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Authentic standards for the reductive-cleavage method. The positional isomers of partially methylated and acetylated or benzoylated 1,5-anhydro-D-mannitol

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Abstract

Described herein is an efficient method for the synthesis of the sixteen positional isomers of methylated and acetylated or benzoylated 1,5-anhydro-D-mannitol. The compounds are generated simultaneously by partial methylation of 1,5-anhydro-D-mannitol and subsequent benzoylation, and the individual isomers are obtained in pure form by high-performance liquid chromatography. Debenzoylation of the latter and acetylation yielded the desired acetates. The ^{1}H NMR spectra of the benzoates and the electron-ionization mass spectra of the acetates and the tetra-*O*-methyl derivative are reported herein as are the linear temperature-programmed gas-liquid chromatography retention indices of the acetates and the tetra-*O*-methyl derivative on three different capillary columns.

Keywords: D-Mannitol, 1,5-anhydro-; Methylated derivatives; Acylated derivatives; Reductive-cleavage

1. Introduction

The reductive-cleavage method [1] for glycosyl-linkage analysis is based upon methylation analysis but departs from it significantly in that reductive cleavage, rather than hydrolysis, of glycosidic linkages in the fully methylated glycan is performed. The products, partially methylated anhydroalditols, are identified by comparison of the GLC retention indices and chemical-ionization (CI) and electron-ionization (EI) mass spectra of their acetates to those of authentic standards. We recently described a general

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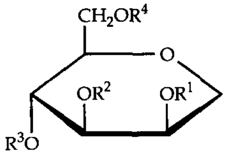
procedure for the synthesis of such standards as illustrated for the synthesis of the eight positional isomers of methylated and acetylated or benzoylated 1,5-anhydro-D-fucitol [2]. The positional isomers were generated simultaneously by partial methylation of 1,5-anhydro-D-fucitol and subsequent benzoylation, and the individual isomers were obtained in pure form by HPLC. Debenzoylation and acetylation afforded the desired acetates.

Described herein is the application of this approach to the synthesis of the sixteen positional isomers of methylated and acetylated or benzoylated 1,5-anhydro-D-mannitol (**1**–**16**). As an aid to those who wish to use the reductive-cleavage method, the ^{1}H NMR spectra of the fifteen methylated and benzoylated positional isomers of 1,5-anhydro-D-mannitol (**2b**–**16b**) are reported, as are the electron-ionization (EI) mass spectra of the corresponding acetates (**2a**–**16a**) and the tetra-*O*-methyl derivative (**1**). In addition, the retention data of the acetates (**2a**–**16a**) and the tetra-*O*-methyl derivative (**1**) on three different GLC columns are reported as linear temperature-programmed gas–liquid chromatography retention indices (LTPGLCRI), a particularly accurate method of identification, but one not used until recently [2] for carbohydrate derivatives useful in glycosyl-linkage analysis.



	R^1	R^2	R^3	R^4
1	Me	Me	Me	Me
2a	Ac	Me	Me	Me
2b	Bz	Me	Me	Me
3a	Me	Ac	Me	Me
3b	Me	Bz	Me	Me
4a	Me	Me	Ac	Me
4b	Me	Me	Bz	Me
5a	Me	Me	Me	Ac
5b	Me	Me	Me	Bz
6a	Ac	Ac	Me	Me
6b	Bz	Bz	Me	Me
7a	Ac	Me	Ac	Me
7b	Bz	Me	Bz	Me
8a	Ac	Me	Me	Ac
8b	Bz	Me	Me	Bz
9a	Me	Ac	Ac	Me
9b	Me	Bz	Bz	Me
10a	Me	Ac	Me	Ac
10b	Me	Bz	Me	Bz
11a	Me	Me	Ac	Ac
11b	Me	Me	Bz	Bz
12a	Ac	Ac	Ac	Me
12b	Bz	Bz	Bz	Me
13a	Ac	Ac	Me	Ac
13b	Bz	Bz	Me	Bz
14a	Ac	Me	Ac	Ac
14b	Bz	Me	Bz	Bz
15a	Me	Ac	Ac	Ac
15b	Me	Bz	Bz	Bz
16a	Ac	Ac	Ac	Ac
16b	Bz	Bz	Bz	Bz

2. Results

Synthesis.—The tetra-*O*-methyl (**1**), tetra-*O*-acetyl (**16a**) and tetra-*O*-benzoyl (**16b**) derivatives of 1,5-anhydro-D-mannitol were prepared from the latter by total methylation [3], acetylation, and benzoylation, respectively. The remaining fourteen partially methylated and benzoylated positional isomers (**2b–15b**) were prepared by partial methylation [4] of 1,5-anhydro-D-mannitol, followed by benzoylation in situ [2]. The resultant mixture of partially methylated 1,5-anhydro-D-mannitol benzoates was then separated by semipreparative reversed-phase HPLC using a Rainin C_{18} column (Table 1). The individual components were isolated and, after removal of solvent, were identified by ^{1}H NMR spectroscopy. Fractions containing compounds that were not resolved (**2b** and **3b**; **14b** and **15b**) were applied to a Regis silica gel column and, in all cases, complete separation was achieved (Table 1). A portion of each benzoate was then debenzoylated (NaOMe in MeOH), and the product was acetylated, affording the partially methylated 1,5-anhydro-D-mannitol acetate in chromatographically pure form.

Table 1
Reversed-phase and normal-phase capacity factors (k') of compounds **2b–16b** [a]

Compound [b] (Position of benzoyl)	k'	
	Reversed-phase [c]	Normal-phase [d]
4b (4-)	0.66	
5b (6-)	0.80	
2b (2-)	1.05	5.95 [e]
3b (3-)	1.05	8.43
11b (4,6-)	2.26	
9b (3,4-)	2.30	
7b (2,4-)	2.46	
6b (2,3-)	2.57	
8b (2,6-)	2.76	
10b (3,6-)	2.80	
12b (2,3,4-)	3.45	
14b (2,4,6-)	3.57	17.0 [f]
15b (3,4,6-)	3.57	13.8
13b (2,3,6-)	3.78	
16b (2,3,4,6-)	4.26	

[a] Capacity factors (k') were calculated from the equation $k'(x) = (t_{r(x)} - t_m)/t_m$, where $k'(x)$ is the capacity factor of interest (x), $t_{r(x)}$ is the absolute retention time of the compound of interest (x), and t_m is the dead time. Dead time was estimated from the equation $t_m = (0.5Ld_c^2)/F$, where 0.5 is a unitless constant, L is the length of the column in centimeters, d_c is the column diameter in centimeters, and F is the column flow rate in mL/min.

[b] Compounds listed in the order in which they eluted from the C_{18} column.

[c] Reversed-phase HPLC was conducted using a 5-μm particle-size Rainin Dynamax Microsorb semipreparative C_{18} column (1.0×25.0 cm) which was equilibrated in 55:45 MeCN–H_2O at 3.0 mL/min. After injection, the column was eluted for 5 min, then programmed with a linear gradient to 95:5 MeCN–H_2O over 20 min.

[d] Normal-phase HPLC was conducted using a 5-μm particle-size Regis silica gel column (0.46×25.0 cm). After injection, the column was eluted isocratically until all compounds had passed through the column.

[e] The column was equilibrated in 80:20 hexane–EtOAc at 3.0 mL/min.

[f] The column was equilibrated in 90:10 hexane–EtOAc at 3.0 mL/min.

Table 2
^{1}H NMR data (δ in ppm, J in Hz in brackets) for partially methylated 1,5-anhydro-D-mannitol benzoates **2b–16b** [a,b]

Compound (posn of Bz)	H-1e	H-1a	H-2	H-3	H-4 [c]	H-5	H-6	H-6′	O-Me
2b	4.15 *dd*	3.60 *br d*	5.55 *m*	3.41 *dd*	3.49 *t*	3.35 *ddd*	3.69*dd*	3.65 *dd*	3.44,3.45,3.56
(2-)	(2.1,13.1)	(13.1)		(3.5,9.2)	(9.4)	(2.1,5.3,9.6)	(2.1,10.5)	(5.3,10.5)	
3b	4.15 *dd*	3.49 *br d*	3.78 *m*	5.10 *dd*	3.74 *t*	3.41 *m*	3.67 *dd*	3.63 *dd*	3.39, 3.43, 3.49
(3-)	(2.3, 12.8)	(12.7)		(3.4, 9.8)	(9.7)		(2.1, 10.4)	(5.3, 10.4)	
4b	4.25 *dd*	3.44 – 3.48	3.73 *m*	3.51 *dd*	5.37 *t*	3.64 *ddd*	3.55 *dd*	3.44 – 3.48	3.33, 3.42, 3.51
(4-)	(2.6, 12.8)	complex		(3.4, 9.3)	(9.5)	(2.4, 7.7, 9.5)	(7.7, 10.5)	complex	
5b	4.22 *dd*	3.33 *br d*	3.66 *m*	3.32 *dd*	3.58 *t*	3.45 *ddd*	4.62*dd*	4.46 *dd*	3.47, 3.52, 3.55
(6-)	(2.3, 13.0)	(13.0)		(3.4, 9.2)	(9.3)	(1.9, 5.1, 9.4)	(1.9, 11.9)	(5.1, 11.9)	
6b	4.23 *dd*	3.80 *br d*	5.64 *m*	5.35 *dd*	3.88 *t*	3.5 *dt* [d]	3.72 *br d*		3.48, 3.50
(2,3-)	(2.1, 13.2)	(13.1)		(3.6, 9.8)	(9.6)	(9.8, 3.4)	(3.2)		
7b	4.29 *dd*	3.76 *br d*	5.67 *m*	3.67 *dd*	5.49 *t*	3.74 *ddd*	3.60*dd*	3.54 *dd*	3.36, 3.38
(2,4-)	(2.2, 13.2)	(13.1)		(3.4, 9.5)	(9.8)	(2.6, 7.0, 9.8)	(7.0, 10.6)	(2.6, 10.6)	
8b	4.16 *dd*	3.65 *br d*	5.61 *m*	3.48 *dd*	3.67 *t*	3.54 *ddd*	4.69*dd*	4.57 *dd*	3.48, 3.59
(2,6-)	(2.0, 13.0)	(13.1)		(3.4, 9.2)	(9.4)	(2.0, 4.3, 9.7)	(2.0, 11.8)	(4.3, 11.8)	
9b	4.27 *dd*	3.65 *br d*	3.91 *m*	5.33 *dd*	5.71 *t*	3.78 *ddd*	3.59*dd*	3.53 *dd*	3.36, 3.45
(3,4-)	(2.2, 12.9)	(12.8)		(3.3, 10.0)	(9.9)	(2.4, 7.0, 9.9)	(7.0, 10.5)	(2.4, 10.5)	
10b	4.19 *dd*	3.54 *br d*	3.84 *m*	5.16 *dd*	3.85 *t*	3.62 *ddd*	4.66*dd*	4.53 *dd*	3.41,3.52
(3,6-)	(2.1,12.9)	(12.5)		(3.4,9.8)	(9.6)	(2.0,5.6,9.6)	(2.0,11.8)	(5.6,11.8)	
11b	4.25 *dd*	3.48 *br d*	3.76 *m*	3.56 *dd*	5.61 *t*	3.83 *ddd*	4.55*dd*	4.38 *dd*	3.44,3.51
(4,6-)	(2.7,12.8)	(12.8)		(3.2,9.3)	(9.3)	(2.9,6.0,9.3)	(2.9,12.1)	(6.0,12.1)	
12b	4.35 *dd*	3.93 *br d*	5.73 *m*	5.59 *dd*	5.83 *t*	3.88 *ddd*	3.64*dd*	3.60 *dd*	3.39
(2,3,4-)	(2.0,13.3)	(13.3)		(3.6,10.0)	(9.9)	(2.7,6.2,9.9)	(6.2,10.6)	(2.7,10.6)	
13b	4.23 *dd*	3.85 *br d*	5.68 *m*	5.40 *dd*	3.97 *t*	3.72 *ddd*	4.72*dd*	4.65 *dd*	3.52
(2,3,6-)	(2.1,13.1)	(13.0)		(3.4,9.7)	(9.6)	(2.1,4.1,9.7)	(2.1,11.9)	(4.1,11.9)	
14b	4.27 *dd*	3.77 *br d*	5.69 *m*	3.71 *dd*	5.77 *t*	3.91 *ddd*	4.68*dd*	4.39 *dd*	3.40
(2,4,6-)	(2.3,13.1)	(12.7)		(3.4,9.5)	(9.6)	(2.7,5.1,9.7)	(2.7,12.1)	(5.1,12.1)	
15b	4.27 *dd*	3.66 *br d*	3.94 *m*	5.36 *dd*	5.91 *t*	3.95 *ddd*	4.59*dd*	4.45 *dd*	3.46
(3,4,6-)	(2.3,12.8)	(12.7)		(3.3,10.0)	(9.9)	(2.9,5.8,9.9)	(2.9,12.1)	(5.8,12.1)	
16b	4.35 *dd*	3.96 *br d*	5.77 *m*	5.62 *dd*	6.10 *t*	4.05 *ddd*	4.72*dd*	4.47 *dd*	
(2,3,4,6-)	(2.1,13.4)	(13.1)		(3.5,10.1)	(9.9)	(2.5,4.6,9.8)	(2.5,12.2)	(4.6,12.2)	

[a] Additional resonances were observed for benzoyl hydrogens at δ 7.28–8.14.

[b] Multiplicities include *br*, broad; *d*, doublet; *dd*, doublet of doublets; *dt*, doublet of triplets; *m*, multiplet; *t*, triplet.

[c] The resonances assigned as a triplet (*t*) and a doublet of triplets (*dt*) were actually a doublet of doublets (*dd*) and doublet of doublets of doublets (*ddd*), respectively, with a pair of coupling constants having nearly equal magnitude.

[d] Resonance partially obscured.

*^{1}H NMR spectra of partially methylated 1,5-anhydro-*D*-mannitol benzoates* (**2b**–**16b**). —Given in Table 2 are ^{1}H NMR spectral data for compounds **2b**–**16b**. The individual components of the mixture were easily identified based upon a straightforward analysis of the chemical shifts and coupling constants of the ring hydrogen resonances. All resonances displayed the multiplicities and coupling constants expected for a tetrahydropyran derivative of the *manno* configuration in the $^4C_1$ conformation and, in addition, the positions of substitution of benzoyl groups were readily discerned based upon the large downfield shift of the respective ring hydrogen resonances.

*Mass spectra of the methylated 1,5-anhydro-*D*-mannitol acetates* (**1**, **2a**–**16a**).— Compounds **1** and **2a**–**16a** were analyzed by chemical-ionization (CI) mass spectrometry with ammonia as the reagent gas and by EI mass spectrometry. The CI (NH_3) mass spectra of all compounds displayed the expected $(M+H)^+$ and $(M+NH_4)^+$ ions, which, because of the their unique molecular weights, readily identifies them as anhydrohexitol derivatives. The EI mass spectra (Fig. 1) of the compounds (**1**, **2a**–**16a**) readily identified them as 1,5-anhydrohexitol derivatives since all displayed characteristic fragment ions for loss [5] of either the exocyclic methoxymethyl ($MeOCH_2$, M − 45)

Table 3
Linear temperature-programmed gas–liquid chromatography retention indices (LTPGLCRI) of compounds **1** and **2a**–**16a** [a]

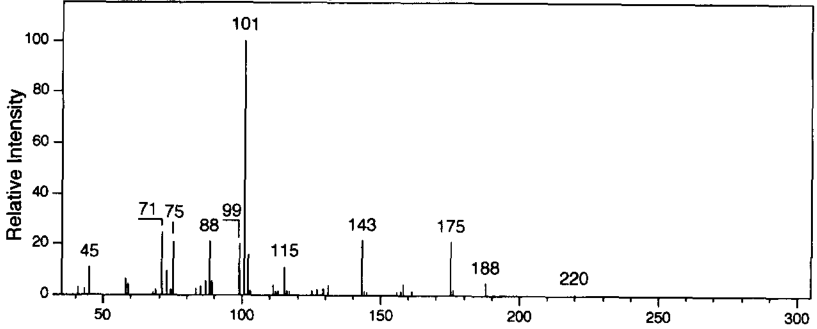
Compound (Position of acetyl)	Stationary phase		
	DB-5	DB-17	RT$_x$-200
1 (none)	1422.70 [b]	1716.41	1633.33
2a (2-)	1503.73	1817.15	1744.71
3a (3-)	1545.54	1867.44	1840.98
4a (4-)	1600.00	1943.04	1982.19
5a (6-)	1603.36	1950.45	1915.04
6a (2,3-)	1641.79	1977.37	2032.71
7a (2,4-)	1671.77	2040.91 [c]	2064.03
9a (3,4-)	1676.29	2039.32 [c]	2103.72
8a (2,6-)	1682.91	2041.65 [c]	2050.39
10a (3,6-)	1717.79	2091.92	2120.07
12a (2,3,4-)	1745.51	2119.37	2217.92
11a (4,6-)	1752.77	2140.54	2213.16
13a (2,3,6-)	1816.68	2196.88	2348.94
15a (3,4,6-)	1820.22	2231.55	2320.29
14a (2,4,6-)	1820.22	2231.55	2302.99
16a (2,3,4,6-)	1885.42	2300.00	2449.37

[a] Unless noted otherwise, indices were determined using a mixture of all compounds co-injected with the homologous series of *n*-alkanes from $C_{11}H_{24}$ to $C_{26}H_{54}$. Values were calculated from the equation $\mathrm{LTPGLCRI}_{(x)} = 100n + [100\cdot\Delta n\cdot(t_{R(x)} - t_{R(n)})/(t_{R(n+\Delta n)} - t_{R(n)})]$, where $\mathrm{LTPGLCRI}_{(x)}$ is the linear temperature programmed gas–liquid chromatography retention index of the compound of interest (x), n is the carbon number of the *n*-alkane standard eluting just before the compound of interest (x), Δn is the difference in carbon number between the *n*-alkane standard eluting just before and just after the compound of interest (x), $t_{R(x)}$ is the absolute retention time of the compound of interest (x), and $t_{R(n)}$ and $t_{R(n+\Delta n)}$ are the absolute retention times of the *n*-alkanes eluting just before and just after the compound of interest (x).

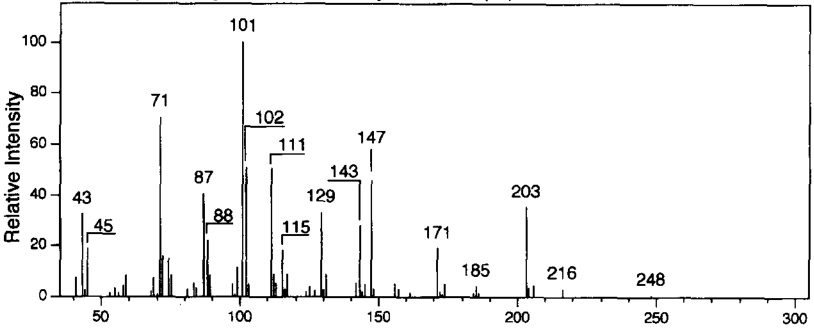
[b] Values are listed according to increasing retention index on the DB-5 column.

[c] Retention index determined separately.

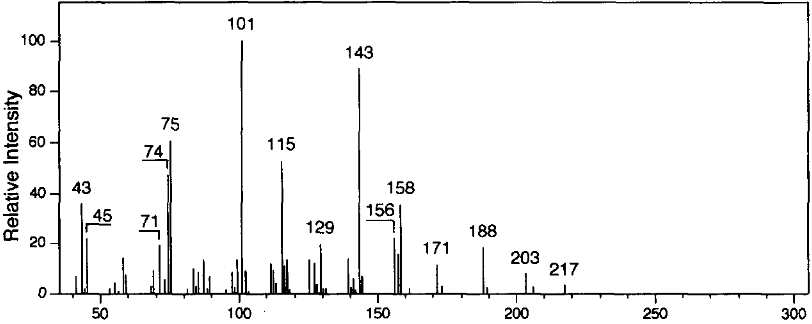
1,5-Anhydro-2,3,4,6-tetra-*O*-methyl-D-mannitol (**1**)



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



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



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

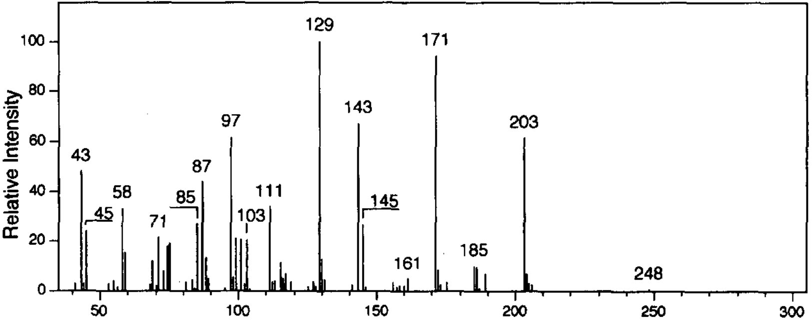
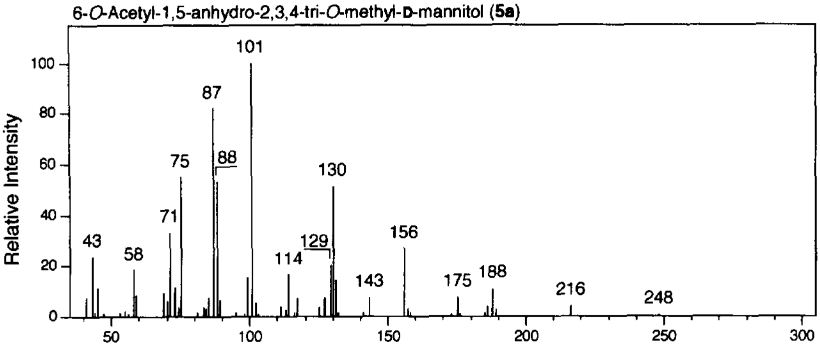
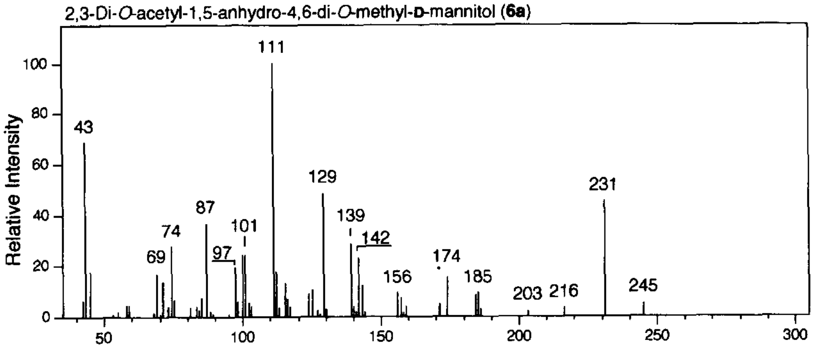


Fig. 1. Electron-ionization mass spectra (EIMS) of the partially methylated 1,5-anhydro-D-mannitol acetates (compounds **1** and **2a–16a**).

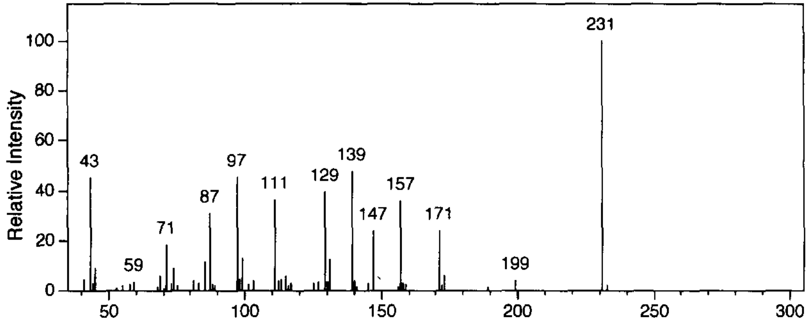
6-*O*-Acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-mannitol (**5a**)



2,3-Di-*O*-acetyl-1,5-anhydro-4,6-di-*O*-methyl-D-mannitol (**6a**)



2,4-Di-*O*-acetyl-1,5-anhydro-3,6-di-*O*-methyl-D-mannitol (**7a**)



2,6-Di-*O*-acetyl-1,5-anhydro-3,4-di-*O*-methyl-D-mannitol (**8a**)

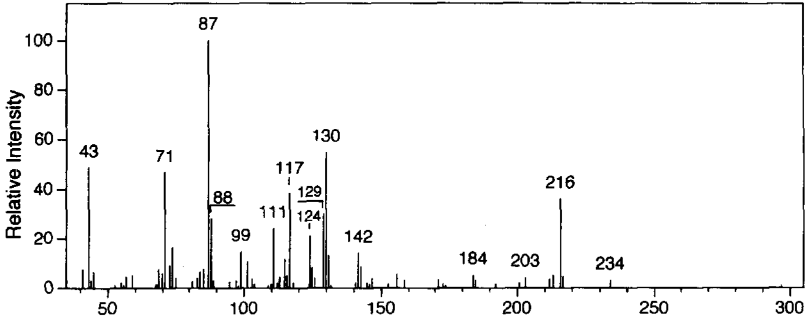
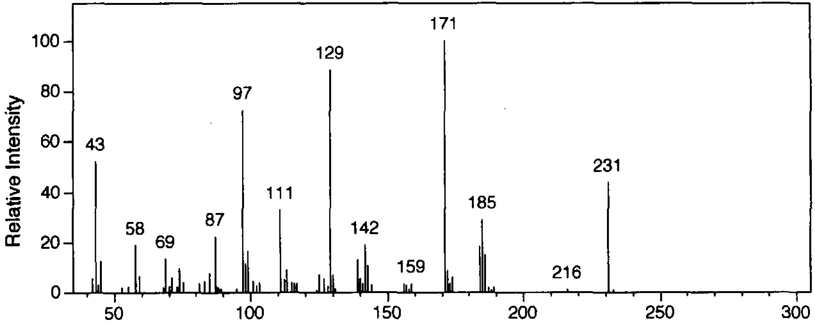
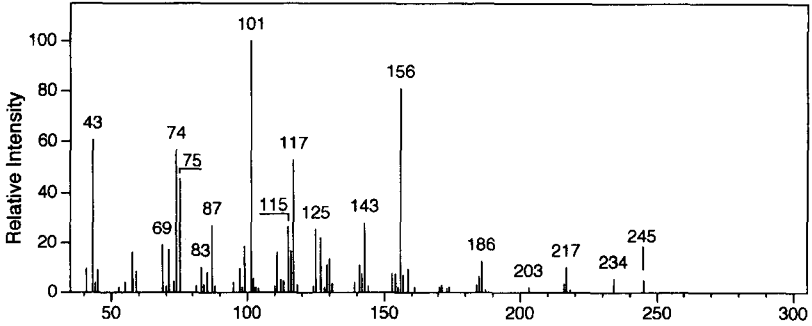


Fig. 1. (continued).

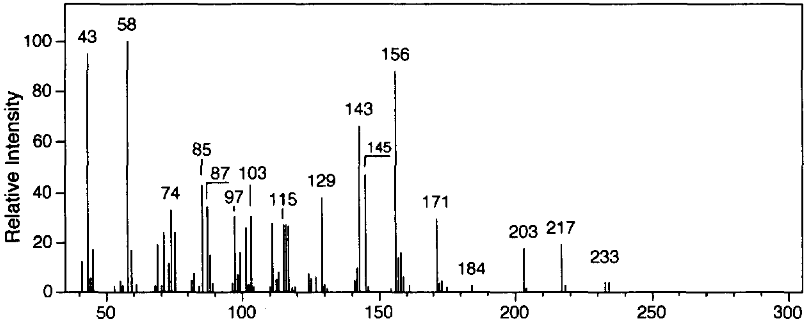
3,4-Di-*O*-acetyl-1,5-anhydro-2,6-di-*O*-methyl-D-mannitol (**9a**)



3,6-Di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methyl-D-mannitol (**10a**)



4,6-Di-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methyl-D-mannitol (**11a**)



2,3,4-Tri-*O*-acetyl-1,5-anhydro-6-*O*-methyl-D-mannitol (**12a**)

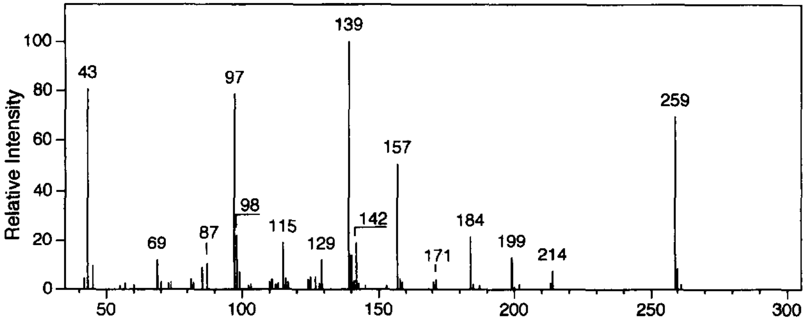
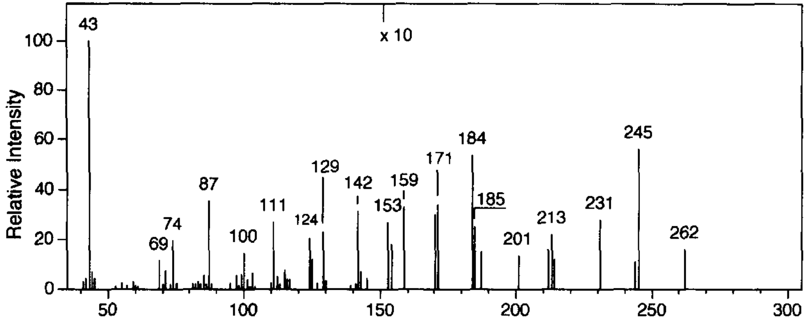
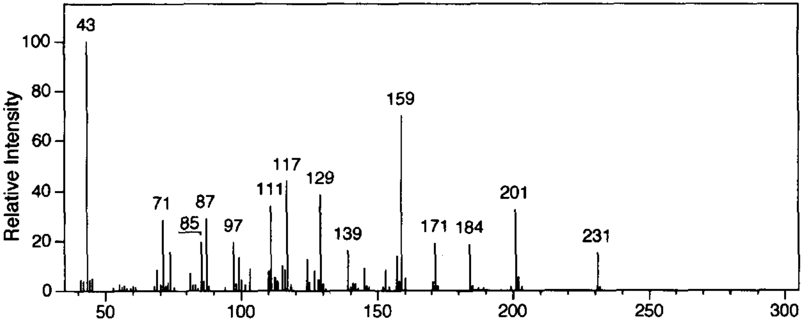


Fig. 1. (continued).

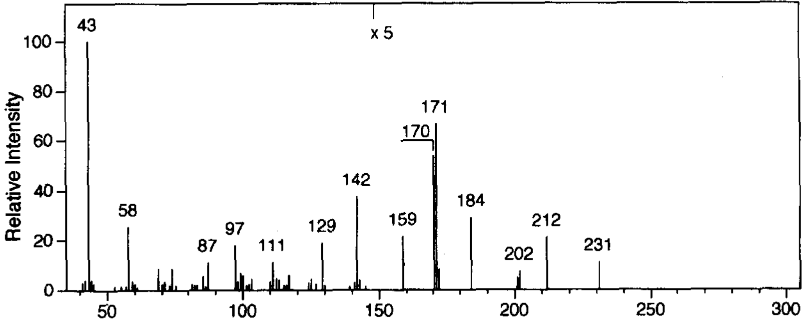
2,3,6-Tri-*O*-acetyl-1,5-anhydro-4-*O*-methyl-D-mannitol (**13a**)



2,4,6-Tri-*O*-acetyl-1,5-anhydro-3-*O*-methyl-D-mannitol (**14a**)



3,4,6-Tri-*O*-acetyl-1,5-anhydro-2-*O*-methyl-D-mannitol (**15a**)



2,3,4,6-Tetra-*O*-acetyl-1,5-anhydro-D-mannitol (**16a**)

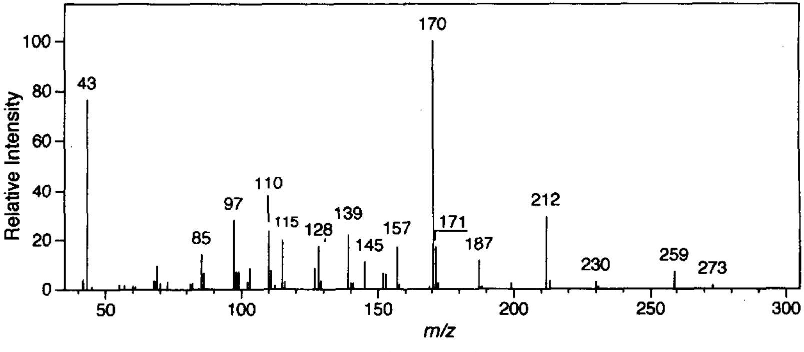


Fig. 1. (continued).

or exocyclic acetoxymethyl ($AcOCH_2$, M − 73) group. Although fragmentation pathways for 1,5-anhydrohexitol derivatives have not been established, it is clear from inspection of their mass spectra that they are diagnostically different and fragmentation patterns appear to be similar to that of methyl hexopyranoside derivatives as reported by Kotchetkov and Chizhov [6].

*GLC retention indices of methylated 1,5-anhydro-*D*-mannitol acetates* (**1**, **2a–16a**).—Given in Table 3 are the linear temperature-programmed gas–liquid chromatography retention indices [7] (LTPGLCRI) values for compounds **1** and **2a–16a** determined on three different capillary columns [2]: one (DB-5), a relatively nonpolar stationary phase (5% phenyl–95% methyl polysiloxane); one (DB-17), a more polarizable stationary phase (50% phenyl–50% methyl polysiloxane), and one (RT_x-200), a relatively polar stationary phase (50% trifluoropropyl–50% methyl polysiloxane). Analyses were performed in triplicate on each column using a mixture of all sixteen compounds and a mixture of *n*-alkanes from $C_{11}H_{24}$ to $C_{26}H_{54}$ as retention index standards [7]. All standard deviations were less than 0.1.

3. Discussion

This is another of a series of papers describing the synthesis and spectral characterization of authentic standards for the reductive-cleavage method. The goal of these studies is to provide such data for standards representing all possible combinations of position(s) of linkage and ring form for the most frequently encountered sugars. For such an effort to be successful, a simple, rapid method for the synthesis of these compounds was needed, and, moreover, a method of synthesis was needed wherein the ring form (furan, pyran) of the anhydroalditol product was not in question. As illustrated previously for the synthesis of methylated and acetylated positional isomers of 1,5-anhydro-D-fucitol [2] and 1,5-anhydro-D-galactitol [8] and herein for the methylated and acetylated positional isomers of 1,5-anhydro-D-mannitol, these requirements are easily met by a strategy involving partial methylation of the corresponding 1,5-anhydroalditol. This strategy also has the advantage of providing both the benzoyl and acetyl derivatives of the standards, as both types of derivatives are useful for structural analysis.

Equally as important as the development of an efficient synthetic scheme was the decision to report retention data as retention indices on three different stationary phases. As the data base of reductive-cleavage standards has grown, it has become apparent that the probability is great of two or more compounds having very similar retention data on one and even two different stationary phases. As described by Elvebak [9], combining the accuracy and precision of retention indices [10] with the superior differentiation power of a three-stationary phase approach [11] ensures that retention data will be reliable and unambiguous.

4. Experimental

General.—Reagents and solvents were prepared as previously described [2]. All alkane standards were obtained from Aldrich Chemical Company. A stock solution of the homologous series of alkanes from $C_{11}H_{24}$ to $C_{26}H_{54}$ was prepared by combining 20–30 mg of each alkane and diluting to 10 mL with hexane.

Instrumentation.—HPLC was performed using a Beckman model 338 System Gold chromatograph. Reversed-phase chromatography was performed on a 5-μm particle-size Rainin Dynamax Microsorb semipreparative C_{18} reversed-phase column (1.0 × 25.0 cm) equipped with a guard column (1.0 × 5.0 cm) having the same packing. Normal-phase chromatography was performed on a 5-μm particle-size Regis Spherisorb S5W Hi-Chrom analytical silica gel column (0.46 × 25.0 cm). The system was fitted with a 2.0-μm stainless steel in-line filter frit installed between the solvent mixing chamber and the injector and a 0.50-μm stainless steel filter frit installed between the injector and the guard column. UV–vis detection was performed at 254 nm and 280 nm for reversed-phase and normal-phase chromatography, respectively.

Analytical GLC was performed on a Hewlett–Packard 5890 gas–liquid chromatograph equipped with two split/splitless injection ports, two flame-ionization detectors, and a Perkin–Elmer Nelson 1020X Dual Channel Personal Integrator. The columns used were a J & W Scientific DB-5 fused silica capillary column (0.25 mm × 30 m, 0.25-μm film thickness, 5% phenyl–95% methyl polysiloxane stationary phase), a J & W DB-17 fused silica capillary column (0.25 mm × 30 m, 0.25-μm film thickness, 50% phenyl–50% methyl polysiloxane stationary phase) and a Restek RT_x-200 fused silica capillary column (0.25 mm × 30 m, 0.25-μm film thickness, 50% trifluoropropyl–50% methyl polysiloxane stationary phase). Each column was fitted with a J & W deactivated fused silica capillary guard column (0.25 mm × 1 m) via a press-tight connector (either J & W or Restek). Chromatography on the DB-5 and RT_x-200 columns was performed simultaneously by fitting these columns and a guard column into a two-way (Y) press-tight capillary column splitter (Restek); the guard column was installed in the injection port. The injector and detector temperatures were set at 250°C and 275°C, respectively. Helium was used as the carrier gas at measured linear velocities (methane injection, oven temperature 80°C) of 26.1, 28.4 and 27.8 cm/s, respectively, for the DB-5, DB-17 and RT_x-200 columns. The temperature program for all columns, which was optimized according to the guidelines set forth by Krupcik et al. [12] was 80–250°C at 2°C/min with no initial hold time.

GLC–MS analyses were performed using a Finnegan MAT 95 high-resolution double-focusing, reversed-geometry mass spectrometer equipped with a Hewlett–Packard 5890A Series II gas chromatograph and a DEC model 2100 workstation. Chemical-ionization mass spectra were acquired with NH_3 as the reagent gas at a source temperature of 180°C, and NH_3 was introduced at a pressure of 4×10^{-4} torr as indicated on the source ionization gauge. For CI spectra, the instrument was scanned from m/z 60–650 at 1 s/decade. Electron-ionization mass spectra were obtained at an ionization energy of 70 eV and at a source temperature of 200°C. For EI spectra, the instrument was scanned from m/z 20–650 at 1 s/decade. The accelerating voltage was 5 kV. Both CI and EI mass spectra were acquired at a resolution of 1,000 (10% valley definition).

^{1}H NMR spectra were recorded on a Varian VXR-500S NMR spectrometer in $CDCl_3$ as the solvent and were referenced to internal tetramethylsilane.

*Partially methylated 1,5-anhydro-*D*-mannitol benzoates* (**2b–16b**).—1,5-Anhydro-D-mannitol (80 mg), prepared by the method of Ness et al. [13], was dissolved in 4 mL of dry Me_2SO in a flame-dried 10-mL conical flask. Three 1-mL aliquots of this solution were removed and added separately to flame-dried 10-mL conical flasks. To each reaction was added 0.75, 1.5, 2.5, and 3.5 equiv, respectively, of lithium methylsulfinylmethanide. The reaction mixtures were then benzoylated as described previously [2].

Separation of the above mixture of benzoates (**2b–16b**) was accomplished by reversed-phase and normal-phase HPLC (Table 1) using a semipreparative C_{18} column and an analytical silica column, respectively. Aliquots (20 μL) of the mixture were applied to the C_{18} column, which was equilibrated in 55:45 MeCN–H_2O at 3.0 mL/min. After injection, the column was eluted for 5 min, then programmed with a linear gradient to 95:5 MeCN–H_2O over 20 min. The individual components from eight or more applications were collected and combined and, after removal of solvent by evaporation under vacuum, were dissolved in $CDCl_3$ and identified by ^1H NMR spectroscopy.

Compounds **2b** and **3b**, and **14b** and **15b**, which did not separate under reversed-phase HPLC conditions, were each isolated in pure form by normal-phase HPLC (Table 1). The contents of the NMR tubes containing the mixtures of components were quantitatively transferred to separate 10-mL conical vials and the $CDCl_3$ was removed by evaporation under vacuum. The compounds were redissolved in 200 μL of EtOAc. Aliquots (20 μL) of the mixtures were applied to the analytical silica gel column which was equilibrated in hexane-EtOAc at 3.0 mL/min (see Table 1 for eluent concentrations specific to each set of compounds). The column was then eluted isocratically until all compounds had passed through the column. The individual components were collected and combined and, after removal of solvent by evaporation under vacuum, were dissolved in $CDCl_3$ and identified by ^1H NMR spectroscopy.

*Methylated 1,5-anhydro-*D*-mannitol acetates* (**1**, **2a–16a**).—Approximately one-third to one-half of each pure benzoate, obtained as described above, was debenzoylated and acetylated as described previously [2] to afford the partially methylated 1,5-anhydro-D-mannitol acetate standards in pure form. The pure standards were then chromatographed individually on the three aforementioned GLC columns under the conditions already described, except that the temperature of the columns was programmed from 80–250°C at 6°C/min. In this way, the relative orders of elution of the standards on each column were determined. In order to expedite acquisition of their mass spectra, further studies used mixtures of the standards prepared by acetylation of portions of the partial methylation reactions that were saved. The latter mixtures were also found to contain the tetra-*O*-methyl derivative (**1**), whose position of elution was verified by chromatography of an independently synthesized sample.

*Determination of LTPGLCRI values of methylated 1,5-anhydro-*D*-mannitol acetates* (**1**, **2a–16a**).—In order to ensure that the mixture of standards contained only the title compounds, aliquots of the individual pure standards were combined such that the integral of the area (flame-ionization detection) of each component was at least 5% of the area of the most abundant component. An aliquot of the stock solution of *n*-alkanes from $C_{11}H_{24}$ to $C_{26}H_{54}$ was diluted 20-fold with hexane, then amounts of the alkane standard solution and the methylated anhydroalditol acetate standard solution were chosen for injection such that their area responses were comparable. Typically, a 1.0-μL

Hamilton syringe was loaded sequentially with 0.05 μL $CHCl_3$, 0.05 μL air, 0.5 μL methylated anhydroalditol acetate standard solution, 0.05 μL air, 0.1 μL alkane standard solution, and 0.1 μL air, and then the contents were injected. The manner of loading the syringe is that recommended by the manufacturers of the GLC columns. Immediately after injection, the temperature program for the column was begun. LTPGLCRI values were determined in triplicate on each of the columns using the equation given in Table 3.

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