of 2,3,4,6-tetra-*O*-methylgalactose (**16**). Derivatives of the epimeric 2-deoxy-2-*C*-formylpentofuranosides (**17**) were only recognized in the case of **18** formed on reduction and acetylation. These derivatives (**18**) were also formed in a similar manner from methyl 2-amino-2-deoxy-3,4,6-tri-*O*-methyl-α-D-glucopyranoside. The major product (**12**) from the deamination furnished derivatives **13–15** which were analyzed by g.l.c.–m.s. The mass spectra clearly established the nature of the reducing and non-reducing units but, as discussed in more detail below, linkage types were indicated with less certainty.



12 X = CHO
13 X = $CHDOCD_3$
14 X = CHDOAc
15 X = CN



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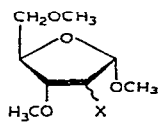
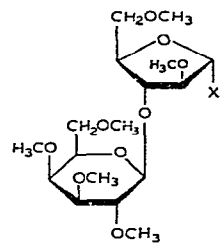
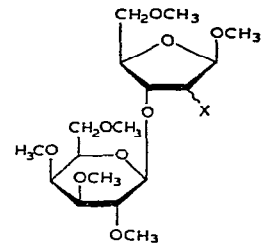


17 X = CHO
18 X = CHDOAc



19 X = CHO
20 X = CHDOH
21 X = $CHDOCD_3$
22 X = CHDOAc
23 X = CN

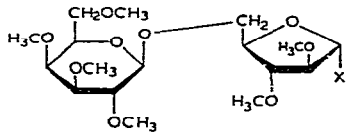


24 X = CHO
25 X = $CHDOCD_3$
26 X = CHDOAc
27 X = CN

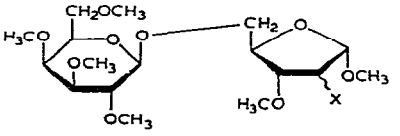
N-Deacetylation of the per-*O*-methylated disaccharide methyl glycosides **9** and **11** followed by nitrous acid deamination was carried out in a similar manner, but, in each case, product analysis was complicated by the formation of isomeric 2,5-anhydromannose and 2-*C*-formylpentofuranoside derivatives which were inseparable by g.l.c. Thus, the (1→4)-linked disaccharide **9** presumably furnished a mixture of **19** and **24**, and analogous products (**28** and **32**) would be formed from the (1→6)-linked disaccharide (**11**). Samples of the deamination products were reduced with sodium borodeuteride, hydrolyzed, and reduced with sodium borohydride, and the resulting alditols were acetylated and the partially methylated alditol acetates were examined by

*Vercellotti *et al.*[17] have recently reported that treatment of 2,5-anhydro-D-mannose leads to the formation of an acetylated oxime acetate, which furnishes the acetylated aldononitrile on subsequent heating. The conditions used in our experiments were such that the intermediate formation of oxime acetates *en route* to aldononitriles would not have been detected.

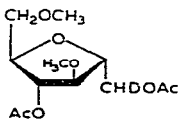
g.l.c.–m.s. Each disaccharide furnished 2,3,4,6-tetra-*O*-methylgalactitol diacetate and a suitably labelled di-*O*-acetyl-2,5-anhydro-di-*O*-methylmannitol (**36** and **37**, respectively). However, no direct evidence has yet been obtained for branched-chain alditol derivatives formed from ring-contracted glycosides.



28 X = CHO
29 X = $CHDOCD_3$
30 X = CHDOAc
31 X = CN



32 X = CHO
33 X = $CHDOCD_3$
34 X = CHDOAc
35 X = CN



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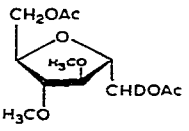

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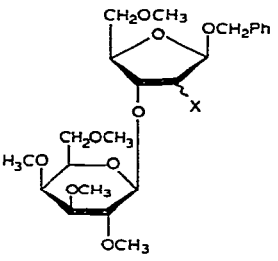
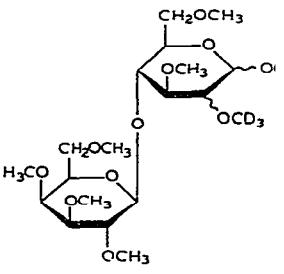


38 X = CHO
39 X = CHDOH

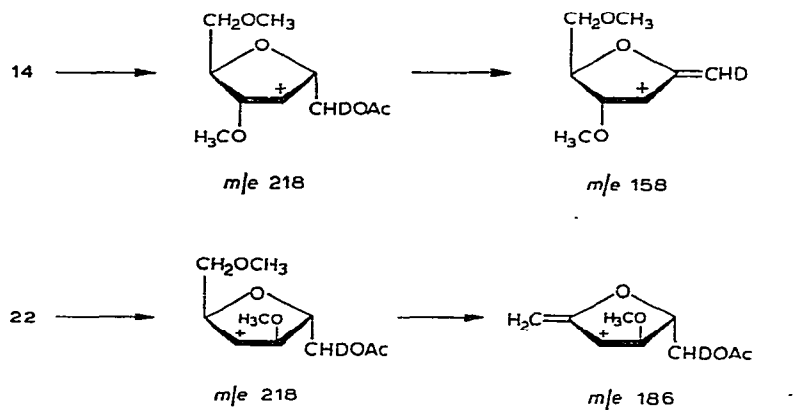


40

That the alternative ring-contraction leading to the 2-deoxy-2-*C*-formylpentofuranosides accompanies the major glycoside-cleavage reaction was directly demonstrated for the benzyl glycoside (**10**) which was *N*-deacetylated and treated with nitrous acid. The reaction mixture (containing **19** and **38**) was reduced with sodium borodeuteride, and the products (**20** and **39**) were separated by t.l.c. The 2,5-anhydromannitol derivative **20** was converted into the trideuteriomethyl ether (**21**) and the acetate (**22**), to furnish samples for g.l.c.–m.s. which were uncontaminated by ring-contracted glycosides. The mixture of benzyl glycosides was hydrogenolyzed over palladium–charcoal, the reducing sugars were treated with methanolic hydrogen chloride at room temperature, and the resulting methyl glycosides were trideuteriomethylated. G.l.c.–m.s. analysis of the fully methylated disaccharides thus obtained revealed six components, four of which were sufficiently separated to give readily interpretable mass spectra. The major component had the same retention time as the isomeric 2,5-anhydromannitol derivative **21**, and was most probably **25** (as a mixture of C-2 epimers). Two other peaks gave the same mass spectra and were probably the corresponding β-D-glycosides. The remaining recognizable component gave a mass spectrum consistent with the presence of a permethylated methyl hexopyranosyl-(1→4)-hexoside (**40**) containing a single trideuteriomethyl group at O-2. This compound, which must have originated as a benzyl glycoside in the deamination reaction, represented not more than ~3% of the total mixture formed from the parent benzyl

glycoside **10**. The compound probably originates as a product of solvolytic displacement rather than rearrangement of the intermediate diazonium ion formed in the nitrous acid deamination.

In the foregoing experiments, no significant fragment ions were observed in the high-mass range of the spectra of **25** and its epimers, *e.g.*, M − 31 (OCH_3), which would confirm the presence of the methyl glycosides. Furthermore, the fragment ions (bA_1, *etc.*) derived from the permethylated methyl 2-deoxy-2-hydroxymethylpentofuranoside moieties had the same *m/e* values as those of the corresponding, permethylated 2,5-anhydromannitol moieties formed from the isomeric compound. Mass spectrometry, therefore, cannot be used to identify the presence of isomeric components when these are formed in admixture, *e.g.*, **21** and **25** from the methylated disaccharide **9**, or **29** and **33** from the methylated disaccharide **11**.



Scheme 3

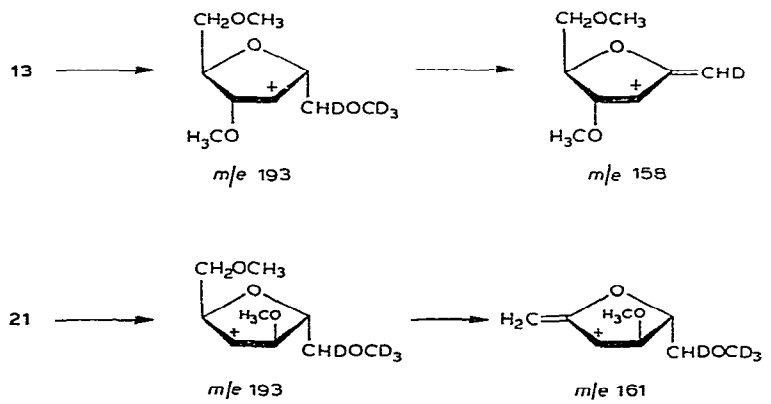
Relative retention times for the derivatives of the variously linked, per-*O*-methylated 2,5-anhydro-*O*-β-D-galactopyranosyl-D-mannoses are shown in Table I. The mass spectra clearly indicated the nature of both sugar units, but only very weak peaks were detected in the high-mass range and conclusions as to linkage type could only be drawn in those instances where the fragmentation pathways of ions derived from the "non-reducing" moiety provided relevant information. The three aldononitriles (**15**, **23**, and **31**) were easily recognized by their retention times, but the mass spectra contained no obvious features indicative of linkage type. Of the per-*O*-methylated disaccharide alditol acetates, the (1→3)- (**14**) and (1→4)-linked (**22**) isomers were inseparable by g.l.c., but the fragment ions shown in Scheme 3 enable inferences to be made on the sites of substitution. Of the permethylated 2,5-anhydro-β-D-galactopyranosyl-D-mannitols (**13**, **21**, and **29**), the (1→6)-linked derivative (**29**), although probably not separated from the isomeric 2-deoxy-2-*C*-methoxymethylglycoside (**33**), was well-separated (in g.l.c.) from **13** and **21** which, isotopic labelling aside, are the same compound. Nevertheless, the mass spectra of the isotopically

TABLE I

G.L.C. OF DERIVATIVES FORMED FROM PRODUCTS OF *N*-DEACETYLATION–DEAMINATION OF PER-*O*-METHYLATED DISACCHARIDE GLYCOSIDES

Per-O-methylated disaccharide	*Intermediate compounds*	*Derivatives*	*Relative retention times*[a] *(column B at 210°)*
8	12	13	1.19
		14	2.40
		15	2.14
9	19+24	21+25	1.19
		22+26	2.41
		23+27	1.99
11	28+32	29+33	1.62
		30+34	3.73
		31+35	3.00
10	19+38	20 → 21	1.19
		20 → 22	2.41
		39 → 25	1.19
		39 → epimers of 25	0.98, 1.42
		39 → 40	1.87

[a]Retention times are relative to nona-*O*-methyl-lactitol. In further examination by g.l.c.–m.s., the mass spectra of each disaccharide derivative showed prominent fragment ions in the aA, bA, and abJ$_1$ series, which established the nature of the sugar moieties. The experimental section records further details only when the spectra provide information on linkage types.



Scheme 4

distinct derivatives **13** and **21** showed sufficient differences in the abundances of fragment ions at *m/e* 158 and 161 (Scheme 4) to be diagnostic of linkage type. In unpublished experiments on other compounds of known structure, we have shown that this distinction between 3-*O*- and 4-*O*-substituted 2,5-anhydrommanitol units formed from the deamination reaction sequence is reliable. The permethylated

2,5-anhydromannitol derivatives are the most thermally stable of those examined, and g.l.c.–m.s. can be used for the analysis of the corresponding trisaccharide derivatives.

The 3,4,6-tri- and 3,4-di-*O*-methyl derivatives of 2,5-anhydro-D-mannitol were synthesized as their acetates, to serve as convenient reference substances. The syntheses were by standard procedures from 1-*O*-trityl- and 1,6-di-*O*-trityl-D-mannitol, respectively. 1-*O*-Acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol was obtained as the major product of *N*-deacetylation and deamination of methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-α(and -β)-D-glucopyranoside, followed by reduction and acetylation. The isomeric methyl 2-*C*-acetoxymethyl-2-deoxypentofuranosides, formed as minor products from the alternative ring-contraction, were difficult to separate. For products from the α-D-glycoside, partial separation of one component was obtained, and the mass spectrum of this substance showed fragment ions for M−31 (OCH_3) and M−(31+60) ($OCH_3+CH_3CO_2H$) which differentiated the glycoside from the isomeric 2,5-anhydro-D-mannitol derivative, for which fragment ions were observed as M−32 (CH_3OH) and M−(32+60) ($CH_3OH+CH_3CO_2H$).

The experiments reported here show that nitrous acid deamination of per-*O*-methylated amino-oligosaccharides affords products which may be converted into derivatives suitable for characterization by g.l.c.–m.s. The problems encountered with the formation of methyl 2-deoxy-2-*C*-formylpentofuranosides isomeric with 2,5-anhydro-D-mannose derivatives arise only from the use of methyl glycosides as model compounds. The alternative ring-contraction will give products of different degrees of polymerization when the aminoglycosidic linkage undergoing reaction is part of a longer, methylated oligosaccharide chain, such as those derived from glycoproteins.

EXPERIMENTAL

General methods. — Evaporations were carried out under diminished pressure at bath temperatures of 40° or less. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter at 20 ±2°. N.m.r. spectra were recorded at 60 MHz with a Varian EM-360 spectrometer, and unless stated otherwise, for solutions in chloroform-*d* containing 1% of tetramethylsilane as internal standard. Microanalyses were determined by A. B. Gygli, Toronto, Ontario.

G.l.c. was performed, at temperatures indicated, with Perkin–Elmer 990 and Tracor 560 chromatographs, using columns of Gas-Chrom Q coated with (*A*) 3% of silicone-polyester copolymer ECNSS-M, (*B*) 3% of silicone gum OV-225, and (*C*) 3% of silicone gum XE-60; (*D*) an OV-225 S.C.O.T. column; and (*E*) a column of Chromosorb W-HP coated with 3% of silicone gum OV-1. For g.l.c.–m.s., columns were attached *via* a Watson–Biemann separator to a Perkin–Elmer–Hitachi RMU-6 mass spectrometer, operated with an inlet temperature of 250°, an ionization potential of 70 eV, and an ion-source temperature of ~250°.

*Methylation of methyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranoside* (**1**). — The glycoside **1** was prepared independently, but by the route

recently reported by Matta and Barlow[4]. The physical constants of all intermediate compounds were in agreement with those reported by these authors[4], but those for **1** were rather different, although our sample also gave satisfactory elemental and n.m.r. spectroscopic analyses. Our sample of **1** had m.p. 247–249° and $[\alpha]_D$ +86° (*c* 0.5, water); lit.[4], m.p. 236–239°, $[\alpha]_D$ +57° (water). The glycoside **1** (90 mg) was methylated by successive additions of methyl sulfate and aqueous 30% sodium hydroxide. The final reaction mixture was heated at 80° for 2 h to destroy methyl sulfate, the cooled solution was neutralized with sulfuric acid, and sodium sulfate was precipitated by the addition of ethanol (3 vol.). The filtrate was concentrated to remove ethanol, and the resulting solution was extracted continuously with dichloromethane to furnish a solid (68 mg) that was recrystallized twice from ether to give methyl 2-acetamido-2-deoxy-4,6-di-*O*-methyl-3-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**8**, 54 mg), m.p. 157–159°, $[\alpha]_D$ +80° (*c* 1.0, chloroform); n.m.r. data: δ 2.13 (s, 3 H, NAc), 3.40–3.63 (m, 21 H, OMe), 4.62 (d, 1 H, *J* 3.5 Hz, H-1), 4.25 (d, 1 H, *J* 7 Hz, H-1′); *m/e* (% relative abundance) 481 (0.3, M^+), 450 (1, $M-OCH_3$[baA$_1$]), 418 (0.05, $M-OCH_3-CH_3OH$[baA$_2$]), 306 (42, abJ$_1$), 246 (34, bA$_1$), 219 (11, aA$_1$), 214 (97, bA$_2$), 187 (53, aA$_2$), 182 (37, bA$_3$), 101 (100), and 88 (100).

Anal. Calc. for $C_{21}H_{39}NO_{11}$: C, 52.39; H, 8.11; N, 2.74. Found: C, 52.21; H, 8.11; N, 2.74.

A sample of **1** was methylated by the Hakomori procedure[13], and the per-methylated (*N* and *O*) disaccharide, after purification by chromatography on silica gel, gave a mass spectrum that included peaks at *m/e* 495 (1.5, M^+), 464 (1.3, $M-OCH_3$[baA$_1$]), 432 (0.3, $M-OCH_3-CH_3OH$[baA$_2$]), 320 (10, abJ$_1$), 260 (99, bA$_1$), 228 (32, bA$_2$), 219 (10, aA$_1$), 187 (41, aA$_2$), 101 (77), and 88 (100).

*Methyl 2-acetamido-2-deoxy-4-*O*-β-*D*-galactopyranosyl-β-*D*-glucopyranoside* (**2**) *and per-*O*-acetyl* (**5**) *and per-*O*-methyl* (**9**) *derivatives.* — *N*-Acetyl-β-lactosamine hepta-acetate[5] (2.5 g) was kept in dichloromethane (215 ml) containing ferric chloride (2.5 g) for 3.5 h. The mixture was diluted with dichloromethane (400 ml), and the solution was washed with ice–water, dried, and concentrated to give a syrupy oxazoline, whose i.r. spectrum showed bands at 1760 (C=O), 1680 (C=N), and 1280 (C–OAc), but none at 1580 cm^{-1} (amide II), and for which t.l.c. showed only traces of impurities. Methanol (1.5 ml) and *p*-toluenesulfonic acid (35 mg) were added to the oxazoline (1.5 g) in nitromethane–toluene (1:1, 50 ml), and the solution was kept at 40° for 1 h, neutralized with aqueous sodium hydrogen carbonate, dried, and concentrated to a syrup. The syrup was chromatographed on silica gel (50 × 3 cm), and elution with benzene–acetone (1:1) gave a syrup which crystallized from ether to give the hexa-*O*-acetyl derivative **5** (1.21 g), m.p. 151–153°, $[\alpha]_D$ −16° (*c* 1.0, dichloromethane); n.m.r. data: δ 1.90–2.20 (m, 21 H, NAc and OAc), 3.40 (s, 3 H, OMe), 4.40 (d, 1 H, *J* 7 Hz, H-1′), 4.50 (d, 1 H, *J* 9 Hz, H-1), and 5.90 (d, 1 H, NH).

Anal. Calc. for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.01; N, 2.16. Found: C, 49.79; H, 6.09; N, 2.04.

M Barium methoxide (~5 ml) was added dropwise to **5** (710 mg) in methanol (25 ml) until the solution was permanently alkaline. The solution was kept at 40° for

45 min, and was then filtered, stirred with Amberlite IR-120(H^+) resin to remove barium ions, filtered, and concentrated to a syrup which was crystallized from methanol to give the disaccharide glycoside **2** (340 mg), m.p. 250–252°, $[\alpha]_D$ −34° (*c* 1.0, water); lit.[18] m.p. 244°, $[\alpha]_D$ −23° (water); n.m.r. data (D_2O): δ 1.90 (s, 3 H, NAc) and 3.30 (s, 3 H, OCH_3).

Compound **2** (120 mg) was treated with methyl sulfate and 30% aqueous sodium hydroxide, as described above, and the product (78 mg) was crystallized from hexane–ethyl acetate to give methyl 2-acetamido-2-deoxy-3,6-di-*O*-methyl-4-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**9**, 65 mg), m.p. 184–185°, $[\alpha]_D$ −21° (*c* 1.0, chloroform); n.m.r. data: δ 2.06 (s, 3 H, NAc), 3.45–3.63 (m, 21 H, OMe), 4.39 (d, 1 H, *J* 7 Hz, H-1′), and 4.71 (d, 1 H, *J* 7 Hz, H-1); *m/e* 481 (0.8, M^+), 450 (0.2, $M-OCH_3$[baA$_1$]), 418 (0.24, $M-OCH_3-CH_3OH$[baA$_2$]), 332 (5, baB$_1$), 306 (19, abJ$_1$), 246 (100, bA$_1$), 219 (32, aA$_1$), 214 (7.5, bA$_2$), 188 (7.5, bB$_2$), 187 (55, aA$_2$), 182 (13, bA$_3$), 101 (100), and 88 (7.5).

Anal. Calc. for $C_{21}H_{39}NO_{11}$: C, 52.39; H, 8.11; N, 2.91. Found: C, 52.44; H, 7.82; N, 2.96.

A sample of **2** was methylated by the Hakomori procedure[13], and the per-methylated disaccharide gave a mass spectrum that included peaks at *m/e* 495 (0.5, M^+), 464 (0.5, $M-OCH_3$[baA$_1$]), 432 (0.5, $M-OCH_3OH$[baA$_2$]), 346 (23, baB$_1$), 320 (7, abJ$_1$), 260 (57, bA$_1$), 228 (5, bA$_2$), 219 (30, aA$_1$), 187 (63, aA$_2$), 101 (78), 88 (88), and 45 (100).

*Benzyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside* (**3**) *and per-*O*-acetyl* (**6**) *and per-*O*-methyl* (**10**) *derivatives.* — Benzyl alcohol (0.7 ml) and *p*-toluenesulfonic acid (15 mg) were added to the oxazoline (400 mg, prepared as described in the previous experiment) in nitromethane–toluene (1:1, 15 ml), and the solution was kept at 100° for 2 h. The solution was neutralized with aqueous sodium hydrogen carbonate, dried, and concentrated to a syrup. The syrup was chromatographed on silica gel (50 × 3 cm); elution with light petroleum–ethyl acetate (1:1) removed benzyl alcohol, and elution with ethyl acetate afforded a syrup which was crystallized from ether–dichloromethane to give the hexa-*O*-acetyl derivative **6** (219 mg), m.p. 102–103°, $[\alpha]_D$ −27° (*c* 0.5, dichloromethane); n.m.r. data: δ 2.0–2.20 (m, 21 H, NAc and OAc), 5.80 (d, 1 H, NH), and 2.50 (s, 5 H, Ar).

Anal. Calc. for $C_{33}H_{43}NO_{17}$: C, 54.62; H, 5.93; N, 1.93. Found: C, 54.54; H, 6.12; N, 1.71.

Treatment of **6** (159 mg) with barium methoxide, as described for **5**, furnished a syrup which was crystallized from methanol to give the disaccharide glycoside **3** (104 mg), m.p. 247–249°, $[\alpha]_D$ −26° (*c* 1.0, water); n.m.r. data (D_2O): δ 2.30 (s, 3 H, NAc), 5.20 (d, 1 H, *J* 7 Hz, H-1), and 7.60 (s, 5 H, Ar).

Anal. Calc. for $C_{21}H_{31}NO_{11}$: N, 2.96. Found: N, 2.86.

Glycoside **3** (320 mg) was methylated with methyl sulfate and 30% aqueous sodium hydroxide, as described previously, and the reaction product was purified by chromatography on silica gel. Elution with acetone–ethyl acetate (1:1) afforded a syrup (190 mg) which crystallized from ethyl acetate–hexane to give benzyl 2-

acetamido-2-deoxy-3,6-di-*O*-methyl-4-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**10**, 176 mg), m.p. 177–178°, $[\alpha]_D$ −50° (*c* 1.0, dichloromethane); n.m.r. data: δ 2.01 (s, 3 H, NAc), 3.47–3.65 (m, 18 H, OMe), 4.42 (d, 1 H, *J* 7 Hz, H-1′), 4.62, 4.99 (AB quartet, 2 H, *J* 12 Hz, Bzl), and 7.40 (s, 5 H, Ar); *m/e* 557 (0.1, M^+), 450 (0.8, $M-OCH_3$[baA_1]), 418 (0.1, $M-OCH_3-CH_3OH$[baA_2]), 408 (4.5, baB_1), 382 (6.5, abJ_1), 322 (25, bA_1), 219 (38, aA_1), 187 (66, aA_2), 182 (10, bA_3), 135 (57), 115 (49), and 105 (100).

Anal. Calc. for $C_{27}H_{43}NO_{11}$: C, 58.16; H, 7.72; N, 2.51. Found: C, 58.16; H, 7.75; N, 2.34.

Methyl 2-acetamido-2-deoxy-6-O-β-D-galactopyranosyl-α-D-glucopyranoside (**4**) *and per-O-acetyl* (**7**) *and per-O-methyl* (**11**) *derivatives.* — A mixture of tetra-*O*-acetyl-α-D-galactopyranosyl bromide (0.8 g), methyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-trityl-α-D-glucopyranoside[8] (1 g), nitromethane (7 ml), Drierite (1 g), and silver perchlorate (0.44 g) was stirred at room temperature for 30 min and at 80° for 10 min, and then filtered. The filtrate was diluted with chloroform, washed with aqueous sodium hydrogen carbonate and water, dried, and concentrated. The resulting syrup was chromatographed on silica gel with 1-propanol–benzene (1:1), and crystallization from ethanol then furnished the hexa-*O*-acetyl derivative **7** (0.67 g), m.p. 185–186°, $[\alpha]_D$ +63° (*c* 1.0, chloroform); n.m.r. data: δ 2.10 (m, 21 H, NAc and OAc), 3.42 (s, 3 H, OMe), 5.18 (d, 1 H, *J* 8 Hz, H-1′), and 5.40 (d, 1 H, *J* 3 Hz, H-1).

Anal. Calc. for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.01; N, 2.16. Found: C, 49.84; H, 6.12; N, 2.09.

Treatment of **7** (0.5 g) with barium methoxide, as described previously, afforded a solid which was recrystallized twice from ethanol to give the disaccharide glycoside **4** (0.3 g), m.p. 217–218°, $[\alpha]_D$ +83° (*c* 1.0, water); n.m.r. data (D_2O): δ 1.95 (s, 3 H, NAc), 3.34 (s, 3 H, OMe), 4.52 (d, 1 H, *J* 8 Hz, H-1′), and 4.78 (d, 1 H, *J* 4 Hz, H-1).

Anal. Calc. for $C_{15}H_{27}NO_{11}$: C, 45.34; H, 6.80; N, 3.53. Found: C, 45.18; H, 6.93; N, 3.67.

Glycoside **4** (400 mg) was methylated with methyl sulfate and 30% aqueous sodium hydroxide, as described previously, and the product was crystallized from ethanol to give methyl 2-acetamido-2-deoxy-3,4-di-*O*-methyl-6-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**11**, 360 mg), m.p. 215–217°, $[\alpha]_D$ +56° (*c* 1.0, chloroform); n.m.r. data: δ 2.12 (s, 3 H, NAc), 3.60 (m, 21 H, OMe), 4.40 (d, 1 H, *J* 7 Hz, H-1′), 4.71 (d, 1 H, *J* 3 Hz, H-1); *m/e* 481 (0.3, M^+), 450 (1, $M-OCH_3$[baA_1]), 418 (0.7, $M-OCH_3-CH_3OH$[baA_2]), 353 (5, baD_1), 306 (42, baJ_1), 246 (4, bA_1), 219 (3, aA_1), 214 (2, bA_2), 187 (7, aA_2), 182 (4, bA_3), 115 (73), 101 (82), and 88 (100).

Anal. Calc. for $C_{21}H_{39}NO_{11}$: C, 52.39; H, 8.11; N, 2.91. Found: C, 52.39; H, 8.01; N, 2.78.

A sample of **4** was methylated by the Hakomori procedure[13], and the per-methylated disaccharide gave a mass spectrum that included peaks at *m/e* 464 (0.2,

$baA_1[M-OCH_3]$), 432 (0.2, $baA_2[M-OCH_3-CH_3OH]$), 353 (5, baD_1), 320 (45, baJ_1), 260 (4, bA_1), 228 (4, bA_2), 219 (0.4, aA_1), 187 (5, aA_2), and 88 (100).

Partially methylated 2,5-anhydro-D-mannitol acetates. — (*a*) *Derivatives of the 3,4,6-trimethyl ether.* 2,5-Anhydro-D-mannitol (0.5 g) and trityl chloride (1.5 g) in pyridine were stirred at room temperature for 3 days. Water (1 ml) was added, stirring was continued for 1 h, and the solution was poured into water (200 ml) and then extracted with dichloromethane. The organic solution was washed with water, dried, and concentrated to a foamy residue (1.6 g), which was chromatographed on silica gel (80 g). Elution with light petroleum–ethyl acetate (1:1) afforded 2,5-anhydro-1,6-di-*O*-trityl-D-mannitol (0.3 g) as an amorphous solid whose physical constants, m.p. 78–85° (variable), $[\alpha]_D$ +18° (*c* 1.0, pyridine), differed from those reported earlier; lit.[19] m.p. 149°, $[\alpha]_D$ +61° (pyridine); n.m.r. data: δ 3.0–3.5 (m, 4 H, H-1,1′,6,6′), 4.10 (s, 2 H, vanishes on D_2O exchange, OH), 3.9–4.3 (m, 4 H, H-2,3,4,5), and 7.1–7.6 (m, 30 H, Ar). Further elution of the column with ethyl acetate gave 2,5-anhydro-1-*O*-trityl-D-mannitol (0.38 g) as a syrup, $[\alpha]_D$ +49° (*c* 1.7, chloroform); n.m.r. data: δ 3.1–3.4 (m, 2 H, H-1,1′), 3.5–3.8 (m, 2 H, H-6,6′), 4.0 (s, 3 H, vanishes on D_2O exchange, OH), 3.8–4.3 (m, 4 H, H-2,3,4,5), and 7.1–7.9 (m, 15 H, Ar). The monotrityl ether (100 mg) was conventionally methylated with methyl iodide and sodium hydride in *N*,*N*-dimethylformamide[20], and elution of the crude product from a short column of silica gel with dichloromethane furnished syrupy 2,5-anhydro-3,4,6-tri-*O*-methyl-1-*O*-trityl-D-mannitol (68 mg), $[\alpha]_D$ +13° (*c* 1.1, chloroform); n.m.r. data: δ 3.38 (s, 3 H, OMe), 3.40 (s, 6 H, OMe), and 7.1–7.6 (m, 15 H, Ar). The foregoing trityl ether (65 mg) was treated with 2% methanolic hydrogen chloride at room temperature for 30 min. The product was worked up in the usual way and, without further purification, was acetylated with acetic anhydride in pyridine. The product was chromatographed on silica gel with light petroleum–ethyl acetate (1:1) to give syrupy 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol (21 mg), which was homogeneous in t.l.c. and g.l.c. (columns *A* at 140° and *C* at 150°), and had $[\alpha]_D$ +34° (*c* 2.0, chloroform); n.m.r. data: δ 2.10 (s, 3 H, OAc), 3.43 (s, 9 H, OMe), 3.37 (d, 2 H, *J* 5.8 Hz, H-6,6′), 3.61–3.85 (m, 2 H, H-3,4), 3.95–6.22 (m, 4 H, H-1,1′,2,5); *m/e* 216 (26, $M-CH_3OH$), 203 (10, $M-CH_2OCH_3$), 188 (3, $M-CH_3CO_2H$), 175 (3, $M-CH_2OAc$), 45 (45), and 43 (100).

(*b*) *Derivatives of the 3,4-dimethyl ether.* 2,5-Anhydro-1,6-di-*O*-trityl-D-mannitol (672 mg) was methylated with methyl iodide and sodium hydride in *N*,*N*-dimethylformamide[20], and the product was recrystallized from ethanol to give 2,5-anhydro-3,4-di-*O*-methyl-1,6-di-*O*-trityl-D-mannitol (542 mg), m.p. 163–164°, $[\alpha]_D$ +4.3° (*c* 1.2, chloroform); n.m.r. data: δ 3.15–3.40 (m, 10 H, 2×OMe, H-1,1′,6,6′), 3.80 (narrow multiplet, H-3,4), 4.13 (broad multiplet, 2 H, H-2,5), 7.15–7.60 (m, 30 H, Ar); *m/e* 676 (0.5, M^+), 599 (3, M−Ph), 433 (5, M−Tr), 403 (11, $M-CH_2OTr$), 243 (100, Tr^+), and 165 (38, Ph_2C^+).

Anal. Calc. for $C_{46}H_{44}O_5$: C, 81.60; H, 6.51. Found: C, 81.60; H, 6.49.

2,5-Anhydro-3,4-di-*O*-methyl-1,6-di-*O*-trityl-D-mannitol (200 mg) was detritylated, as described previously, and acetylation of the product, followed by

chromatography on silica gel with light petroleum–ethyl acetate (1:1), furnished syrupy 1,6-di-*O*-acetyl-3,4-di-*O*-methyl-D-mannitol (70 mg), which was homogeneous in t.l.c. and g.l.c. (columns *A* at 140° and *C* at 150°), and had $[\alpha]_D$ +42° (*c* 2.6, chloroform); n.m.r. data: δ 2.11 (s, 6 H, OAc), 3.42 (s, 6 H, OMe), 3.72 (s, 2 H, H-3,4), and 4.24 (s, 6 H, H-1,1′2,5,6,6′); *m/e* 276 (0.4, M^+), 216 (25, $M-CH_3CO_2H$), 203 (4, $M-CH_2O_2CCH_3$), and 43 (100).

N-Deacetylation–deamination of methyl 2-acetamido-2-deoxy-3,4,6-tri-O-methyl-α-D-glucopyranoside and the β-D-glycoside. — Methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-α-D-glucopyranoside[21] (10 mg) was heated under reflux in hydrazine (1 ml, containing 1% of hydrazine sulfate) in an inert atmosphere for 70 h. The cooled solution was concentrated under reduced pressure, toluene being added for two further evaporations to ensure removal of the last traces of hydrazine. The residue was dissolved in water (1 ml) containing sodium nitrite (100 mg), and M sulfuric acid was added dropwise, with cooling, to give a solution of pH 3.5–4.0. The solution was kept for 30 min at room temperature and extracted with dichloromethane, and the extract was dried (washing with sodium hydrogen carbonate was deliberately omitted) and concentrated to give syrupy 2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannose which has a positive optical rotation*. The syrup was immediately reduced with sodium borodeuteride (5 mg) in methanol–water (3:2, 1 ml) for 2 h. Dowex 50 X8 (H^+) resin was added to destroy excess of hydride and to remove sodium ions, and the filtered solution was concentrated four times with 2% methanolic acetic acid to remove boric acid as methyl borate. The resulting alcohol was acetylated with acetic anhydride in pyridine to give a product which gave a single peak in g.l.c. (columns *A* and *C*) and whose mass spectrum was indistinguishable from that of synthetic 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol. However, on column *A* at 130°, a minor component (relative retention time, 0.94) appeared as a shoulder on the leading edge of the main peak. The mass spectrum of this component showed fragment ions at *m/e* 218 ($M-OCH_3$, A_1) and 158 ($M-OCH_3-CH_3CO_2H$, A_2) consistent with those expected of a methyl 2-*C*-acetoxymethyl-2-deoxy-3,5-di-*O*-methylpentofuranoside.

A similar series of reactions was performed with methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranoside[21], and analysis of the product by g.l.c.–m.s. on column *D* at 170° showed a major component, indistinguishable from 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol, and a minor component (~5% of the total product) which separated sufficiently (relative retention time, 0.91) to provide a mass spectrum consistent with that of a methyl 2-*C*-acetoxymethyl-2-deoxy-3,5-di-*O*-methylpentofuranoside.

N-Deacetylation–deamination of methyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-glucopyranoside (**1**). — Compound **1** (100 mg) was heated under reflux

*In an earlier investigation, Grant reported that the reaction gave only optically inactive 5-methoxymethyl-2-furaldehyde[22].

with hydrazine (30 ml) containing 1% of hydrazine sulfate for 2 days. Hydrazine was removed by evaporation under reduced pressure in the presence of toluene. The residue in water (4 ml) was adsorbed on a column (100 ml) of Amberlite IR-120(H^+) resin, and the column was eluted with water to remove unreacted acetamido sugar. Elution with 10% aqueous ammonia (400 ml) then afforded crude amino sugar (100 mg), and t.l.c. showed the absence of acetamido sugar. The amino sugar in water (1 ml) was treated with acetic acid (0.2 ml) and sodium nitrite (0.1 g) at room temperature for 90 min. Argon was bubbled through the solution for 15 min and the last traces of nitrous acid were removed by the addition of urea. The solution was diluted with water (3 ml), acetic acid was added to bring the pH to 3.5, sodium cyanoborohydride (100 mg) was added, and the solution was kept for 18 h. The solution was brought to pH 7 by the addition of sodium hydrogen carbonate and concentrated. The residue was dissolved in methanol, acetic acid (3 drops) was added, and the solution was concentrated, and the whole operation was repeated three times. Treatment of the residue with acetic anhydride and pyridine afforded crude syrup (180 mg) which was chromatographed on silica gel with ethyl acetate–light petroleum (1:1) to give fractions *A* (19 mg) and *B* (78 mg).

Fraction *A* was shown by t.l.c. (light petroleum–ethyl acetate, 1:1) to contain two components (R_F 0.35 and 0.20). G.l.c. (column *A* at 190°) revealed compounds having the retention times of methyl 2-*C*-acetoxymethyl-3,5-di-*O*-acetyl-2-deoxy-α-D-*ribo*-pentofuranoside and the D-*arabino* isomer (40%), 1,3,4,6-tetra-*O*-acetyl-2,5-anhydro-D-mannitol* (12%), and hexa-*O*-acetylgalactitol (48%). The identities of the ring-contracted glycosides were confirmed by g.l.c.–m.s. (column *C* at 180°), including direct comparison with samples similarly derived from deamination of methyl 2-amino-2-deoxy-α-D-glucopyranoside[9].

Fraction *B* was homogeneous in t.l.c. (light petroleum–ethyl acetate, 1:1) and was assigned the structure 1,4,6-tri-*O*-acetyl-2,5-anhydro-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-mannitol on the basis of the mass spectrum, which showed the fragment ions reported by Dmitriev *et al.*[10], and of the n.m.r. data: δ 2.00, 2.08, 2.13, 2.20 [4 s (ratios, 1:2:3:1), 21 H, OAc], 3.90–4.40 (m, 10 H, H-1,1*,2,3,5,5′,6,6*,6′,6′*), 4.64 (d, 1 H, *J* 7 Hz, H-1′), and 5.05–5.45 (m, 4 H, H-2′,3′,4,4′). However, the observed optical rotation, $[\alpha]_D$ +9° (*c* 0.32, chloroform), differed from that (+90°) reported by Dmitriev *et al.*[10].

N-*Deacetylation–deamination of* **8** *followed by derivative formation.* — Glycoside **8** (~10 mg) was heated under reflux in hydrazine (5 ml) containing 1% of hydrazine sulfate in an inert atmosphere for 4–5 days. Hydrazine was removed by co-distillation with toluene under reduced pressure. The residue (aminoglycoside, acetylhydrazide, and hydrazine sulfate) was dissolved in water (2 ml), sodium nitrite (250 mg) was added, and M sulfuric acid was added dropwise, with external cooling, to bring the

*The origin of this compound is not clear, as care was taken to avoid inadvertent hydrolysis of disaccharide derivatives. It is possible that **1** was contaminated by ~2% of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside which escaped detection, for example, when both the glycoside **1** and the product of *N*-deacetylation were examined by ^{13}C-n.m.r. spectroscopy.

solution to pH 4. The solution was kept at room temperature for 40 min, and was then extracted with dichloromethane (6 × 4 ml). The extracts were washed once with water, dried, and concentrated to a syrup. Samples of the syrup were converted into derivatives by the following three procedures. For comparative purposes, all derivatives of disaccharide products from *N*-deacetylation–deamination of per-*O*-methylated glycosides **8**–**11** were examined by g.l.c. isothermally at 210° on column *B* (see Table I).

(*a*) *Per-O-methylated alditols.* The deamination products (~10 mg) were reduced with sodium borodeuteride (20 mg) in ethanol–water (3:2, 0.5 ml). Excess of reagent was decomposed, and sodium ions were removed, by shaking with Dowex 50 X8 (H^+) resin (200–400 mesh). The resin was removed by filtration and washed thoroughly with methanol, and the combined filtrates were repeatedly concentrated with methanol to remove boric acid as methyl borate. The resulting syrup (6 mg) was then alkylated with trideuteriomethyl iodide and sodium hydride in methyl sulfoxide[13], and the products were purified by column chromatography on Sephadex LH-20 (elution with dichloromethane) and on silica gel (elution with ethyl acetate–acetone, 2:1). G.l.c. examination of the products at 150° (isothermal) on column *E* showed a monosaccharide derivative having the retention time of hexa-*O*-methylgalactitol. G.l.c.–m.s. (column *C*; 150°, 2 min, 2°/min → 200°, hold) showed (*i*) a minor component (eluted at 157°) whose retention time and mass spectra were identical with those of an isotopically labelled hexa-*O*-methylgalactitol similarly prepared from 2,3,4,6-tetra-*O*-methyl-D-galactose, and (*ii*) a major component (eluted at 200°) which was assigned structure **13** on the basis of fragment ions at *m/e* 253 (82, abJ_1), 219 (3, aA_1), 193 (38, bA_1), 187 (8, aA_2), 161 (17, bA_2), 158 (30, bA_2), 101 (95), 88 (100), 49 (32, $CHDOCD)_3$, and 45 (80, CH_2OCH_3).

(*b*) *Alditol acetates.* Deamination products were reduced with sodium borodeuteride and then acetylated with acetic anhydride in pyridine. G.l.c.–m.s. (column *C*; 150°, 2 min, 4°/min → 200°, hold) showed (*i*) two minor components (eluted at 170° and 172°) both of whose mass spectra included fragment ions at *m/e* 218 ($M-OCH_3$) and 158, consistent with those expected of methyl 2-*C*-acetoxymethyl-2-deoxy-3,5-di-*O*-methylpentofuranosides; (*ii*) a further minor component (eluted at 197°) whose retention time and mass spectrum were indistinguishable from those of a derivative similarly prepared from 2,3,4,6-tetra-*O*-methyl-D-galactose; and (*iii*) a major component (eluted at 200°, 34 min) which was assigned the structure **14** on the basis of fragment ions at *m/e* 278 (44, abJ_1), 219 (5, aA_1), 218 (32, bA_1), 187 (5, aA_2), 158 (9, bA_2), 101 (93), and 88 (100).

(*c*) *Aldononitriles.* Deamination products were heated with hydroxylamine hydrochloride in pyridine, and again after addition of acetic anhydride[23]. The reaction mixture, after work-up, was examined by g.l.c.–m.s. (column *B*, 200°) which showed (*i*) a minor component, which was indistinguishable from an authentic sample of 5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactononitrile, and (*ii*) a major component which was assigned structure **15**.

N-*Deacetylation–deamination of* **9** *and* **11**, *followed by derivative formation.* —

Samples of the glycosides **9** and **11** were *N*-deacetylated and then deaminated on treatment with nitrous acid, as described for glycoside **8**. Portions of the deamination products were converted into derivatives, as described previously, and examined by g.l.c. (Table I) and by g.l.c.–m.s. In no case were derivatives of the methyl 2-*C*-formyl-2-deoxypentofuranosides separated from those of the isomeric 2,5-anhydro-mannoses. Portions of the deamination products were reduced with sodium borodeuteride, hydrolyzed, reduced with sodium borohydride, and acetylated. Analysis of the partially methylated alditol acetates by g.l.c.–m.s. showed acetates from 2,3,4,6-tetra-*O*-methylgalactitol (from **9** and **11**); 2,5-anhydro-3,6-di-*O*-methylmannitol (from **9**) with significant fragment ions at *m/e* 232, 203, 200, 143, and 140; and 2,5-anhydro-3,4-di-*O*-methylmannitol (from **11**) which was indistinguishable from a synthetic sample.

N-*Deacetylation–deamination of* **10**. — The glycoside **10** (120 mg) was heated under reflux in hydrazine (40 ml) containing hydrazine sulfate (300 mg) for 4 days. Hydrazine was removed by co-distillation with toluene under reduced pressure. T.l.c. in dichloromethane–acetone (1:1) showed only a small proportion of unchanged **10**. Sodium nitrite (0.5 g) was added to the crude product in water (10 ml), and M sulfuric acid (~3 ml) was added slowly, with cooling, to bring the solution to pH 4. The solution was kept at room temperature for 1 h, and was then extracted with dichloromethane (5 × 15 ml). The dried solution was concentrated, and the residue was reduced with sodium borodeuteride (50 mg) in methanol–water (1:1, 4 ml) for 1.5 h. The solution was treated with Dowex 50(H^+) resin, to decompose excess of hydride and to remove sodium ions, and the filtered solution was concentrated with methanol to remove boric acid. T.l.c. of the product in dichloromethane–acetone (3:2) showed a minor component (R_F 0.8) and a major component (R_F 0.5), and the remaining product was chromatographed on silica gel (20 × 1.5 cm) in dichloromethane–acetone to give fractions *A* (5 mg) and *B* (55 mg).

Fraction *A* had $[\alpha]_D$ −74° (*c* 0.45, chloroform), and the n.m.r. spectrum showed the presence of aromatic protons (δ 7.13). The syrup in ethanol (0.5 ml) was shaken in hydrogen at atmospheric pressure over 5% palladium–charcoal for 6 h. T.l.c. revealed a new product (R_F 0.1), and the solution was filtered and concentrated. The residue was kept in 0.5% methanolic hydrogen chloride (4 ml) at room temperature for 7 h, giving a new product (R_F 0.65). The acid was neutralized, and the solution concentrated to a syrup which was methylated with trideuteriomethyl iodide and sodium hydride in methyl sulfoxide[13]. The resulting product was examined by g.l.c.–m.s. (column *B* at 220°), and the presence of 6 peaks was demonstrated. Peak 3, the major component, had the same retention time as the permethylated 2,5-anhydro-3(or 4)-*O*-β-D-galactopyranosyl-D-mannitol (**21**), and the essential identity of the mass spectra of peaks 1, 3, and 4 indicated that they were probably stereoisomers of the general structure **25**. Peaks 2 and 5 did not separate cleanly and their mass spectra indicated that mixtures of substances were present. Peak 6 was assigned structure **40**, as its mass spectrum showed peaks characteristic of a per-*O*-methylated methyl hexopyranosyl-(1→4)-hexoside carrying a single trideuteriomethyl group at O-2:

m/e 308 (1.5, bF_1^2), 282 (13.5, baJ_1), 222 (13.5, bA_1), 219 (4.5, aA_1), 190 (11.5, bA_2), 187 (12.5, aA_2), 101 (100), and 88 (100).

The n.m.r. spectrum of fraction *B* showed weak signals at δ 1.9 (NAc) and 7.1 (aromatic); on standing, the syrup deposited unreacted, crystalline benzyl glycoside **19** (19 mg). For purification, the residual syrup was acetylated with acetic anhydride in pyridine, and the product was chromatographed on silica gel (dichloromethane–acetone, 3:2) to give syrupy acetate **22**, which gave a single peak in g.l.c. (column *B*) and whose mass spectrum showed fragment ions at *m/e* 278 (55, abJ_1), 219 (6, aA_1), 218 (14, bA_1), 187 (6, aA_2), 186 (11, bA_2), 101 (100), and 88 (96). The acetate was treated with barium methoxide, and a sample of the resulting alcohol (**20**) was alkylated with trideuteriomethyl iodide and sodium hydride in methyl sulfoxide[13] to give the permethylated derivative (**21**). This derivative was examined by g.l.c.–m.s. (column *B*, 210°), and the mass spectrum showed fragment ions at *m/e* 253 (100, abJ_1), 219 (2, aA_1), 193 (75, bA_1), 187 (17, aA_1), 161 (69, bA_2), 158 (24, bA_2), 101 (89), 88 (94), 49 (69, $CHDOCD_3$), and 45 (78, CH_2OCH_3).

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