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Authentic standards for the reductive-cleavage method: the positional isomers of partially methylated and acetylated or benzoylated 1,5-anhydro-L-arabinitol

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Abstract

Described herein is the synthesis of the eight positional isomers of methylated and acetylated or benzoylated 1,5-anhydro-L-arabinitol. The compounds were generated simultaneously from 1,5-anhydro-L-arabinitol by sequential partial methylation and benzoylation and isolated in pure form by high-performance liquid chromatography. The desired acetates were obtained by debenzoylation and acetylation of the pure isomers. 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: Positional isomers; 1,5-Anhydro-L-arabinitol; Anhydroalditols; Acetate; Benzoyl

1. Introduction

In the reductive-cleavage method [1], the glycosidic linkages in fully methylated glycans are reduced and the partially methylated anhydroalditols so obtained are either converted to their benzoates, which are separated by HPLC and characterized by ^{1}H NMR spectroscopy [2], or to their acetates, which are separated by GLC and analyzed by mass spectrometry [3]. This method is capable of simultaneously establishing the composition of glycans as well as the ring form and position(s) of linkage of each monosaccharide residue provided that chromatographic and/or spectroscopic data for the products are available. A major goal of our recent work, therefore, has been to provide such data for standards representing all possible combinations of ring form and position(s) of linkage for the commonly encountered sugars. We have previously described a general procedure for the synthesis of such standards, as illustrated for the eight positional isomers of methylated and acetylated or benzoylated 1,5-anhydro-D-fucitol [4]. Described herein is the application of this approach to the synthesis of all standards derivable from L-arabinopyranosyl residues, namely, the eight positional isomers of methylated and acetylated or benzoylated 1,5-anhydro-L-arabinitol (**1**–**8**). As an aid to those who wish to use the reductive-cleavage method, the ^{1}H NMR spectra of the seven methylated and benzoylated positional

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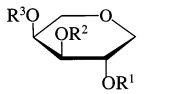
isomers of 1,5-anhydro-L-arabinitol (**2b–8b**) are reported, as are the electron-ionization (EI) mass spectra of the corresponding acetates (**2a–8a**) and the tri-*O*-methyl derivative (**1**). In addition, the retention data of the acetates (**2a–8a**) and the tri-*O*-methyl derivative (**1**) on 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 [4] for carbohydrate derivatives useful in glycosyl-linkage analysis.



	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

2. Results

Synthesis.—The tri-*O*-methyl (**1**), tri-*O*-acetyl (**8a**) and tri-*O*-benzoyl (**8b**) derivatives of 1,5-anhydro-L-arabinitol were prepared from the latter [5,6] by total methylation [7], acetylation, and benzoylation, respectively. The remaining six partially methylated and benzoylated positional isomers (**2b–7b**) were prepared by partial methylation of 1,5-anhydro-L-arabinitol, followed by benzoylation in situ [4]. The resultant mixture of partially methylated 1,5-anhydro-L-arabinitol 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. A portion of each benzoate was then debenzoylated (NaOMe in MeOH), and the product was acetylated, affording the partially methylated 1,5-anhydro-L-arabinitol acetate in chromatographically pure form.

^{1}H NMR spectra of partially methylated 1,5-anhydro-L-arabinitol 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 L-arabino 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-L-arabinitol 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 (Fig. 1) were

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

Compound (position of benzoyl)	Capacity factor (k')[b]
2b (2-)	2.29
3b (3-)	2.46
4b (4-)	2.38
5b (2,3-)	4.16
6b (2,4-)	4.35
7b (3,4-)	4.08
8b (2,3,4-)	5.18

[a] Reversed-phase HPLC was conducted using a 5-μm particle-size Rainin Dynamax, Microsorb semipreparative C_{18} column (1 × 25 cm) equilibrated in 50:50 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.

[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 [8].

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

Compound	H-1e	H-1a	H-2[b]	H-3	H-4[b]	H-5e	H-5a	O-Me
2b	3.96 dd (2.6, 12.4)	3.67–3.77 complex	5.29 m	3.67–3.77 complex	3.67–3.77 complex	3.67–3.77 complex	3.67–3.77 complex	3.46, 3.58
3b	3.92 dd (3.3, 12.0)	3.58 dd (5.8, 12.0)	3.65–3.71 complex	5.39 dd (3.0, 6.6)	3.79 dt (6.3, 3.1)	3.85 dd (6.6 11.4)	3.65–3.71 complex	3.41, 3.49
4b	4.07 dd (4.3, 11.6)	3.31 dd (8.2, 11.6)	3.63–3.67 complex	3.50 dd (3.3, 8.0)	5.53 dt (2.2, 3.8)	4.02 dd (4.4, 12.3)	3.62 dd (1.6, 12.3)	3.47, 3.52
5b	4.16dd (3.6, 12.0)	3.69 dd (6.3, 12.0)	5.51 dt (3.7, 6.6)	5.57 dd (2.9, 7.3)	3.89 dt (5.9, 2.9)	3.97 dd (6.1, 11.6)	3.75 dd (2.7, 11.6)	3.46
6b	4.14 dd (3.6, 12.0)	3.63 dd (6.1, 12.0)	5.39 dt (3.6, 6.5)	3.78–3.84 complex	5.58 dt (6.4, 3.2)	4.03 dd (6.4, 11.9)	3.78–3.84 complex	3.53
7b	4.12 dd (4.0, 11.9)	3.55 dd (7.4, 11.9)	3.82 dt[c] (4.0, 7.5)	5.48 dd (3.5, 7.6)	5.62 dt (5.6, 3.1)	4.05 dd (5.2, 12.2)	3.83–3.87 complex	3.51
8b	4.33 dd (4.0, 11.7)	3.68 dd (7.6, 11.7)	5.67 dt[c] (4.3, 8.1)	5.68–5.75 complex	5.68–5.75 complex	4.18 dd (4.3, 12.6)	3.92 dd (1.9, 12.6)	

[a] Additional resonances were observed for benzoyl hydrogens at δ 7.41–8.11.
[b] The resonances assigned as a doublet of triplets (dt) were actually a doublet of doublets of doublets (ddd) with a pair of coupling constants of almost equal magnitude.
[c] Resonance partially obscured.

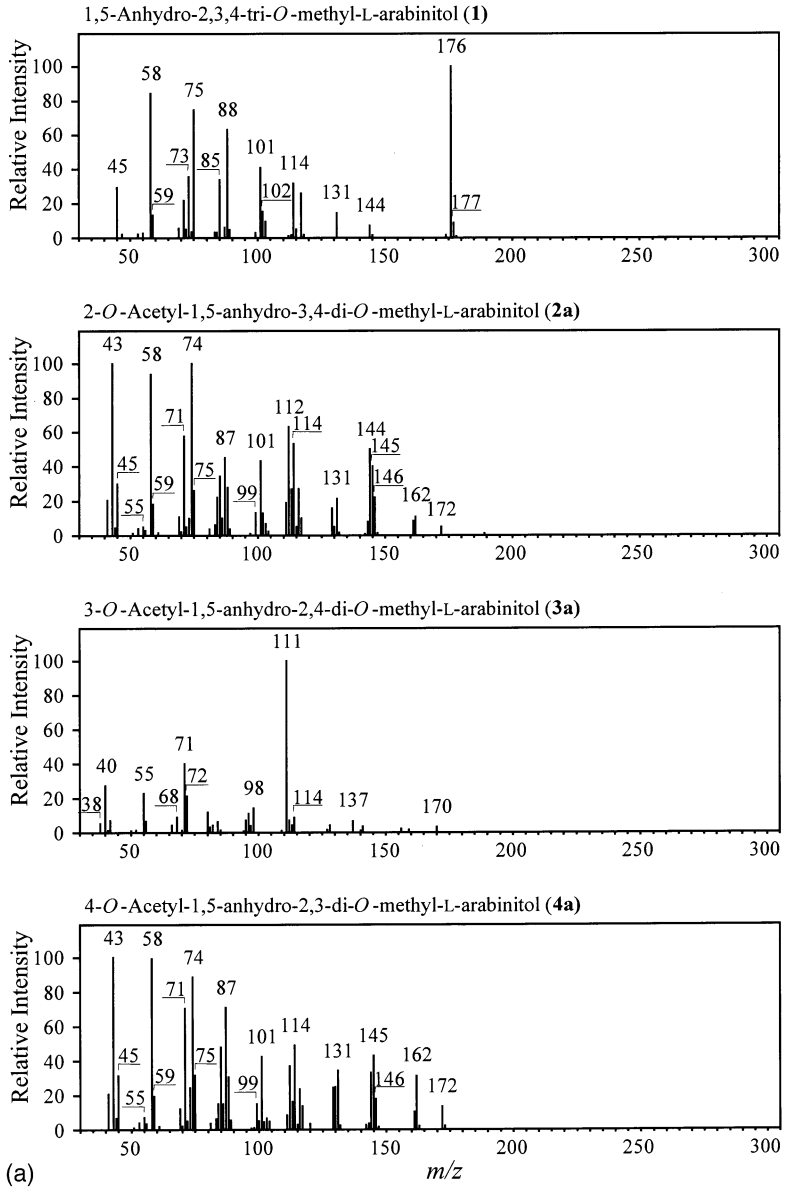


Fig. 1. Electron-ionization mass spectra of the methylated 1,5-anhydro-L-arabinitol acetates (compounds **1** and **2a–8a**).

very similar to those of the corresponding *xylo* isomers [9], as was expected. It should be noted that two pairs of the compounds, namely the 2-*O*-acetyl and 4-*O*-acetyl derivatives (**2a** and **4a**, respectively) and the 2,3-di-*O*-acetyl and 3,4-di-*O*-acetyl derivatives (**5a** and **7a**, respectively) gave very similar, if not identical, mass spectra. Although fragmentation pathways for derivatives of this type have not been established, it is apparent that they depend more on the sequence of O-methyl and O-acetyl groups on the tetrahydropyran ring than on their relative configuration.

*GLC retention indices of methylated 1,5-anhydro-*L*-arabinitol acetates* (**1**, **2a–8a**).— Given in Table 3 are the LTPGLCRI [10] for compounds **1** and **2a–8a** determined on three different capillary columns [4], one (DB-5), a relatively nonpolar stationary phase (5% phenyl–95% methyl polysiloxane); one (DB-17),a more polar 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 eight compounds and a mixture of *n*-alkanes from $C_{11}H_{24}$ to $C_{26}H_{54}$ as retention index standards

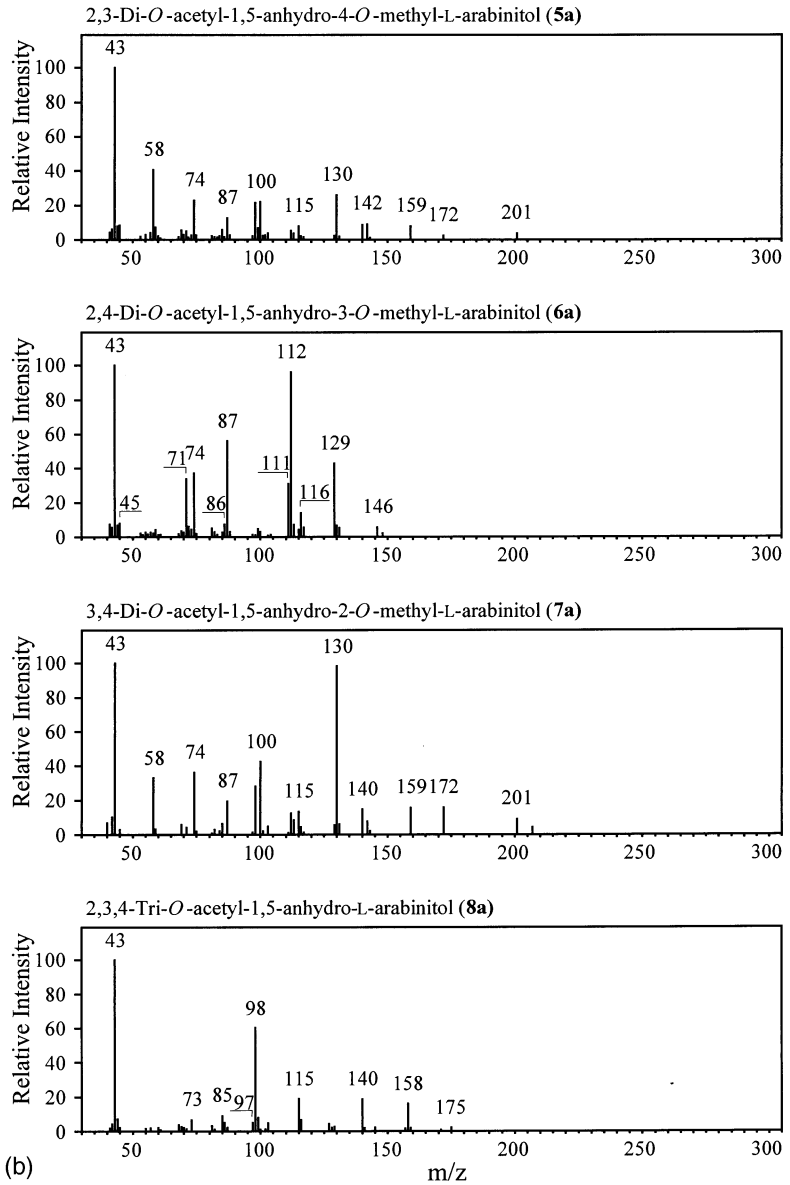


Fig. 1. (*Continued*)

[10]. All standard deviations were less than 0.1.

3. Discussion

This is one of a series of papers describing the synthesis and spectral characterization of authentic standards for the reductive-cleavage method. To expedite the preparation of these compounds, a simple, rapid method for their synthesis was needed. Moreover, a method of synthesis was needed wherein the ring form (furan, pyran) of the anhydroalditol product was not in question. As illustrated previously, these requirements are easily met by a strategy involving either partial methylation of the corresponding anhydroalditol and subsequent benzoylation [4] or partial benzoylation of the corresponding anhydroalditol followed by methylation [11]. In the present case, sequential partial methylation and benzoylation gave the required eight positional isomers, so the strategy involving reversal of methylation and benzoylation was not required.

For the benefit of those who use the data presented herein to establish the position(s) of linkage of arabinopyranosyl 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 readily distinguishable by mass spectrometry. This ambiguity arises because the sequence of O-acetyl and O-methyl groups on the tetrahydropyran ring is identical within each pair of isomers. Since the individual isomers within each pair are structurally different, however, they are readily distinguishable based on their LTPGLCRI (Table 3). Moreover, the compounds in question are also distinguishable by ^{1}H NMR spectroscopy of their benzoates (Table 2), so if such residues were encountered in an analysis, the products could be identified after isolation by HPLC [2].

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

Compound (position of acetyl)	Stationary phase		
	DB-5	DB-17	RT_x-200
1 (none)	1211.91[b]	1443.91	1404.29
4a (4-)	1328.09	1592.56	1594.02
3a (3-)	1335.53	1605.79	1615.06
2a (2-)	1349.58	1600.00	1645.97
7a (3,4-)	1462.65	1757.60	1859.53
6a (2,4-)	1473.87[c]	1766.72	1835.26
5a (2,3-)	1474.09[c]	1762.60	1878.98
8a (2,3,4-)	1578.67	1890.83	2062.25

[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.

[c] Retention index determined separately.

4. Experimental

General.—Reagents, solvents and materials were prepared as previously described [4]. 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 [4]. 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 and conditions were the same as previously described [4]. 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 ionization mass spectra and electron-ionization mass spectra were acquired as previously described [4]. ^{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-L-*arabinitol benzoates* (**2b–8b**).—A mixture of the seven positional isomers was prepared from 1,5-anhydro-L-arabinitol [5,6] by sequential partial methylation [12] and benzoylation in situ as previously described for the isomers of 1,5-anhydro-D-fucitol [4]. 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, these were dissolved in $CDCl_3$ and identified by ^{1}H NMR spectroscopy.

*Methylated 1,5-anhydro-*L*-arabinitol 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 [4] to afford the partially methylated 1,5-anhydro-L-arabinitol 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 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 tri-*O*-methyl derivative (**1**), whose position of elution was verified by chromatography of an independently synthesized sample.

*Determination of LTPGLCRI of methylated 1,5-anhydro-*L*-arabinitol 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. Typically, a 1.0-μL Hamilton syringe was loaded sequentially with 0.05 μL $CHCl_3$, 0.05 μL air, 0.1 μL alkane standard solution, 0.05 μL air, 0.5 μL methylated anhydroalditol acetate standard solution, and 0.1 μL air, then 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 were determined in triplicate on each of the columns using the equation given in Table 3.

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