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

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Abstract

The eight positional isomers of methylated and benzoylated 1,5-anhydroribitol were generated simultaneously from 1,5-anhydroribitol by sequential partial methylation and benzoylation, and the four meso isomers and two enantiomeric pairs of isomers so-formed were isolated in chemically pure form by high-performance liquid chromatography. The corresponding acetates were obtained by debenzoylation of the pure isomers and acetylation. Reported herein are the ^{1}H NMR spectra of the benzoates and the electron-ionization mass spectra of the acetates and the tri-*O*-methyl derivative. Also reported for the acetates and the tri-*O*-methyl derivative are their linear temperature-programmed gas–liquid chromatography retention indices on three different capillary columns.

Keywords: Reductive-cleavage method; Positional isomers; 1,5-Anhydroribitol

1. Introduction

Reductive-cleavage analysis [1,2] is a powerful chemical technique that allows the unambiguous determination of the identity, ratio, linkage position(s) and, in some cases, sequence [3,4] of the monosaccharide residues in polysaccharides. Unlike methylation analysis [5–7], the ring forms of the monosaccharide residues are retained in reductive-cleavage analysis since glycosidic linkages are cleaved by an ionic hydrogenation process. The partially methylated anhydroalditols so-obtained are subsequently analyzed as their benzoates or acetates [3].

In order for the reductive-cleavage method to be of routine use in carbohydrate structural analysis, complete sets of authentic standards are needed. Indeed, we have previously described a method [8] for the rapid synthesis of such standards and have used the method to prepare a wide variety of partially methylated and acetylated or benzoylated anhydroalditols. Described herein is the application of this method to the synthesis of standards derivable from D-ribopyranose residues, namely, the partially methylated and benzoylated (**2b**–**8b**) or acetylated (**2a**–**8a**) derivatives of 1,5-anhydroribitol. The ^{1}H NMR spectra of the benzoates, as well as the GLC retention indices and EI mass spectra of the acetates (**2a**–**8a**) and the tri-*O*-methyl derivative (**1**) are reported herein.

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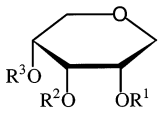
2. Results

Synthesis.—The tri-*O*-methyl (**1**) derivative of 1,5-anhydroribitol was prepared from the latter [9] by total methylation [10]. The six partially methylated and benzoylated positional isomers (**2b**–**7b**) as well as the tri-*O*-benzoyl derivative (**8b**) were prepared by partial methylation of 1,5-anhydroribitol, followed by benzoylation in situ [8].



	R^1	R^2	R^3
1	Me	Me	Me
2a	Ac	Me	Me
2b	Bz	Me	Me
3a	Me	Ac	Me
3b	Me	Bz	Me
4a	Me	Me	Ac
4b	Me	Me	Bz
5a	Ac	Ac	Me
5b	Bz	Bz	Me
6a	Ac	Me	Ac
6b	Bz	Me	Bz
7a	Me	Ac	Ac
7b	Me	Bz	Bz
8a	Ac	Ac	Ac
8b	Bz	Bz	Bz

The resultant mixture of partially methylated 1,5-anhydroribitol benzoates was then separated by semipreparative reversed-phase HPLC using a Rainin C_{18} column (Table 1).

Table 1
Reversed-phase HPLC capacity factors of compounds **2b**–**8b** [a]

Compound (position of benzoyl)	Capacity factor (k') [b]
2b, **4b** (2-, and 4-)	1.87
3b (3-)	2.13
6b (2,4-)	4.52
5b, **7b** (2,3-, and 3,4-)	4.91
8b (2,3,4-)	5.83

[a] Reversed-phase HPLC was conducted using a 5-μm particle-size Rainin Dynamax Microsorb semipreparative C_{18} column (1 × 25 cm) equilibrated in 1:1 MeCN–water at 3.0 mL/min. After injection, the column was eluted for 5 min, then programmed with a linear gradient to 19:1 MeCN–water over 20 min.

[b] 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 the compound of interest (x), $t_{r(x)}$ 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 [11].

The individual components were isolated, and after removal of solvent, were identified by ^{1}H NMR spectroscopy. A portion of each benzoate was then debenzoylated (NaOMe in MeOH), and the product was acetylated, affording the partially methylated 1,5-anhydroribitol acetates (**2a**–**7a**), as well as the tri-*O*-acetyl derivative (**8a**) in chromatographically pure form.

^{1}H NMR spectra of partially methylated 1,5-anhydroribitol benzoates (**2b**–**8b**).—Given in Table 2 are ^{1}H NMR spectral data for compounds **2b**–**8b**. The individual components of the mixture (see Table 2) 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 ribo configuration in the $^4C_1$ conformation. The positions of substitution of benzoyl groups were readily discerned based upon the large downfield shift of the respective ring hydrogen resonances. Since 1,5-anhydroribitol is a meso compound, two pairs of spectroscopically indistinguishable enantiomeric compounds (**2b**/**4b** and **5b**/**7b**) were formed in the partial methylation/benzoylation reaction sequence, and these enantiomeric pairs were not separable by HPLC under the conditions employed. In addition, compounds **3b**, **6b**, and **8b** are also meso compounds, so each contains three pairs of enantiotopic hydrogens (H-1e and H-5e, H-1a and H-5a, and H-2 and H-4). Each pair of enantiotopic hydrogens gave one signal in the NMR spectrum with an integrated area corresponding to two protons.

Mass spectra of the methylated 1,5-anhydroribitol acetates (**1**, **2a**–**8a**).—Compounds **1** and **2a**–**8a** 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 their unique molecular weights, readily identifies them as anhydropentitol derivatives. The EI mass spectra of the compounds are shown in Fig. 1. Although fragmentation pathways for derivatives of this type have not been established, it is apparent that they are diagnostically differ-

Table 2
^{1}H NMR data (δ in ppm, J in Hz in brackets) for partially methylated 1,5-anhydroribitol benzoates **2b**–**8b** [a,b]

Compound (position of benzoyl)	H-1e	H-1a	H-2	H-3	H-4	H-5a	H-5e	*O*-Me
2b, **4b** [c] (2-, and 4-)	3.68–3.85 complex	3.68–3.85 complex	3.46–3.49 complex	4.03 br t	5.12 ddd (2.5, 5.0, 8.5)	3.68–3.85 complex	3.68–3.85 complex	3.59, 3.48
3b (3-)	3.87 br dd (5.0, 10.5)	3.62 app t (10.5)	3.48 ddd (2.5, 5.0, 10.5)	6.12 br t	3.48 ddd (2.5, 5.0, 10.5)	3.62 app t (10.5)	3.87 br dd (5.0, 10.5)	3.40
5b, **7b** [d] (2,3-, and 3,4-)	4.01 dd (5.0, 11.0)	3.86 app t [e] (10.5)	5.29 ddd (3.0, 5.0, 10.0)	6.10 br s	3.67 ddd (2.5, 4.5, 10.0)	3.76 app t [e] (10.3)	3.94 dd (4.5, 11.0)	3.43
6b (2,4-)	3.93 dd (3.5, 11.5)	4.00 br dd (7.0, 11.5)	5.34 dt (3.5, 7.0)	3.99 br s	5.34 dt (3.5, 7.0)	4.00 br dd (7.0, 11.5)	3.93 dd (3.5, 11.5)	3.58
8b (2,3,4-)	4.03 dd (3.5, 12.0)	4.11 br dd (7.0, 12.0)	5.51 dt (3.5, 7.0)	5.93 br s	5.51 dt (3.5, 7.0)	4.11 br dd (7.0, 12.0)	4.03 dd (3.5, 12.0)	

[a] Additional resonances were observed for benzoyl hydrogens at δ 7.41–8.11.
[b] Multiplicities include: br, broad; dd, doublet of doublets; ddd, double doublet of doublets; t, triplet; dt, doublet of triplets.
[c] Compounds **2b** and **4b** were analyzed as a mixture of enantiomers; the assignments are given for **4b**.
[d] Compounds **5b** and **7b** were analyzed as a mixture of enantiomers; the assignments are given for **5b**.
[e] The resonances assigned as an apparent triplet (app t) were actually doublets of doublets with a pair of coupling constants having nearly equal magnitude.

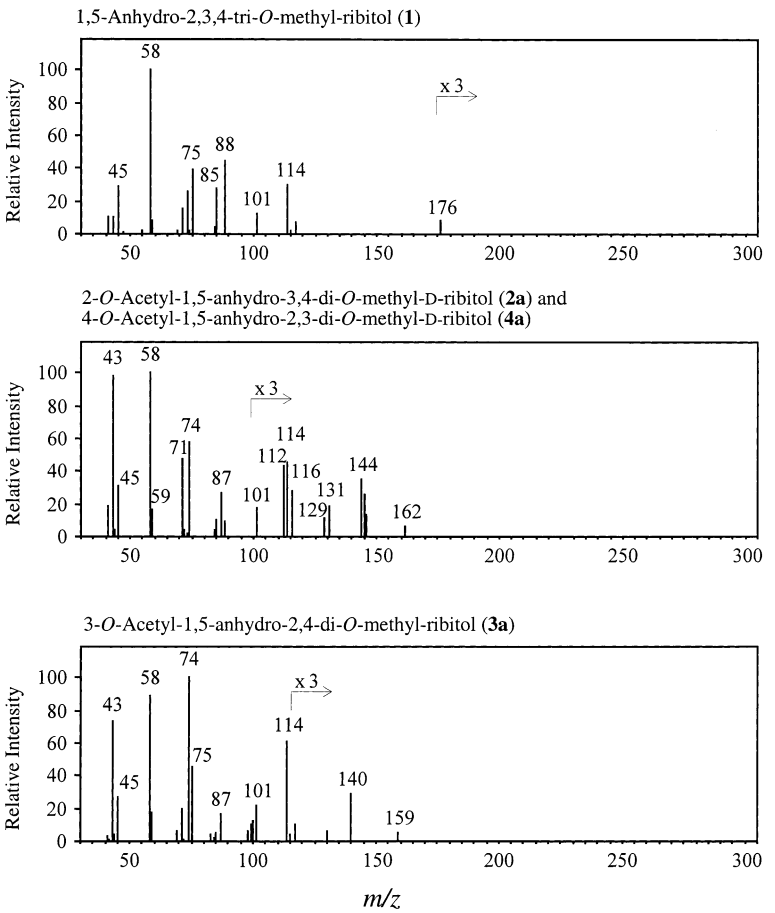


Fig. 1. Electron-ionization mass spectra of the methylated 1,5-anhydroribitol acetates (compounds **1** and **2a**–**8a**).

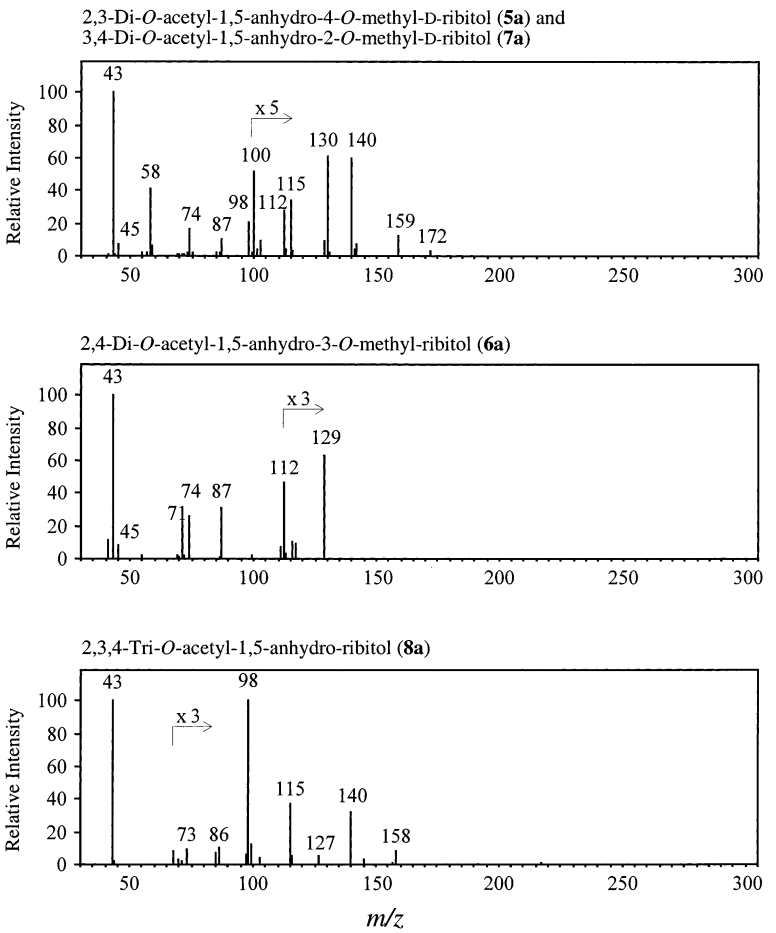


Fig. 1. (*Continued*)

ent. Furthermore, the spectra presented in Fig. 1 are qualitatively indistinguishable from those of the corresponding arabino [12] and xylo [13] derivatives, indicating that fragmentation depends more upon the relative positions of *O*-methyl and *O*-acetyl groups than on their relative configurations. It should be noted, however, that the ribo, arabino, and xylo positional isomers are readily distinguishable based on their GLC retention indices and, should it be necessary, by ^{1}H NMR spectroscopy of the corresponding benzoyl derivatives ([12,13] and herein).

GLC retention indices of methylated 1,5-anhydroribitol acetates (**1**, **2a**–**8a**).—Given in Table 3 are the linear temperature-programmed gas–liquid chromatography retention index [14] (LTPGLCRI) values for compounds **1** and **2a**–**8a** determined on three different capillary columns [8], one (DB-5) a relatively nonpolar stationary phase (1:19 phenyl–methyl polysiloxane), one (DB-17) a more polar stationary phase (1:1 phenyl–methyl polysiloxane), and one (RT_x-200) a relatively polar stationary phase (1:1 trifluoropropyl–methyl polysiloxane). Analyses were performed in triplicate on each column using a mixture of all six compounds and a mixture of *n*-alkanes $C_{11}H_{24}$ to $C_{26}H_{54}$ as retention index standards [14].

3. Discussion

This is one 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. The present report describes the synthesis of such standards for D-ribopyranosyl residues, which have been identified as compo-

Table 3
Linear temperature-programmed gas–liquid chromatography retention indices (LTPGLCRI) of compounds **1** and **2a**–**8a** [a]

Compound	Stationary phase		
(position of acetyl)	DB-5	DB-17	RT_x-200
1 (none)	1223.26 [b]	1434.56	1415.21
3a (3-)	1308.86	1555.64	1546.01
2a, **4a** (2-, and 4-)	1344.54	1576.09	1607.44
5a, **7a** (2,3-, and 3,4-)	1439.24	1695.96	1774.38
6a (2,4-)	1496.07	1750.19	1881.86
8a (2,3,4-)	1589.41	1868.39	2076.65

[a] 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 $LTPGLCRI_{(x)} = 100n + [100\Delta n(t_{r(x)} - t_{r(n)})/(t_{r(n+\Delta n)} - t_{r(n)})]$ where $LTPGLCRI_{(x)}$ 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.

nents in the lipopolysaccharides of gram-negative bacteria [15] as well as plant saponins [16,17].

For the benefit of those who use the data presented herein to establish the identity and position(s) of linkage of ribopyranosyl residues in samples of unknown structure, it should be pointed out that two pairs of compounds, namely the 2-*O*-acetyl (**2a**) and 4-*O*-acetyl (**4a**) derivatives and the 2,3-di-*O*-acetyl (**5a**) and 3,4-di-*O*-acetyl (**7a**) derivatives, are not distinguishable by spectroscopic means. Using these standards, it is therefore not possible to distinguish between 2- and 4-linked ribopyranosyl residues or between 2,3- and 3,4-linked ribopyranosyl residues. However, if such residues were encountered, the products could be distinguished by conducting reductive cleavage in the presence of a deuterated reducing agent [18] and characterizing the products, as their benzoates, by ^{1}H NMR spectroscopy.

4. Experimental

General.—Reagents, solvents and materials were prepared as previously described [8]. 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 × 25 cm) as previously described [8]. Analytical GLC was performed on a Hewlett–Packard 5890 gas–liquid chromatograph equipped with two flame ionization detectors, and HP 3365 Series II Chemstation recording software. The columns and conditions were the same as previously described [8]. GLC–MS analyses were performed using a Finnegan MAT 95 high resolution double focusing, reverse-geometry mass spectrometer equipped with a Hewlett–Packard 5890A Series II gas chromatograph and a DEC model 2100 workstation. Chemical- and electron-ionization mass spectra were acquired as previously described [8]. ^{1}H NMR spectra were recorded at 22 °C on a Varian VXR-500S NMR spectrometer in $CDCl_3$ as the solvent and were referenced to residual $CHCl_3$ at δ 7.27.

Partially methylated 1,5-anhydroribitol benzoates (**2b**–**8b**).—A mixture of the seven positional isomers was prepared from 1,5-anhydroribitol [9] as described for 1,5-anhydro-D-fucitol [8]. Separation of the mixture of benzoates (**2b**–**8b**) was accomplished by reversed-phase HPLC (Table 1) using a semipreparative C_{18} column. 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 ^{1}H NMR spectroscopy.

Methylated 1,5-anhydroribitol acetates (**1**, **2a**–**8a**).—Approximately one-third to one-half of each pure benzoate, obtained as described above, was debenzoylated and

acetylated as described previously [8] to afford the partially methylated 1,5-anhydroribitol acetate standards in pure form. The pure standards were then chromatographed individually on the three GLC columns under the conditions described previously, except that the temperature of the columns was programmed from 80 to 300 °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 tri-*O*-methyl derivative (**1**), whose position of elution was verified by chromatography of an independently synthesized sample prepared by methylation [10] of the parent compound.

Determination of LTPGLCRI values of methylated 1,5-anhydroribitol acetates (**1**, **2a**–**8a**).—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. The sample was injected manually and the individual components separated using a temperature program from 80 to 300 °C at 2 °C/min. LTPGLCRI values were determined in triplicate on each of the columns using the equation given in Table 3.

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