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ANALYSIS OF THE LINKAGE POSITIONS IN D-FRUCTOFURANOSYL RESIDUES BY THE REDUCTIVE-CLEAVAGE METHOD*

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ABSTRACT

The suitability of the reductive-cleavage method for analysis of the linkage positions in D-fructofuranosyl residues of D-fructans was examined by using sucrose, chicory-root inulin, and *Aerobacter levanicum* levan as models. Permethylation, and reductive cleavage with triethylsilane in the presence of either boron trifluoride etherate or trimethylsilyl trifluoromethanesulfonate, gave the expected methylated derivatives of 2,5-anhydro-D-mannitol and 2,5-anhydro-D-glucitol. With either catalyst, nonreducing (terminal) D-fructofuranosyl groups and D-fructofuranosyl residues linked at O-1 gave derivatives having the *manno* configuration as the major product, whereas D-fructofuranosyl residues linked at O-6, and at both O-1 and O-6, gave derivatives having the *gluco* configuration as the major product. The independent synthesis, and n.m.r.- and mass-spectral characterization, of the methylated 2,5-anhydro-D-mannitol and 2,5-anhydro-D-glucitol derivatives formed from these residues by reductive cleavage are reported.

INTRODUCTION

The reductive-cleavage method for determination[1] of polysaccharide structure is based upon methylation analysis, but departs from it significantly with regard to the types of fragments formed by cleavage of the fully methylated polysaccharide. Standard methylation analysis suffers the disadvantage that it is incapable of distinguishing between 4-linked aldopyranosyl and 5-linked aldofuranosyl residues in a polysaccharide. This deficiency also applies to 5- and 6-linked ketohexosyl residues. In the reductive-cleavage technique, however, the linkage positions and ring form of each monosaccharide residue are established simultaneously; regiospecific, ionic hydrogenation of all glycosidic carbon–oxygen bonds in the fully methylated polysaccharide affords a mixture of anhydroalditol derivatives

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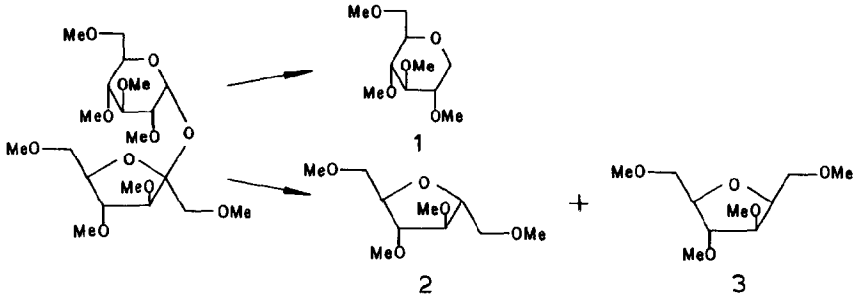
that is subsequently acetylated *in situ*, and the acetates are subjected to analysis by g.l.c.–m.s.

In a continuing program to investigate the usefulness of this approach, we have chosen to examine structurally well-characterized polysaccharides as models. In a previous report[2], a series of yeast D-mannans containing 2-, 3-, 6-, 2,6-, and 3,6-linked D-mannopyranosyl residues were examined, and found to give the expected derivatives of 1,5-anhydro-D-mannitol. We now describe the results obtained from an investigation of carbohydrates containing D-fructofuranosyl residues. Specifically, we have examined sucrose, chicory-root inulin, and *Aerobacter levanicum* levan. In all cases, the expected derivatives of 2,5-anhydro-D-mannitol and 2,5-anhydro-D-glucitol were formed. The independent synthesis, and n.m.r.- and mass-spectral characterization, of these derivatives are also reported.

RESULTS

Sucrose. — The structures of per-*O*-methylated sucrose and its expected reductive-cleavage products are shown in Scheme 1. Based on studies with model D-glucosides, it was expected that anhydroalditols would be formed exclusively, *via* the formation and reduction of cyclic oxonium ions[3]. Per-*O*-methylated sucrose



Scheme 1

provided an easy test of this assumption as it applies to the D-fructofuranosyl moiety, because acyclic products (alditols) arising *via* the formation and reduction of acyclic oxonium ions would be readily identified by g.l.c.–m.s. analysis. The D-glucopyranosyl group was thus expected to give rise to a single product, namely, 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**). The D-fructofuranosyl group can, however, give rise to two anhydroalditols, namely, 2,5-anhydro-1,3,4,6-tetra-*O*-methyl-D-mannitol (**2**) and 2,5-anhydro-1,3,4,6-tetra-*O*-methyl-D-glucitol (**3**). Compounds **2** and **3** are the result of net overall retention and inversion, respectively, of the stereochemistry at C-2 in the D-fructofuranosyl group. Shown in Fig. 1 is the gas–liquid chromatogram obtained when the reductive cleavage of permethylated sucrose was conducted with $Me_3SiO_3SCF_3$ as the catalyst (hereafter termed catalyst X). As expected, the methylated D-glucopyranosyl group gave rise

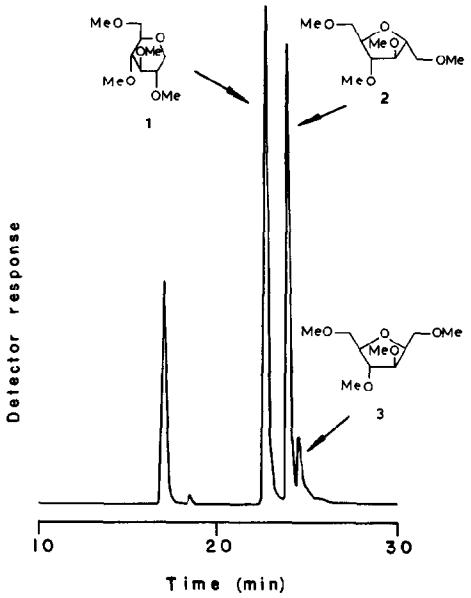


Fig.1. Gas–liquid chromatogram (Column 2, programmed from 80 to 230° at 6°/min) of the methylated anhydroalditols derived by reductive cleavage of per-*O*-methylated sucrose with X as the catalyst. (The component eluted at 17 min was identified as hexamethyldisiloxane.)

to a single anhydroalditol, namely, 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**), and the D-fructofuranosyl group gave two anhydroalditols, identified as 2,5-anhydro-1,3,4,6-tetra-*O*-methyl-D-mannitol (**2**) and 2,5-anhydro-1,3,4,6-tetra-*O*-methyl-D-glucitol (**3**). Integration of the g.l.c. peaks (see Table I) revealed that **2** and **3** were produced in the ratio of ~5:1 when X was the catalyst. Moreover, the combined mole fraction of **2** + **3** (0.50) was, within experimental error, equal to the mole fraction of **1** (0.49) when X was the catalyst. With $BF_3 \cdot Et_2O$ as the

TABLE I

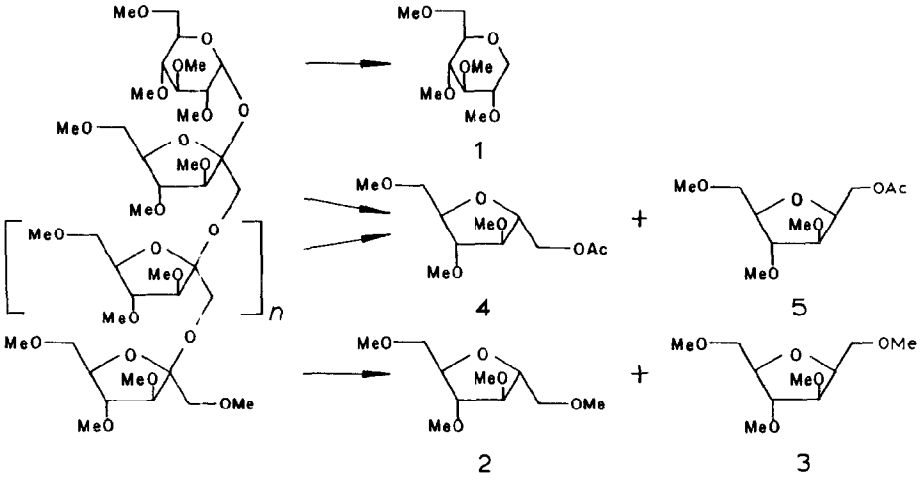
MOLE FRACTIONS OF PRODUCTS DERIVED BY REDUCTIVE CLEAVAGE OF PER-*O*-METHYLATED SUCROSE, INULIN, AND LEVAN

D-*Fructan*	*Catalyst*	*Mole fraction*[a] *of compound*							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sucrose	BF_3	0.41	0.41	0.17	—	—	—	—	—
	X[b]	0.49	0.42	0.08	—	—	—	—	—
Inulin	BF_3	0.04	0.05	0.01	0.65	0.24	—	—	—
	X	0.05	0.04	0.01	0.67	0.22	—	—	—
Levan	BF_3	—	0.08	0.03	0.26	—	0.53	0.03	0.07
	X	—	0.09	0.04	0.25	—	0.54	0.03	0.05

[a]Analyzed as their acetyl derivatives, where appropriate. [b]$Me_3SiO_3SCF_3$.

catalyst, **2** and **3** were formed in the ratio of ~5:2, but the combined mole fraction of **2** and **3** (0.58) was greater than the mole fraction of **1** (0.41). This deviation from the theoretical values was found to be due to the preferential, evaporative loss of **1** during processing.

Inulin. — Chicory-root inulin is a well characterized, (2→1)-linked, D-fructofuranose polymer terminated at its "reducing end" by a D-glucosyl group. The structures of per-*O*-methylated inulin and its expected reductive-cleavage products are shown in Scheme 2. The nonreducing (terminal) D-fructofuranosyl group is expected to give rise to both **2** and **3**, as was observed for sucrose. The internal,



Scheme 2

(2→1)-linked, D-fructofuranosyl residues should also give rise to two products, namely, 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol (**4**) and 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-glucitol (**5**). The remaining D-glucopyranosyl group should give rise to a single product (**1**), as was observed for sucrose. Shown in Fig. 2 is the gas–liquid chromatogram obtained when the reductive cleavage of permethylated inulin was conducted with catalyst X. All residues gave the expected products, as established by g.l.c.–m.s. analysis and by comparison to independently synthesized standards. Integration of these peaks, and correction for molar response[2,4], gave the mole fractions listed in Table I. As expected, based on the results obtained with per-*O*-methylated sucrose, the terminal D-fructofuranosyl group gave both **2** and **3**, with the product having the *manno* configuration (**2**) preponderating. Also as expected, the combined amount of **2** and **3** was equivalent to the amount of **1**, formed from the terminal D-glucopyranosyl group. The internal, (2→1)-linked, D-fructofuranosyl residues also gave rise to two products (**4** and **5**), and again, the product having the *manno* configuration (**4**) was preponderant. The average number of (2→1)-linked D-fructofuranosyl residues in the chain, obtained

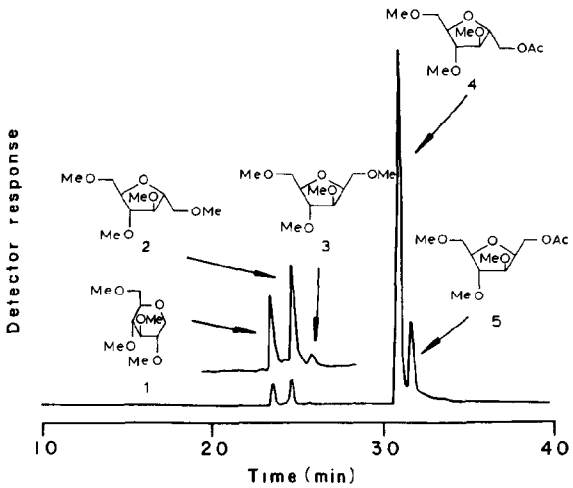
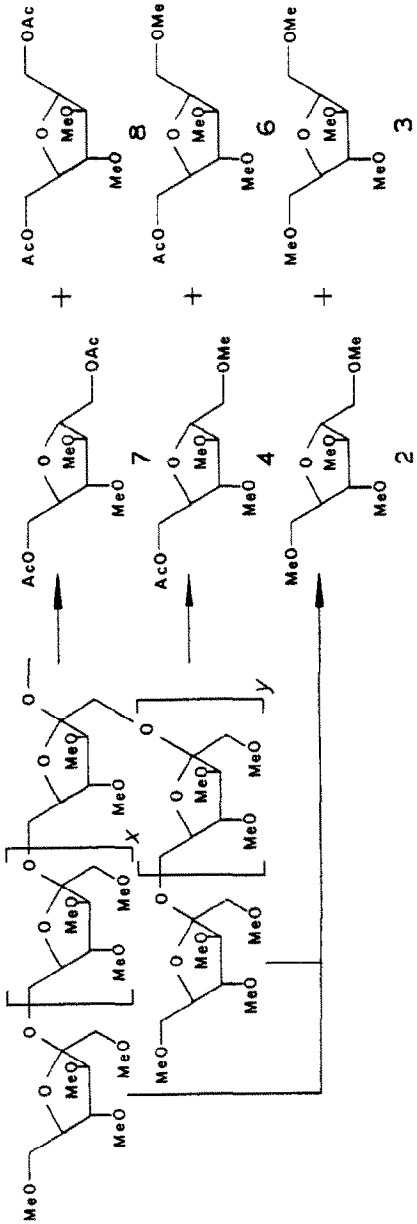


Fig. 2. Gas–liquid chromatogram (Column 2, programmed from 70 to 300° at 6°/min) of the partially *O*-methylated anhydroalditol acetates derived by reductive cleavage of per-*O*-methylated inulin with X as the catalyst.

by dividing the combined mole fraction of **4** and **5** (0.89) by the combined mole fraction of **2** and **3** (0.05), was found to be 18. This degree of polymerization is identical to that reported[5].

Levan. — *Aerobacter levanicum* levan is a D-fructan of high molecular weight that is comprised of a (2→6)-linked D-fructofuranose backbone which is branched at some of the O-1 atoms. The structures of per-*O*-methylated levan and its expected reductive-cleavage products are shown in Scheme 3. Levan contains three different types of D-fructofuranosyl residue, and each is expected to give rise to two products. Nonreducing (terminal) groups should give both **2** and **3**, as was observed with both sucrose and inulin. (2→6)-Linked residues are expected to give 6-*O*-acetyl-2,5-anhydro-1,3,4-tri-*O*-methyl-D-mannitol, which is identical in structure to **4**, and 6-*O*-acetyl-2,5-anhydro-1,3,4-tri-*O*-methyl-D-glucitol (**6**). Finally, branched D-fructofuranosyl residues are expected to give both 1,6-di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-mannitol (**7**) and 1,6-di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol (**8**). The gas–liquid chromatogram of the X-catalyzed, reductive cleavage of per-*O*-methylated levan is shown in Fig. 3. Indeed, all six of the expected products were observed, and they were identified through a comparison, by g.l.c.–m.s. analysis, to independently synthesized standards. As expected, the terminal D-fructofuranosyl groups gave a mixture of **2** and **3**, with the product having the *manno* configuration (**2**) again preponderating. The striking feature of the chromatogram is, however, the preponderance of products having the *gluco* configuration (**6** and **8**) in anhydroalditols derived from D-fructofuranosyl residues linked at O-6 or at both O-1 and O-6. 6-Linked D-fructofuranosyl residues give both **6** and **4**, in the ratio of ~2:1, whereas D-fructofuranosyl residues linked at



Scheme 3

both O-1 and O-6 give **8** and **7** in the ratio of ~2:1 (see Table I). Integration of all peaks in the chromatogram, and correction for molar response[2,4], gives the relative, molar percentages of the three types of residue in the polysaccharide. Averaging the values obtained in the two experiments gave (**2** + **3**) 12, (**4** + **6**) 79, and (**7** + **8**) 9%. This levan therefore contains 12% of terminal, D-fructofuranosyl

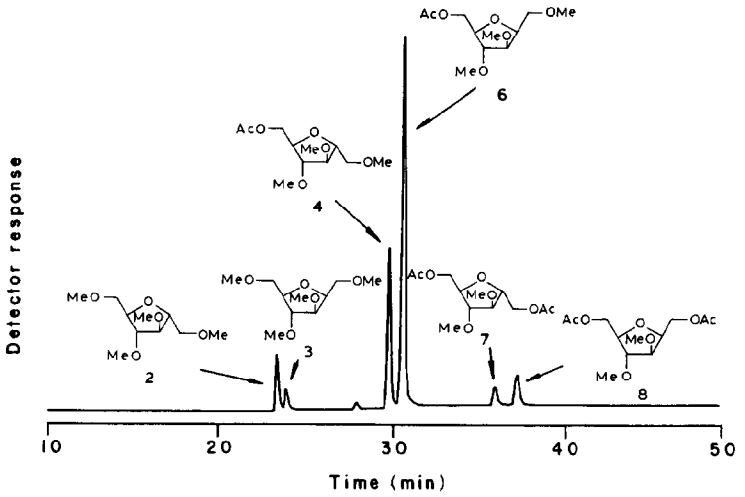


Fig. 3. Gas–liquid chromatogram (Column 2, programmed from 70 to 310° at 6°/min) of the partially *O*-methylated anhydroalditol acetates derived by reductive cleavage of per-*O*-methylated levan with X as the catalyst.

groups, 79% of 6-linked D-fructofuranosyl residues, and 9% of branch-point D-fructofuranosyl residues linked at both O-1 and O-6. These values are in excellent agreement with those reported by Feingold and Gehatia[6], namely, 12% of terminal, 78% of 6-linked, and 10% of 1- and 6-linked units.

DISCUSSION

The D-fructans examined in this study provided convenient experimental models for examining the fate of D-fructofuranosyl residues during reductive cleavage. Moreover, one of the polysaccharides (levan) met the criterion that led to development of the reductive cleavage method, namely, the inability of standard methylation analysis to distinguish between 6-linked ketohexofuranosyl and 5-linked ketohexopyranosyl residues in a polysaccharide. The results obtained in both X- and BF_3-catalyzed, reductive-cleavage reactions of the per-*O*-methylated D-fructans are in full agreement with the published structures. In all of these reactions, the only products detected were the methylated anhydroalditols expected. Although these derivatives are quite volatile, especially the tetra-*O*-methylated anhydroalditols[2], satisfactory quantitative results could be obtained by minimizing the evaporative loss during the processing (see Experimental section).

The D-fructans selected for study also provided a convenient, experimental situation wherein the stereochemistry of reductive cleavage could be established. All of the D-fructofuranosyl residues examined gave two products, *i.e.*, the methylated or partially methylated derivatives of both 2,5-anhydro-D-mannitol and 2,5-anhydro-D-glucitol. The formation of two products from each type of residue was

not, however, a disadvantage. On the contrary, for the D-fructofuranosyl residues examined, the ratios of products having the *manno* and *gluco* configuration were also characteristic of the linkage position. Nonreducing (terminal) D-fructofuranosyl groups and D-fructofuranosyl residues linked only at O-1 gave anhydroalditols having the *manno* configuration as the major product, whereas D-fructofuranosyl residues linked at O-6 or at both O-1 and O-6 gave anhydroalditols having the *gluco* configuration as the major product. The reason for this stereoselectivity is, at present, unknown.

We conclude from these results that reductive cleavage is an effective technique for establishing the ring form of, and the positions of linkage in, D-fructofuranosyl residues.

EXPERIMENTAL

General. — N.m.r. spectra were recorded with a Nicolet NT-300 NMR spectrometer; ^1H spectra, recorded with $CDCl_3$ as the solvent, were referenced to internal tetramethylsilane, and ^{13}C spectra, recorded with $CDCl_3$ as the solvent, were referenced to the central, ^{13}C signal (δ 77.00) of chloroform. Analytical g.l.c. was performed in a Hewlett–Packard F and M Model 810 chromatograph equipped with a flame-ionization detector and the following stainless-steel columns: Column 1 (3.18 mm × 1.83 m), 10% of SP2401 on 100–120 Supelcoport; Column 2 (3.18 mm × 3.66 m), 10% of SP2401 on 100–120 Supelcoport; Column 3 (3.18 mm × 2.68 m), 3% of OV-225 on 100–120 Supelcoport; and Column 4 (27 m), capillary of SE-30. Preparative g.l.c. was performed on a Varian VO-P chromatograph equipped with a thermal conductivity detector and the following stainless-steel columns: Column 5 (6.35 mm × 1.83 m), 10% of SE-30 on Chromosorb, and Column 6 (6.35 mm × 3.66 m), the same. G.l.c.–m.s. analyses were performed on a Finnigan 4000 mass spectrometer equipped with a VG Multispec data-system. Column effluents were analyzed by chemical ionization (c.i.) mass spectrometry (m.s.) (g.l.c.–c.i.m.s.) with ammonia as the reagent gas, wherein characteristic, (M + 1) and (M + 18) ions were detected, and by electron impact (e.i.) m.s. (g.l.c.–e.i.m.s.), in order to verify that eluted components had mass spectra identical to those of independently synthesized standards. All g.l.c. samples were also "spiked" with authentic standards, in order to verify their co-migration. Authentic standards were prepared from 2,5-anhydro-D-mannitol or 2,5-anhydro-1,3-*O*-isopropylidene-6-*O*-trityl-D-glucitol by standard, protecting-group strategies. Intermediates were checked for the completeness of protection, or deprotection, by ^1H-n.m.r. spectroscopy. The final products were isolated in pure form by column chromatography, or preparative g.l.c., as noted. Elemental analyses were performed by M-H-W Laboratories, Inc., Phoenix, AZ, on samples purified by preparative g.l.c. Reductive cleavage was conducted as previously described[2].

Octa-O-methylsucrose. — Sucrose was methylated according to standard procedures[7,8], and the oily product purified on silica gel, with elution with 1:1 (v/v)

hexane–ether (R_F 0.15), was shown to be pure by g.l.c. (Column 1, 220°); $[\alpha]_D^{23}$ +44.9° (*c* 0.51, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 3.0–4.05 [complex, H-2,3,4,5,6,6′ (Glc), H-1,3,4,5,6,6′ (Fru)], 3.38, 3.40, 3.41, 3.43, 3.45, 3.47, 3.54, 3.62 (8 s, 24 H, 8 MeO), and 5.55 (d, 1 H, $J_{1,2}$ 5.5 Hz, H-1); ^{13}C-n.m.r. ($CDCl_3$): δ 58.07, 58.19, 58.31, 58.98, 58.98, 59.24, 60.11, 60.42 (methoxyls), 70.23, 72.21, 74.05, 74.05, 79.19, 79.39, 81.67, 83.10, 83.96, 85.15 [C-2,3,4,5,6 (Glc), C-1,3,4,5,6 (Fru)], 89.28 (C-1, Glc), and 104.21 (C-2, Fru).

Anal. Calc. for $C_{20}H_{38}O_{11}$: C, 52.85; H, 8.43. Found: C, 53.00; H, 8.32.

Permethylated inulin and levan. — Inulin (Pfanstiehl) and levan (Sigma) were methylated twice by standard procedures[7,8], and the products were purified by chromatography on Sephadex LH-20 with 2:1 (v/v) $CHCl_3$–MeOH. Infrared spectra of thin films of the methylated polymers on a NaCl plate showed no hydroxyl absorption.

*2,5-Anhydro-1,3,4,6-tetra-*O*-methyl-*D*-mannitol* (**2**). — Direct methylation[7,8] of 2,5-anhydro-D-mannitol[9] (139 mg) gave **2** (155 mg, 83%) as a volatile product which was pure by g.l.c. (Column 2, 220°); $[\alpha]_D^{23}$ +32.6° (*c* 0.76, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 3.39 (s, 6 H, 2 MeO), 3.40 (s, 6 H, 2 MeO), 3.48 (dd, 2 H, *J* 5.6, 10.1 Hz, H-1,6), 3.52 (dd, 2 H, *J* 5.8, 10.1 Hz, H-1′,6′), 3.72 (m, 2 H, H-3,4), and 4.09 (m, 2 H, H-2,5); ^{13}C-n.m.r. ($CDCl_3$): δ 57.55, 59.25 (methoxyls), 72.80 (C-1,6), 81.30 (C-3,4), and 86.72 (C-2,5); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 221 (22) and 238 (100); g.l.c.–e.i.m.s.: *m/z* 45 (50), 71 (32), 75 (12), 87 (12), 89 (35), 99 (32), 101 (78), 111 (25), 115 (42), 125 (10), 126 (12), 143 (100), 156 (68), 158 (6), 174 (6), 175 (96), 176 (9), and 188 (6).

Anal. Calc. for $C_{10}H_{20}O_5$: C, 54.53; H, 9.15. Found: C, 54.39; H, 8.94.

*2,5-Anhydro-1,3,4,6-tetra-*O*-methyl-*D*-glucitol* (**3**). — 2,5-Anhydro-1,3-*O*-isopropylidene-6-*O*-trityl-D-glucitol[10] (1.0 g, 2.3 mmol) was treated with 30% HBr in acetic acid (1.1 g, 4.6 mmol) at 0° according to a published procedure[11]. After filtration to remove trityl bromide, and repeated addition and evaporation of water under vacuum, the product was dried under high vacuum (363 mg, 98%). The resultant 2,5-anhydro-D-glucitol (42 mg, 0.6 mmol) was methylated[7,8], and the product was purified on silica gel with elution with 3:1 (v/v) hexane–ethyl acetate (R_F 0.32), to produce **3** (24 mg, 36%), which was pure by g.l.c. (Column 6, 150°); $[\alpha]_D^{23}$ +64° (*c* 0.44, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 3.38, 3.385, 3.39, 3.40 (4 s, 12 H, 4 MeO), 3.48 (dd, 1 H, *J* 6.0, 10.0 Hz, H-6), 3.54 (dd, 1 H, *J* 5.8, 10.0 Hz, H-6′), 3.57–3.67 (complex, 3 H, H-1,4,1′), 3.68 (dd, 1 H, *J* 2.1, 4.0 Hz, H-3), 3.91 (dt, 1 H, *J* 3.6, 6.0 Hz, H-5), and 4.09 (ddd, 1 H, *J* 4.0, 5.0, 6.6 Hz, H-2); ^{13}C-n.m.r. ($CDCl_3$): δ 57.25, 57.38, 59.13, 59.13 (methoxyls), 70.65, 73.18 (C-1,6), 79.84, 82.23, 84.86, and 85.79 (C-2,3,4,5); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 221 (34) and 238 (100); g.l.c.–e.i.m.s.: *m/z* 45 (36), 71 (21), 87 (15), 89 (21), 99 (21), 101 (100), 102 (11), 111 (18), 115 (19), 143 (72), 156 (7), 157 (2), 175 (54), 176 (5), and 188 (1).

Anal. Calc. for $C_{10}H_{20}O_5$: C, 54.53; H, 9.15. Found: C, 54.14; H, 8.89.

*1-*O*-Acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-*D*-mannitol* (**4**). — 2,5-Anhydro-

D-mannitol[9] (589 mg) was monotritylated by treating with 1 equiv. of trityl chloride in pyridine[12], to produce 2,5-anhydro-1-*O*-trityl-D-mannitol (1.24 g, 82%), which was methylated[7,8]. Detritylation with gaseous HCl in[12] $CHCl_3$, and acetylation of the product, gave **4**, which was isolated in pure form by preparative g.l.c. (Column 6, 190°); $[\alpha]_D^{23}$ +38.2° (*c* 1.91, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.09 (s, 3 H, AcO), 3.39 (s, 3 H, MeO), 3.40 (s, 6 H, 2 MeO), 3.48 (dd, 1 H, *J* 5.5, 10.1 Hz, H-6), 3.52 (dd, 1 H, *J* 5.8, 10.1 Hz, H-6'), 3.68 (dd, 1 H, *J* 2.9, 3.2 Hz, H-3), 3.73 (dd, 1 H, *J* 2.9, 4.2 Hz, H-4), and 4.09–4.20 (complex, 4 H, H-1,2,5,1'); ^{13}C-n.m.r. ($CDCl_3$): δ 20.79 (C-2 of acetyl), 57.55, 57.55, 59.20 (methoxyls), 64.29, 72.82, 80.40, 81.45, 86.44, 86.70 (C-1,2,3,4,5,6), and 170.70 (C-1 of acetyl); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 249 (11) and 266 (100); g.l.c.–e.i.m.s.; *m/z* 43 (100), 45 (77), 57 (12), 71 (69), 83 (11), 85 (12), 87 (21), 99 (9), 101 (42), 111 (32), 115 (20), 117 (12), 126 (14), 143 (24), 156 (4), 157 (2), 158 (3), 171 (3), 175 (1), 188 (3), 203 (3), 216 (0.5), and 249 (1).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 53.07; H, 8.09.

1-O-Acetyl-2,5-anhydro-3,4,6-tri-O-methyl-D-glucitol (**5**). — 2,5-Anhydro-1,3-*O*-isopropylidene-6-*O*-trityl-D-glucitol[10] (1.0 g, 2.2 mmol) was repeatedly detritylated with hydrogen and palladium-on-carbon in 95% ethanol[13], to produce 2,5-anhydro-1,3-*O*-isopropylidene-D-glucitol (417 mg, 93%). Sequential methylation[7,8], and hydrolysis with M trifluoroacetic acid in 1:1 (v/v) MeOH–H_2O overnight under reflux, gave 2,5-anhydro-4,6-di-*O*-methyl-D-glucitol (398 mg, 99%) after repeated evaporation of added water under vacuum. Sequential tritylation, methylation, and detritylation of the last compound, as described for **4**, gave 2,5-anhydro-3,4,6-tri-*O*-methyl-D-glucitol (188 mg, 50%), which, on acetylation and preparative g.l.c. (Column 6, 170°), gave analytically pure **5**; $[\alpha]_D^{23}$ +31.0° (*c* 0.80, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.08 (s, 3 H, AcO), 3.38 (s, 3 H, MeO), 3.40 (s, 6 H, 2 MeO), 3.47 (dd, 1 H, *J* 6.0, 10.0 Hz, H-6), 3.54 (dd, 1 H, *J* 6.1, 10.0 Hz, H-6'), 3.65 (dd, 1 H, *J* 1.2, 3.4 Hz, H-4), 3.71 (dd, 1 H, *J* 1.2, 3.5 Hz, H-3), 3.96 (dt, 1 H, *J* 3.4, 6.1 Hz, H-5), 4.11–4.19 (complex, 2 H, H-1,1'), and 4.41 (complex, 1 H, H-2); ^{13}C-n.m.r. ($CDCl_3$): δ 20.85 (C-2 of acetyl), 57.35, 57.35, 59.22 (methoxyls), 63.19, 73.12, 78.85, 82.42, 84.89, 85.30 (C-1,2,3,4,5,6), and 170.83 (acetyl C-1); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 249 (13) and 266 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (70), 59 (13), 69 (13), 71 (53), 87 (27), 101 (45), 111 (21), 117 (14), 126 (13), 143 (7), 158 (3), 171 (1.6), 175 (0.6), 188 (3), and 203 (1).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 53.11; H, 8.24.

6-O-Acetyl-2,5-anhydro-1,3,4-tri-O-methyl-D-glucitol (**6**). — 2,5-Anhydro-D-glucitol (161 mg, 0.7 mmol; see **3**) was tritylated[12] with 1.5 equiv. of trityl chloride, to produce a mixture of one di- and two different mono-trityl ethers. The mixture was methylated[7,8] detritylated[12], and acetylated as described for **4**, to produce a mixture (160 mg) of 6-*O*-acetyl-2,5-anhydro-1,3,4-tri-*O*-methyl-D-glucitol (**6**), 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-glucitol (**5**), and 1,6-di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol (**8**). Analytical g.l.c. (Columns 2 and 3), and preparative g.l.c. (Column 6, 175°) separated the two mono-*O*-acetyl derivatives (**5**

and **6**) from the di-*O*-acetyl derivative (**8**), but failed to resolve the two mono-*O*-acetyl derivatives. Compounds **5** and **6** were separated by capillary g.l.c. (Column 4, 50 to 170°, at 20°/min), which indicated that **5** and **6** were present in the ratio of 3:2.0. The ^{1}H-n.m.r. spectrum of the mixture of **5** + **6** was unassignable, owing to excessive overlapping of signals. The ^{13}C-n.m.r. spectrum ($CDCl_3$) of compound **6** (derived by subtracting resonances due to **5**): δ 20.96 (C-2 of acetyl), 57.42, 57.56, 59.28 (methoxyls), 64.84, 70.66, 80.88, 81.18, 84.45, 85.58 (C-1,2,3,4,5,6), 170.04 (C-1 of acetyl); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 249 (11) and 266 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (75), 59 (12), 69 (11), 71 (47), 87 (21), 101 (41), 111 (19), 117 (20), 126 (3), 143 (9), 156 (1.4), 157 (0.6), 158 (0.8), 171 (1.3), 175 (0.6), 188 (0.9), 203 (1.4), and 249 (0.1).

*1,6-Di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-*D*-mannitol* (**7**). — 2,5-Anhydro-D-mannitol[9] (368 mg, 2.2 mmol) was treated with 1.5 equiv. of trityl chloride in pyridine[12] to produce a mixture of 1-trityl and 1,6-ditrityl ethers which were separated by chromatography on alumina (2 × 50 cm), with elution with 1:1 (v/v) hexane–ethyl acetate. The 1,6-ditrityl fraction (R_F 0.31) was sequentially methylated[7,8], detritylated[12], and acetylated as described for **4**, to produce **7** (53 mg, 19%); $[\alpha]_D^{23}$ +44.7° (*c* 1.5, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.10 (s, 6 H, 1 AcO), 3.39 (s, 6 H, 2 MeO), 3.69 (m, 2 H, H-3,4), and 4.19 (broad s, 6 H, H-1,2,5,6,1′,6′); ^{13}C-n.m.r. ($CDCl_3$): δ 20.82 (C-2 of acetyl), 57.58 (methoxyls), 64.14 (C-1,6), 80.63 (C-3,4), 86.25 (C-2,5), and 170.65 (C-1 of acetyl); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 277 (3) and 294 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (17), 69 (11), 71 (38), 101 (17), 111 (22), 115 (10), 117 (23), 124 (15), 143 (10), 156 (3), 171 (2), 203 (2), 216 (13), and 277 (0.1).

Anal. Calc. for $C_{12}H_{20}O_7$: C, 52.16; H, 7.30. Found: C, 52.19; H, 7.37.

*1,6-Di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-*D*-glucitol* (**8**). — 2,5-Anhydro-D-glucitol (116 mg, 0.7 mmol) was prepared as described for **3**, and treated as described for **7**. Preparative g.l.c. (Column 5, 185°) produced analytically pure **8**; $[\alpha]_D^{23}$ +40.1° (*c* 2.05, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.09 (s, 6 H, 1 AcO), 3.37, 3.40 (2 s, 6 H, 2 MeO), 3.63 (dd, 1 H, *J* 1.3, 3.2 Hz, H-4), 3.73 (m, 1 H, H-3), 4.03 (ddd, 1 H, *J* 3.1, 5.3, 6.4 Hz, H-5), 4.10–4.27 (complex, 4 H, H-1,6,1′,6′), and 4.40 (complex, 1 H, H-2); ^{13}C-n.m.r. ($CDCl_3$): δ 20.81, 20.88 (C-2 of acetyl), 57.36, 57.47 (methoxyls), 63.10, 64.57 (C-1,6), 79.02, 81.37, 84.44, 85.08 (C-2,3,4,5), 170.64, and 170.72 (C-1 of acetyls); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 277 (8) and 294 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 71 (37), 86 (34), 87 (9), 100 (9), 101 (10), 111 (12), 117 (21), 124 (6), 130 (3), 143 (3), 156 (1.6), 171 (0.5), 203 (0.3), and 216 (4).

Anal. Calc. for $C_{12}H_{20}O_7$: C, 52.16; H, 7.30. Found: C, 52.06; H, 7.17.

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