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Authentic standards for the reductive-cleavage method. The positional isomers of partially methylated and acetylated or benzoylated 1,4-anhydro-D-xylitol

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Abstract

Described herein is a general method for the synthesis of all positional isomers of methylated and acetylated or benzoylated 1,4-anhydro-D-xylitol. The benzoates are generated simultaneously from 1,4-anhydro-D-xylitol by sequential partial methylation and benzoylation or sequential partial benzoylation and methylation. The individual isomers are obtained in pure form by high-performance liquid chromatography. Debenzoylation and acetylation provided the corresponding acetates. The ^{1}H NMR spectra of the benzoates and the electron ionization mass spectra of the acetates and the tri-*O*-methyl derivative are reported herein as are the linear temperature programmed gas–liquid chromatography retention indices of the acetates and the tri-*O*-methyl derivative on three different capillary columns.

Keywords: Reductive-cleavage method; 1,4-Anhydro-D-xylitol; Acetate esters; Benzoate esters

1. Introduction

The reductive-cleavage method is a very useful chemical method for determining the primary structure of polysaccharides and complex carbohydrates [1–3]. It is convenient, sensitive, broadly applicable, and capable of simultaneously identifying monosaccharide residues, their ring forms, and linkage position(s). In this method, reductive cleavage of glycosidic linkages in the fully methylated glycan is performed to generate partially

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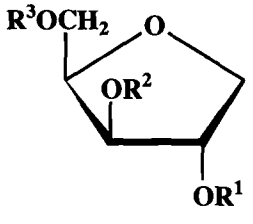
methylated anhydroalditols which are either converted to their benzoates, then separated by HPLC and characterized by ^{1}H NMR spectroscopy or to their acetates, which are separated by gas–liquid chromatography (GLC) and analyzed by electron-ionization (EI) and chemical-ionization (CI) mass spectrometry (MS). Unlike the former method, the latter one is much less laborious and much more sensitive, but depends upon the availability of authentic standards. To avoid the very laborious independent synthesis of authentic standards, as in past work [4–11], we have developed a method [12] by which all partially methylated and acetylated or benzoylated positional isomers of a given anhydroalditol can potentially be synthesized very efficiently. As previously described for the synthesis of the partially methylated and benzoylated or acetylated derivatives of 1,5-anhydro-D-fucitol, this method employs sequential partial methylation of the parent anhydroalditol and benzoylation [12]. The latter derivatives were then separated by HPLC and characterized by ^{1}H NMR spectroscopy; subsequent debenzoylation and acetylation afforded the corresponding acetates.

As described herein, the application of this method to the synthesis of authentic standards derivable from xylofuranose residues failed because one of the positional isomers could not be formed in the partial methylation reaction. In an effort to solve this problem, we explored a route involving reversal of the order of methylation and benzoylation; i.e., the parent anhydroalditol was subjected to partial benzoylation followed by total methylation. Indeed, the latter approach was successful. Therefore, reported herein are the ^{1}H NMR spectra of the seven methylated and benzoylated positional isomers of 1,4-anhydro-D-xylitol (**2b–8b**) and the El mass spectra and GLC retention indices of the corresponding acetates (**2a–8a**) and the tri-*O*-methyl derivative (**1**).



	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**) and tri-*O*-acetyl (**8a**) derivatives of 1,4-anhydro-D-xylitol were prepared from the latter by total methylation [13] and acetylation, respectively.

In an attempt to generate all the partially methylated positional isomers of 1,4-anhydro-D-xylitol, the parent anhydroalditol was deprotonated with 1.5 equiv of lithium methylsulfinylmethanide, and then excess methyl iodide was added [14]. A small portion of the reaction mixture was subjected to acetylation and the resulting *O*-acetyl derivatives were analyzed by GLC and GLC–CIMS (Fig. 1a). It was evident from these results that one of the di-*O*-acetyl-mono-*O*-methyl derivatives, later identified as the 3,5-di-*O*-acetyl-2-*O*-methyl derivative (**7a**), was not formed in this experiment.

In an attempt to derive the 3,5-di-*O*-acetyl-2-*O*-methyl derivative (**7a**), several procedures were explored in which the order of methylation and benzoylation was reversed. It was found that partial benzoylation of 1,4-anhydro-D-xylitol was best carried out in dry pyridine with 1.5 equiv of benzoyl chloride using *N*-methylimidazole as the catalyst. After workup and drying under high vacuum, the product was dissolved in dichloromethane and methylated fully by the addition of trimethyloxonium tetrafluoroborate [15]. A small portion of the mixture was subjected to debenzoylation then

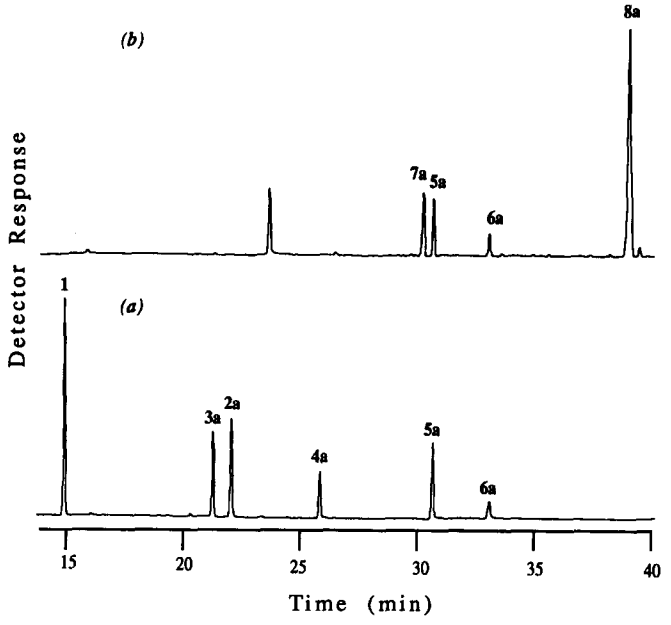


Fig. 1. Gas–liquid chromatogram of the partially methylated 1,4-anhydro-D-xylitol acetates derived from 1,4-anhydro-D-xylitol by sequential partial methylation and acetylation in situ (a), or by sequential partial benzoylation and methylation, then debenzoylation and acetylation (b). The peaks are numbered with the compound numbers. A J&W DB-5 fused silica capillary column (0.25 mm × 30 m, 0.25-μm film thickness) and a guard column (0.25 mm × 1 m) were used. The temperature of the column was programmed from 80°C to 210°C at 2°C/min, with no initial hold.

Table 1
Reversed-phase and normal-phase HPLC capacity factors for partially methylated 1,4-anhydro-D-xylitol benzoates **2b,8b**

Compound	Position of benzoate	Capacity factor (k') [a]	
		Reversed-phase [b]	Normal-phase [c]
2b	2-	2.03	3.07 [d]
3b	3-	1.73	
4b	5-	2.08	5.31 [d]
5b	2,3-	4.10	6.40 [e]
6b	2,5-	4.43	
7b	3,5-	4.10	5.25 [e]
8b	2,3,5-	5.27	

[a] Capacity factors (k') were calculated from the equation $k' = (t_r - t_o)/t_o$, where t_r is the absolute retention time of the compound of interest and t_o is the dead time (5.0 min for reversed-phase and 0.88 min for normal-phase) of the column. The dead time was calculated from the equation $t_o = (0.5\ L\ d_c^2)/F$, where 0.5 is a unitless constant, L is the length of the column in cm, d_c is the column diameter in cm, and F is the column flow rate in mL/min.

[b] Reversed-phase HPLC was conducted on a Rainin Dynamax semipreparative C_{18} column as described in the text.

[c] Normal-phase HPLC was conducted on a Regis Spherisorb silica column as described in the text.

[d] The column was eluted with 85:15 hexane–EtOAc.

[e] The column was eluted with 90:10 hexane–EtOAc.

acetylation and the *O*-acetyl derivatives were again analyzed by GLC and GLC–CIMS (Fig. 1b). In this experiment, the 3,5-di-*O*-acetyl-2-*O*-methyl derivative (**7a**) was present as a major component.

The mixtures of partially methylated 1,4-anhydro-D-xylitol benzoates derived by the two methods were fractionated by semipreparative reversed-phase HPLC using a Rainin C_{18} column. The individual components (see Table 1 for capacity factors) were collected and analyzed by ^{1}H NMR spectroscopy, which revealed that the 3-*O*-benzoyl (**3b**), 2,5-di-*O*-benzoyl (**6b**), and 2,3,5-tri-*O*-benzoyl (**8b**) derivatives were pure. However, the 2-*O*-benzoyl (**2b**) and 5-*O*-benzoyl (**4b**) derivatives were incompletely resolved, and the 2,3-di-*O*-benzoyl (**5b**) and 3,5-di-*O*-benzoyl (**7b**) derivatives were not resolved at all. The latter mixtures were separated by normal-phase HPLC using a Regis Spherisorb silica gel column (Table 1). A portion of each benzoate was then debenzoylated, and the product was acetylated to afford the partially methylated 1,4-anhydro-D-xylitol acetate in chromatographically pure form.

^{1}H NMR spectra of partially methylated 1,4-anhydro-D-xylitol benzoates (**2b–8b**).— Given in Table 2 are ^{1}H NMR spectral data which provide unique and unambiguous assignments for compounds **2b–8b**. The individual components of the mixture (see Table 1) were easily identified based upon a straightforward analysis of the chemical shifts and coupling constants of the ring hydrogen resonances. The positions of benzoyl groups were readily discerned by the large downfield chemical shift of the corresponding ring hydrogen resonances. The assignments were confirmed by NOE and two-dimensional COSY spectra. The NOE experiments were conducted by saturating at each of the H-1 protons and observing the intensity changes of the other H-1 and H-2 protons,

Table 2
^{1}H NMR spectral data (δ in ppm, J in Hz in brackets) for partially methylated 1,4-anhydro-D-xylitol benzoates **2b–8b** [a,b]

Compound	H-1β	H-1α	H-2 [c]	H-3	H-4 [c]	H-5	H-5′	O-Me
2b	4.35 dd	3.94 dd	5.48 dt	3.92 br d	4.24 dt	3.66 dd	3.61 dd	3.51, 3.42
	(4.8, 10.6)	(1.9, 10.7)	(1.2, 4.8)	(4.5)	(4.4, 7.1)	(4.6, 10.2)	(7.1, 10.1)	
3b	4.27 dd	3.81 dd	3.98 dd	5.50 d	4.31 dt	3.65 d	3.65 d	3.35, 3.50
	(5.4, 10.1)	(2.6, 10.1)	(2.6, 5.4)	(3.6)	(3.6, 5.8)	(5.8)	(5.8)	
4b	4.14 dd	3.82 dd	3.92 dt	3.84 br d	4.32 dt	4.61 dd	4.42 dd	3.43, 3.40
	(4.7, 10.0)	(1.9, 10.0)	(1.3, 4.6)	(4.1)	(4.2, 7.5)	(4.2, 11.6)	(7.5, 11.6)	
5b	4.54 dd	3.98 dd	5.54 dt	5.75 dd	4.48 dt	3.69 dd	3.66 dd	3.37
	(5.0, 10.7)	(2.5, 10.7)	(2.0, 4.9)	(1.3, 3.9)	(4.2, 6.7)	(4.5, 10.1)	(6.8, 10.1)	
6b	4.39 dd	4.01 dd	5.52 br d	4.03 d	4.43–4.47	4.65 dd	4.45–4.50	3.55
	(4.6, 10.6)	(1.2, 10.6)	(4.5)	(3.5)	(complex)	(2.9, 10.3)	(complex)	
7b	4.30 dd	3.89 dd	4.03 dd	5.61 br d	4.53 ddd	4.61 dd	4.59 dd	3.53
	(5.1, 10.0)	(2.3, 10.1)	(2.3, 5.1)	(3.6)	(3.6, 4.9, 7.0)	(7.0, 11.4)	(4.9, 11.4)	
8b	4.57 dd	4.05 dd	5.59 br dt	5.87 dd	4.70 dt	4.64 dd	4.61 dd	
	(4.9, 10.8)	(2.3, 10.9)	(2.3, 4.8)	(0.9, 4.1)	(4.1, 5.8)	(6.1, 11.6)	(5.6, 11.6)	

[a] Additional resonances were observed for benzoyl hydrogens at δ 7.40–8.09.
[b] Multiplicities include br, broad; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; dt, doublet of triplets.
[c] The resonance assigned as a doublet of triplets (dt) was actually a doublet of doublets of doublets (ddd), with a pair of coupling constants having nearly equal magnitude.

or by saturating at the H-2 proton and observing the intensity changes of the H-1 protons. In all cases, the H-1 β protons showed strong NOE interactions with both the H-2 and H-1 α protons, while the H-2 or H-1 α protons showed strong interactions only with the H-1 β protons.

*Mass spectra of the methylated 1,4-anhydro-*D*-xylitol acetates* (**1, 2a–8a**).—Compounds **1** and **2a–8a** were analyzed by GLC–CIMS and GLC–EIMS. The CI (ammonia) mass spectra of all compounds displayed the expected $(M + H)^+$ and $(M + NH_4)^+$ ions, which identifies them as anhydropentitol derivatives due to their unique molecular weights. Their EI mass spectra (Fig. 2) are characteristic of 1,4-anhydropentitol derivatives, because all spectra display either an (M − 45) peak or an (M − 73) peak, arising by loss [5,16] of the exocyclic CH_2OCH_3 or CH_2OAc group, respectively. The EI mass spectra of the positional isomers are also diagnostically different.

Some preliminary conclusions can be drawn that permit a distinction to be made among positional isomers. One of the major fragmentation pathways (see Scheme 1) for these derivatives begins with loss of the exocyclic methoxymethyl group (M − 45) or acetoxymethyl group (M − 73), to give fragment ions **9a** and **9b**, respectively. The further elimination of methanol or acetic acid from the (M − 45) ion (**9a**) gives rise to fragment ions **10a** or **11a** (M − 77) and **10b** or **11b** (M − 105), respectively, whereas the elimination or methanol or acetic acid from the (M − 73) ion (**9b**) gives rise to fragment ions **10b** or **11b** (M − 105) and **10c** or **11c** (M − 133), respectively. Some other key ions are those that arise from elimination of methanol (M − 32) or acetic acid (M − 60) from the molecular ion. It should be noted that there are other ions in the spectra of as yet unknown origin that are also diagnostic for identification of a particular positional isomer. One such example is the m/z 71 ion, which is always prominent in the spectra of 3-*O*-methyl derivatives, whereas the m/z 85 ion is always present very intensely in the spectra of 3-*O*-acetyl derivatives.

As summarized in Table 3, there was excellent correlation between the presence or absence of fragment ions at (M − 32), (M − 45), (M − 60), (M − 73), (M − 77), and (M − 105) and the positions of substitution of *O*-methyl and *O*-acetyl groups. The (M − 45) ion (**9a**) is present only in the mass spectra of 5-*O*-methyl derivatives, whereas the (M − 73) ion (**9b**) is always prominent in the spectra of 5-*O*-acetyl derivatives and the (M − 45) ion is absent. Mono-*O*-acetyl derivatives (**2a** and **3a**) that give an (M − 45) ion (**9a**) are readily distinguished by the presence or absence of ions at m/z 85 and m/z 127 (**10a**), which are prominent only in the 3-*O*-acetyl derivative (**3a**). The two di-*O*-acetyl regioisomers (**6a** and **7a**) that give an (M − 73) ion (**9b**) are also easily distinguished by the intensities of the ions at m/z 85 and m/z 127 (**10b**), which are prominent only in the 3,5-di-*O*-acetyl derivative (**7a**).

*GLC retention indices of methylated 1,4-anhydro-*D*-xylitol acetates* (**1, 2a–8a**).—GLC analysis based upon linear temperature programmed gas–liquid chromatography retention indices [17,18] (LTPGLCRI) values provides the most convenient way in which to routinely analyze carbohydrates when the reductive-cleavage method is used. Research in our laboratory has indicated that the use of three different types of columns is necessary in order to generate a unique set of LTPGLCRI values for each authentic standard [19] and that LTPGLCRI values are a much more accurate and reliable way to report retention data than relative retention time values [12,19–21]. Therefore, the

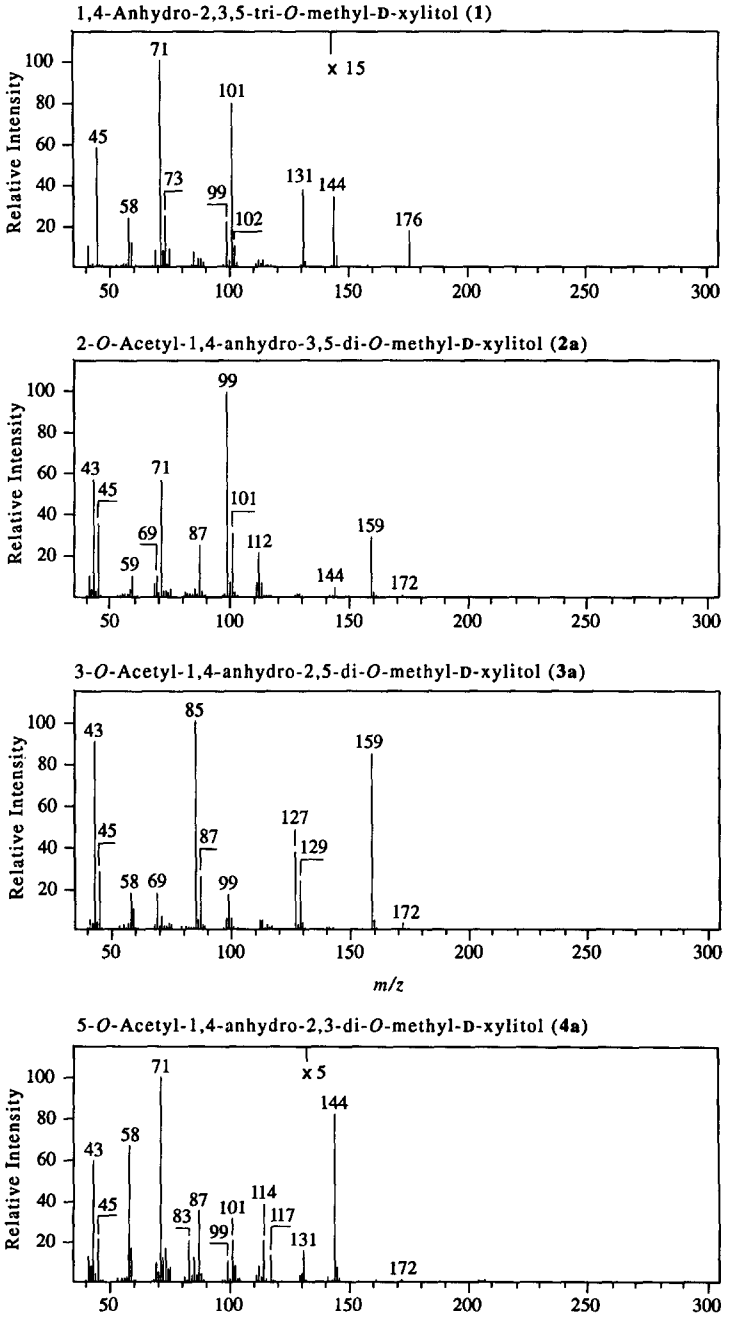


Fig. 2. Electron-ionization mass spectra of the methylated 1,4-anhydro-D-xylitol acetates (compounds **1** and **2a–8a**).

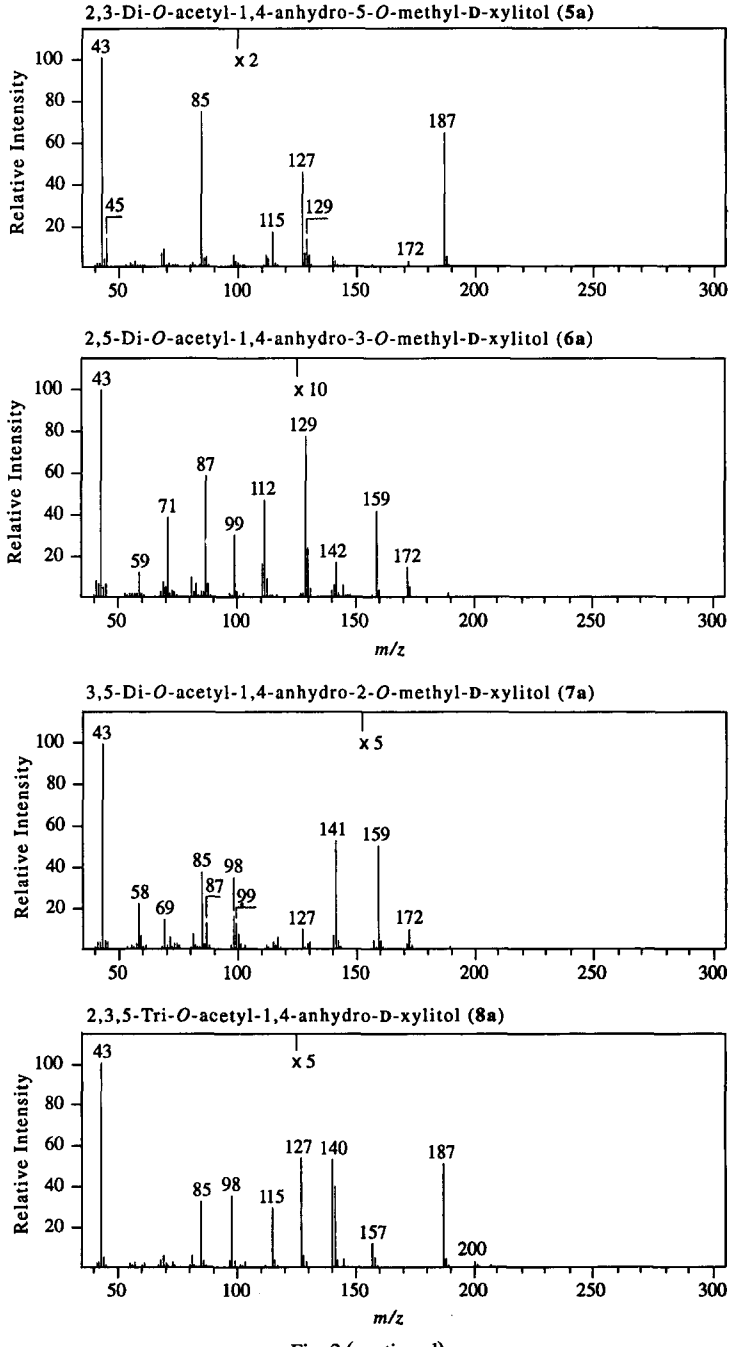
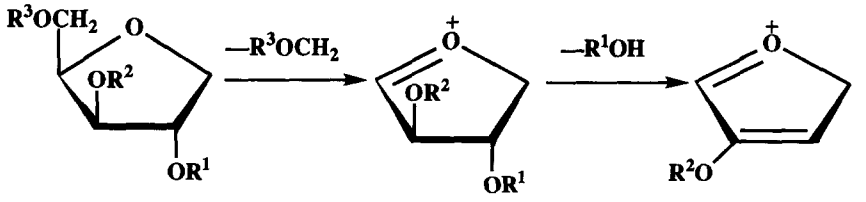
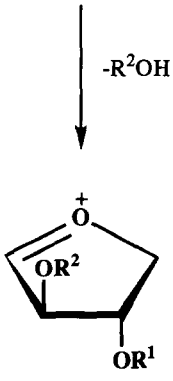


Fig. 2 (continued).



9a: M-45 (R^3=Me)
b: M-73 (R^3=Ac)

10a: M-77 (R^1=R^3=Me)
b: M-105 (R^1=Ac, R^3=Me or R^1=Me, R^3=Ac)
c: M-133 (R^1=R^3=Ac)



11a: M-77 (R^2=R^3=Me)
b: M-105 (R^2=Ac, R^3=Me or R^2=Me, R^3=Ac)
c: M-133 (R^2=R^3=Ac)

Scheme 1.

Table 3
Selected fragments observed in the electron-ionization mass spectra of compounds **1** and **2a–8a**

Compound [a]	Mol wt	(M−32)	(M−45)	(M−60)	(M−73)	(M−77)	(M−105)
1 (None)	176	+	+	− [b]	−	+	+ [c]
2a (2)	204	+	+	+	−	−	+
3a (3)	204	+	+	−	−	+	+
4a (5)	204	+	−	+	+	−	+
5a (2,3)	232	−	+	+	−	−	+
6a (2,5)	232	−	−	+	+	−	−
7a (3,5)	232	−	−	+	+	−	+
8a (2,3,5)	260	−	−	+	+	−	−

[a] Position of the *O*-acetyl group is in parenthesis.
[b] Not observed.
[c] Derived from a fragmentation pathway other than that depicted in Scheme 1.

Table 4
Linear temperature programmed gas–liquid chromatography retention indices (LTPGLCRI) of compounds **1** and **2a–8a** [a]

Compound	Stationary phase		
	DB-5	DB-17	RTx-200
1	1208.41 [b]	1415.37	1407.52
3a	1320.82	1548.61	1598.53
2a	1334.32	1562.33	1595.59
4a	1397.87	1654.80	1713.15
7a	1468.69	1735.64	1828.53
5a	1476.30	1745.22	1865.41
6a	1516.23	1793.91	1896.74
8a	1614.75	1919.85	2080.09

[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 $LTPGLCRI_{(x)} = 100n + [100 \cdot \Delta n \cdot (t_{R(x)} - t_{R(n)})/(t_{R(n+\Delta n)} - t_{R(n)})]$, 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 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), 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.

retention data for compounds **1** and **2a–8a** were obtained on DB-5 (5% phenyl–95% methyl polysiloxane), DB17 (50% phenyl–50% methyl polysiloxane), and RTx-200 (50% trifluoropropyl–50% methyl polysiloxane) capillary columns and their LTPGLCRI values were then calculated (Table 4).

3. Discussion

This work is part of a larger effort whose goal is to provide mass spectral and GLC retention data for authentic standards representing all possible combinations of position(s) of linkage and ring form for the most frequently encountered sugars. The specific goal of the present study was to derive those standards for all possible combinations of position(s) of linkage in D-xylofuranose residues. Even though xylofuranose residues are rare in nature, the availability of standards for such residues will allow them to be readily identified if encountered and will lessen the chances that products arising from such residues will be incorrectly identified. In order to derive such standards, we previously reported [12] a general procedure wherein the parent anhydroalditol was subjected in sequence to partial methylation and benzoylation. However, application of this procedure in the present case failed to give one of the positional isomers (**7a**), presumably, due to substantial differences in reactivities of the hydroxyl groups. If this were indeed the case, we surmised that reversal of the order of methylation and benzoylation, i.e., partial benzoylation followed by complete methylation, should give rise to those positional isomers not formed by the published [12] procedure. The parent anhydroalditol was therefore subjected to benzoylation using limiting reagents [22–24],

and the products were methylated fully by treatment with trimethyloxonium tetrafluoroborate in dichloromethane [15]. Debenzoylation followed by acetylation gave the corresponding acetates, which were analyzed by GLC–MS and found to contain compound **7a** as the most abundant di-*O*-acetyl component. Indeed, this procedure was successful, and we now believe that it should be possible to prepare all partially methylated and acetylated positional isomers of a given anhydroalditol using a combination of these two procedures.

4. Experimental

General.—Dimethyl sulfoxide (Me_2SO) was distilled over Drierite or calcium hydride under vacuum and stored over 3 Å molecular sieves. Iodomethane was fractionally distilled and stored over copper powder at 3°C under nitrogen. Pyridine was distilled over NaOH under vacuum and stored over calcium hydride. Lithium methylsulfinylmethanide, prepared as previously described [10], was standardized [25] and stored at 0°C in 2-mL aliquots in 4-mL screwcap vials fitted with Teflon septa. Acetic anhydride and *N*-methylimidazole were separately distilled and stored over 4 Å molecular sieves under nitrogen at room temperature. Benzoic anhydride was dissolved in $CHCl_3$, the solution was extracted with aq $NaHCO_3$, the $CHCl_3$ layer was evaporated to dryness under vacuum, and the product was recrystallized from petroleum ether. Reagent grade MeOH was refluxed over magnesium methoxide, distilled, and stored at room temperature over 3 Å molecular sieves under N_2. Dichloromethane was refluxed with lithium aluminum hydride and freshly distilled before use. Chloroform (EM Science), D-xylose (Sigma), benzoyl chloride, and trimethyloxonium tetrafluoroborate (Aldrich) were used as obtained without further purification. Dowex-50 (H^+) cation-exchange resin was washed with MeOH, charged by elution with M HCl, then thoroughly rinsed with distilled water.

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.

All HPLC solvents were HPLC grade and were filtered through 0.20-μm pore size membrane filters before use.

Instrumentation.—HPLC was performed using a Beckman model 338 System Gold chromatograph. Reversed-phase HPLC was conducted on a 5-μm particle-size Rainin Dynamax Microsorb Semi-preparative C_{18} column (1 × 25 cm) connected to a guard column (1 × 5 cm) having the same packing. Normal-phase HPLC was performed on a 5-μm particle-size Regis Spherisorb silica column (4.6 mm × 25 cm). Both systems were fitted with a 2.0-μm pore-size stainless steel inline filter frit installed between the solvent mixing chamber and the injector, and a 0.50-μm pore-size stainless steel filter frit installed between the injector and the guard column or the silica column. Chromatography was conducted at a flow rate of 3 mL/min.

Medium-pressure liquid chromatography (MPLC) involved a Rheodyne 7125 injector, an Eldex model B-100-S4 pump, a Scientific Systems Model LP-21 pulse dampener, and a glass column (1.0 × 25 cm) packed with 60 Å silica gel (J.T. Baker, 60–200 mesh). Hexane–ethyl acetate mixtures (70:30 and 85:15) were used as mobile phases.

Gas–liquid chromatograms were obtained using a Hewlett–Packard 5890 gas–liquid chromatograph using the same columns and conditions as previously described [12]. 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–liquid chromatograph and a Digital Equipment Corporation model 2100 workstation. EI mass spectra and CI mass spectra with ammonia as the reagent gas were acquired under the same conditions used previously [12].

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

*Partially methylated 1,4-anhydro-*D*-xylitol benzoates* (**2b–8b**).—1,4-Anhydro-D-xylitol was prepared from D-xylose by the method of Heard et al. [26], and the product was purified as its tribenzoate by MPLC. Debenzoylation (NaOMe–MeOH) afforded the pure anhydroalditol, which was dried under high vacuum before use.

I. Partial methylation followed by benzoylation in situ [12].—1,4-Anhydro-D-xylitol (310 mg) was dissolved in 6 mL of dry Me_2SO in a flame-dried 10-mL conical flask. Two 2-mL aliquots of this solution were removed and added separately to flame-dried 10-mL conical flasks. To each reaction was added 0.7, 1.5, and 2.1 equiv, respectively, of lithium methylsulfinylmethanide, then, after stirring for 120 min, iodomethane (0.15 mL for the first two and 0.2 mL for the third, respectively) was added to each reaction. After stirring for an additional hour, excess CH_3I was removed by blowing dry nitrogen gas above the reaction solution for 30 min. A portion (ca. 10%) of each reaction mixture was saved, and to the remainder of each was added 1.0 mL of dry pyridine, 1.5 g of benzoic anhydride, and 0.2 mL of *N*-methylimidazole. After stirring 30 min, 1 mL of water was added, and the mixture was vigorously stirred overnight to ensure total hydrolysis of excess benzoic anhydride. The reaction mixtures were combined, diluted with 75 mL of deionized water, and extracted with chloroform (3×25 mL). The organic extracts were combined and extracted three times each with 15-mL portions of satd aq $NaHCO_3$, 2 M H_2SO_4, and deionized water. The organic layer was dried (anhyd Na_2SO_4) and then concentrated under vacuum to a syrup. The residue was dissolved in MeCN and filtered through a 0.2-μm pore-size Acrodisc into a 4-mL screwcap vial fitted with a Teflon liner.

II. Partial benzoylation followed by methylation.—After drying under high vacuum in a flame-dried 50-mL conical flask for 6 days, 53.6 mg of 1,4-anhydro-D-xylitol was dissolved in 2 mL of dry pyridine, and then 70 μL (1.5 equiv) of benzoyl chloride and 40 μL of *N*-methylimidazole were added. After stirring for 85 min, cold satd aq $NaHCO_3$ ($\sim$ 5 mL) was added, and the reaction mixture was stirred vigorously overnight. The reaction solution was evaporated to dryness under vacuum to yield a white solid that was partitioned between dichloromethane (15 mL) and water (15 mL). The organic layer was extracted three times with distilled water, dried (anhyd Na_2SO_4), and concentrated to a clear syrup. After drying under high vacuum for 6 days, the syrup was dissolved in 2 mL of dichloromethane, and 118.3 mg of $BF_4 \cdot OMe_3$ (2 equiv) was then added. After stirring vigorously overnight, 2 mL of MeOH was added, the reaction solution was stirred for 15 min, and was then evaporated under vacuum to give a mixture of partially methylated 1,4-anhydro-D-xylitol benzoates as a clear syrup. A small portion of this mixture was debenzoylated (NaOMe in MeOH), and the product was

acetylated with acetic anhydride and *N*-methylimidazole to yield a mixture of partially methylated 1,4-anhydro-D-xylitol acetates, which was analyzed by GLC and GLC–MS.

Separation of the above mixtures of benzoates (**2b–8b**) was accomplished by reversed-phase HPLC (see Table 1) using a semipreparative C_{18} column. Aliquots (20 μL) of the mixtures were applied to the column, which was equilibrated in 50:50 MeCN–H_2O. After injection, the column was eluted for 10 min with 50:50 MeCN–H_2O, followed by a linear gradient to 95:5 MeCN–H_2O over 20 min. The individual components from 12 or more applications were collected, combined and, after removal of the solvent by evaporation under vacuum, were dissolved in $CDCl_3$ and identified by ^1H NMR spectroscopy.

*Methylated 1,4-anhydro-*D*-xylitol acetates* (**1, 2a–8a**).—About one-third of each pure benzoate, obtained as described above, was subjected to debenzoylation (NaOMe, MeOH), then acetylation as previously described [12]. The pure standards so obtained, and the tri-*O*-methyl derivative **1** synthesized independently, were then chromatographed individually on the three aforementioned GLC columns under the conditions already described [12], except that the oven temperature was programmed from 80–200°C at 6°C/min. In this way, the relative orders of elution of the standards on each column were determined.

*Determination of LTPGLCRI values of methylated 1,4-anhydro-*D*-xylitol acetates* (**1, 2a–8a**).—In order to expedite acquisition of their mass spectra and retention time data and to be sure that the mixture of standards contained only the title compounds, further studies used a mixture prepared by combining aliquots of the individual pure standards in a way that the integral of the area (flame ionization detection) of each component was at least 50% of the area of the most abundant component. The methylated anhydroalditol acetate standard solution was then co-injected with the stock solution of *n*-alkanes from $C_{11}H_{24}$ to $C_{26}H_{54}$ in such a way that their area responses were comparable [12]. The LTPGLCRI values were determined in triplicate on each of the columns using the equation depicted in Table 4. All standard deviations were less than 0.1.

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