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Synthesis and characterization of authentic standards for the reductive-cleavage method. The positional isomers of partially methylated and acetylated or benzoylated 1,4-anhydro-L-fucitol

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Abstract

Described herein is the synthesis of all positional isomers of methylated and acetylated or benzoylated 1,4-anhydro-L-fucitol. The benzoates are generated simultaneously from 1,4-anhydro-L-fucitol 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: L-Fucitol, 1,4-anhydro-; Methylated derivatives; Acylated derivatives; Reductive-cleavage

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 based upon methylation analysis [4–6] 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, retain the ring form of their

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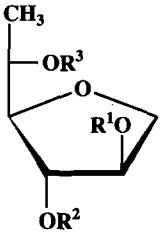


	R^1	R^2	R^3
1	Me	Me	Me
2a	Ac	Me	Me
2b	Bz	Me	Me
3a	Me	Ac	Me
3b	Me	Bz	Me
4a	Me	Me	Ac
4b	Me	Me	Bz
5a	Ac	Ac	Me
5b	Bz	Bz	Me
6a	Ac	Me	Ac
6b	Bz	Me	Bz
7a	Me	Ac	Ac
7b	Me	Bz	Bz
8a	Ac	Ac	Ac
8b	Bz	Bz	Bz

parent sugar residues, and subsequent acetylation then marks the linkage positions. The partially methylated anhydroalditol acetates so obtained are identified by comparison of their GLC retention indices and chemical-ionization (CI) and electron-ionization (EI) mass spectra [7–13] to those of authentic standards. The independent synthesis of each authentic standard is very time consuming and laborious, however, so we have recently developed two efficient procedures for their synthesis [14,15]. Described herein is the application of these procedures to the synthesis of all products derivable from L-fucofuranosyl residues, namely the positional isomers of methylated and acetylated or benzoylated 1,4-anhydro-L-fucitol. Even though such residues are rare in nature, the availability of standards will allow them to be readily identified if encountered and will lessen the chances that products arising from them will be incorrectly identified. As an aid to those who wish to use the reductive-cleavage method, ^{1}H NMR spectra of the seven methylated and benzoylated positional isomers of 1,4-anhydro-L-fucitol (**2b–8b**) are reported, as are the EI mass spectra and the GLC retention indices of the corresponding acetates (**2a–8a**) and the tri-*O*-methyl derivative (**1**).

2. Results and discussion

Synthesis.—The tri-*O*-methyl (**1**), tri-*O*-acetyl (**8a**), and tri-*O*-benzoyl (**8b**) derivatives of 1,4-anhydro-L-fucitol were prepared from the latter by total methylation [16], acetylation, and benzoylation, respectively.

The partially methylated and benzoylated positional isomers (**2b–7b**) were prepared from 1,4-anhydro-L-fucitol by sequential partial methylation [17] (with 1.5 equiv of lithium methylsulfinylmethanide) and benzoylation in situ [14], or by sequential partial benzoylation (with 1.5 equiv of benzoyl chloride) and methylation [15,18]. A small portion of the reaction mixture generated by the former method was subjected to acetylation prior to benzoylation, and a small portion of the benzoate mixture generated by the latter method was subjected to debenzoylation and acetylation and the resulting *O*-acetyl derivatives were analyzed by GLC and GLC–CIMS (see Figs. 1a and 1b, respectively). It was evident from these results that one of the di-*O*-acetyl-mono-*O*-methyl derivatives, later identified as the 2,5-di-*O*-acetyl-3-*O*-methyl derivative (**6a**) was not

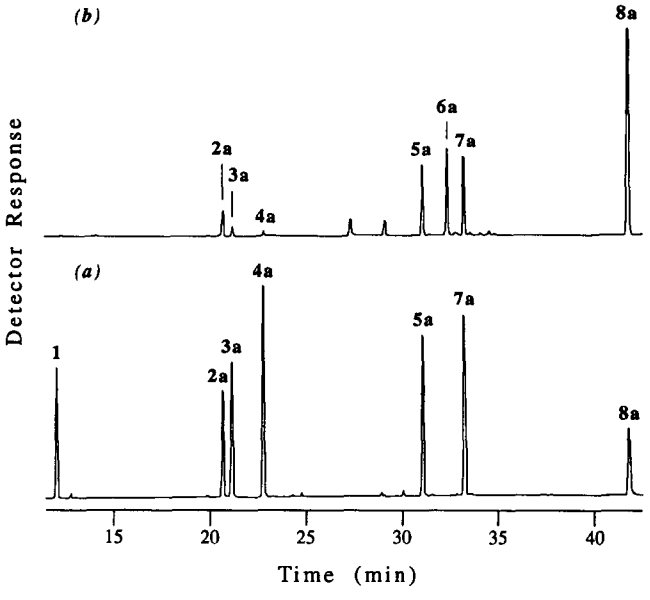


Fig. 1. Gas–liquid chromatogram of the partially methylated 1,4-anhydro-L-fucitol acetates derived from 1,4-anhydro-L-fucitol by sequential partial methylation and acetylation in situ (*a*), or by sequential partial benzoylation and methylation, then debenzoylation and acetylation (*b*). The peaks are numbered with the compound numbers. A Restek RTx-200 fused silica gel 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 to 210°C at 2°C/min, with no initial hold.

formed by the former method (Fig. 1a). However, compound **6a** was formed as a major component by the latter method (Fig. 1b).

The mixtures of partially methylated 1,4-anhydro-L-fucitol 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 2,5-di-*O*-benzoyl (**6b**) and 3,5-di-*O*-benzoyl (**7b**) derivatives were incompletely resolved. The **6b–7b** mixture was therefore 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-L-fucitol acetate in chromatographically pure form.

^{1}H NMR spectra of partially methylated 1,4-anhydro-L-fucitol 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 selective decoupling, NOE, and two-dimensional COSY experiments.

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

Compound	Position of benzoate	Capacity factor (k') [a]	
		Reversed-phase [b]	Normal-phase [c]
2b	2-	2.60	
3b	3-	2.47	4.02 [d]
4b	5-	2.79	4.49 [e]
5b	2,3-	4.58	5.17 [e]
6b	2,5-	4.71	3.18 [e]
7b	3,5-	4.77	3.92 [e]
8b	2,3,5-	5.59	4.49 [e]

[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 gel 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.

Mass spectra of the methylated 1,4-anhydro-L-fucitol acetates (**1, 2a–8a**).—Compounds **1** and **2a–8a** were analyzed by GLC–CIMS and EIMS. The CI (ammonia) mass spectra of all compounds displayed the expected $(M + H)^+$ and $(M + NH_4)^+$ ions, which identifies them as deoxyanhydrohexitol derivatives due to their unique molecular weights. Their EI mass spectra (Fig. 2) are characteristic of 6-deoxy-1,4-anhydrohexitol derivatives, because all spectra display either an $(M - 59)^+$ peak or an $(M - 87)^+$ peak, arising by loss [19,20] of the exocyclic CH_3CHOCH_3 or CH_3CHOAc group, respectively. Inspection of the EI mass spectra reveals diagnostic differences for the positional isomers in terms of the presence or absence of certain ions and their intensity relative to the base peak. Quite interestingly, the base peak is at m/z 43 ($CH_3C{\equiv}O^+$) for all 5-*O*-acetyl derivatives, or at m/z 59, $(CH_3O{=}CHCH_3)^+$, for all 5-*O*-methyl derivatives.

The fragmentation patterns for derivatives of this type have not been established, but 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 methoxyethyl group (M − 59) or acetoxyethyl group (M − 87) to give fragment ions **9a** and **9b**, respectively. The further elimination of methanol or acetic acid from the (M − 59) ion (**9a**) gives rise to fragment ions **10a** or **11a** (M − 91) and **10b** or **11b** (M − 119), respectively, whereas the elimination of methanol or acetic acid from the (M − 87) ion (**9b**) gives rise to fragment ions **10b** or **11b** (M − 119) and **10c** or **11c** (M − 147), 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

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

Compound	H-1α	H-1β	H-2	H-3	H-4 [c]	H-5 [c]	H-6	O-Me
2b	4.14 *br d* (11.0)	4.06 *dd* (4.1, 11.0)	5.37 *br d* (4.0)	3.80 *br d* (4.9)	3.72 *t* (5.3)	3.55 *quint* (6.3)	1.23 *d* (6.3)	3.51, 3.42
3b	4.08 *br d* (9.8)	3.96 *dd* (4.4, 9.8)	3.94 *br d* (4.6)	5.37 *br d* (3.8)	3.90 *dd* (4.1, 6.0)	3.62 *quint* (6.4)	1.23 *d* (6.4)	3.44, 3.46
4b	4.04 *br d* (10.1)	3.89 *dd* (4.6, 10.1)	3.82–3.86 (complex)	3.68 *br d* (4.1)	3.82–3.86 (complex)	5.35 *quint* (6.4)	1.42 *d* (6.4)	3.31, 3.41
5b	4.21 *dd* (2.1, 10.9)	4.25 *dd* (4.5, 10.9)	5.54 *dt* (2.0, 4.4)	5.62 *dd* (1.2, 4.8)	3.95 *t* (4.8)	3.73 *dq* (4.8, 6.4)	1.31 *d* (6.4)	3.47
6b	4.18 *br d* (11.0)	4.12 *dd* (4.2, 10.9)	5.40 *br d* (4.1)	3.87 *br d* (4.6)	3.90 *t* (4.9)	5.43 *quint* (6.4)	1.46 *d* (6.5)	3.52
7b	4.13 *dd* [d] (1.6, 10.2)	4.60 *dd* (4.6, 10.1)	4.00 *br d* (4.6)	5.43 *br d* (3.2)	4.15 *dd* [d] (3.2, 5.8)	5.51 *quint* (6.4)	1.48 *d* (6.4)	3.42
8b	4.26 *dd* (1.8, 10.8)	4.34 *dd* (4.9, 10.8)	5.60 *dt* (2.3, 4.6)	5.62–5.66 complex	4.20 *t* (4.7)	5.62–5.66 complex	1.54 *d* (6.6)	

[a] Additional resonances were observed for benzoyl hydrogens at δ 7.30–7.8.
[b] Multiplicities include *br*, broad; *d*, doublet; *dd*, doublet of doublets; *t*, triplet; *dq*, doublet of quartets; *quint*, quintet.
[c] The resonances assigned as a triplet (*t*) and quintet (*quint*) were actually a doublet of doublets (*dd*) and a doublet of quartets (*dq*), respectively, with a pair of coupling constants having nearly equal magnitude.
[d] Resonance partially obscured.

yet unknown origin that are also diagnostic for identification of a particular positional isomer. For example, the m/z 71 ion is always prominent in the spectra of 3-*O*-methyl derivatives whereas the m/z 85 ion is always prominent in the spectra of 3-*O*-acetyl derivatives. The same correlations have been made for the partially methylated and acetylated derivatives of 1,4-anhydro-D-xylitol [15].

Summarized in Table 3 are the results obtained from a comparison of the relative intensities of the aforementioned fragment ions. The values listed provide an indication of the relative prominence of a given ion and on this basis they are useful for distinguishing between the various positional isomers. The (M − 59) ion (**9a**) is always prominent in the mass spectra of 5-*O*-methyl derivatives, and the m/z 59 ion is the base peak. In contrast, the (M − 87) ion (**9b**) is prominent only in the spectra of 5-*O*-acetyl derivatives and the m/z 43 ion is the base peak. Mono-*O*-acetyl derivatives (**2a** and **3a**) that give an (M − 59) ion (**9a**) are readily distinguished by the relative intensities of the ions at m/z 159 (**9a**), m/z 127 (**10a** or **11a**), m/z 99 (**10b** or **11b**), and m/z 85, which are much more prominent in the spectrum of the 3-*O*-acetyl derivative (**3a**). The two di-*O*-acetyl regioisomers (**6a** and **7a**) that give an (M − 87) ion (**9b**) are also easily distinguished by the intensities of the ions at m/z 159 (**9b**), m/z 127 (**10b** or **11b**), and m/z 85, which, again, are more prominent in the spectrum of the 3,5-di-*O*-acetyl derivative (**7a**).

GLC retention indices of methylated 1,4-anhydro-L-fucitol acetates (**1, 2a–8a**).—GLC analysis based upon linear temperature-programmed gas–liquid chromatography retention indices [21,22] (LTPGLCRI) values provides the most convenient way in which to

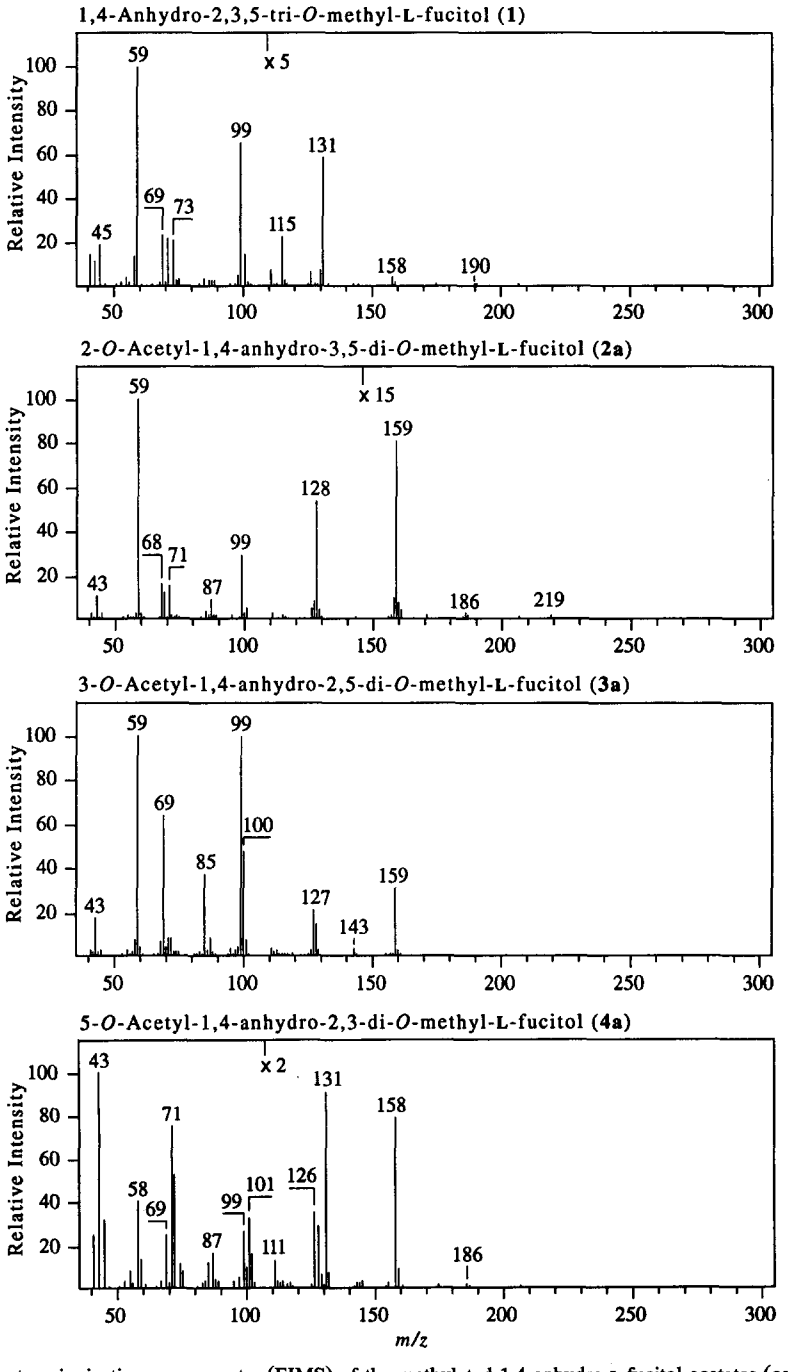


Fig. 2. Electron-ionization mass spectra (EIMS) of the methylated 1,4-anhydro-L-fucitol acetates (compounds **1** and **2a–8a**).

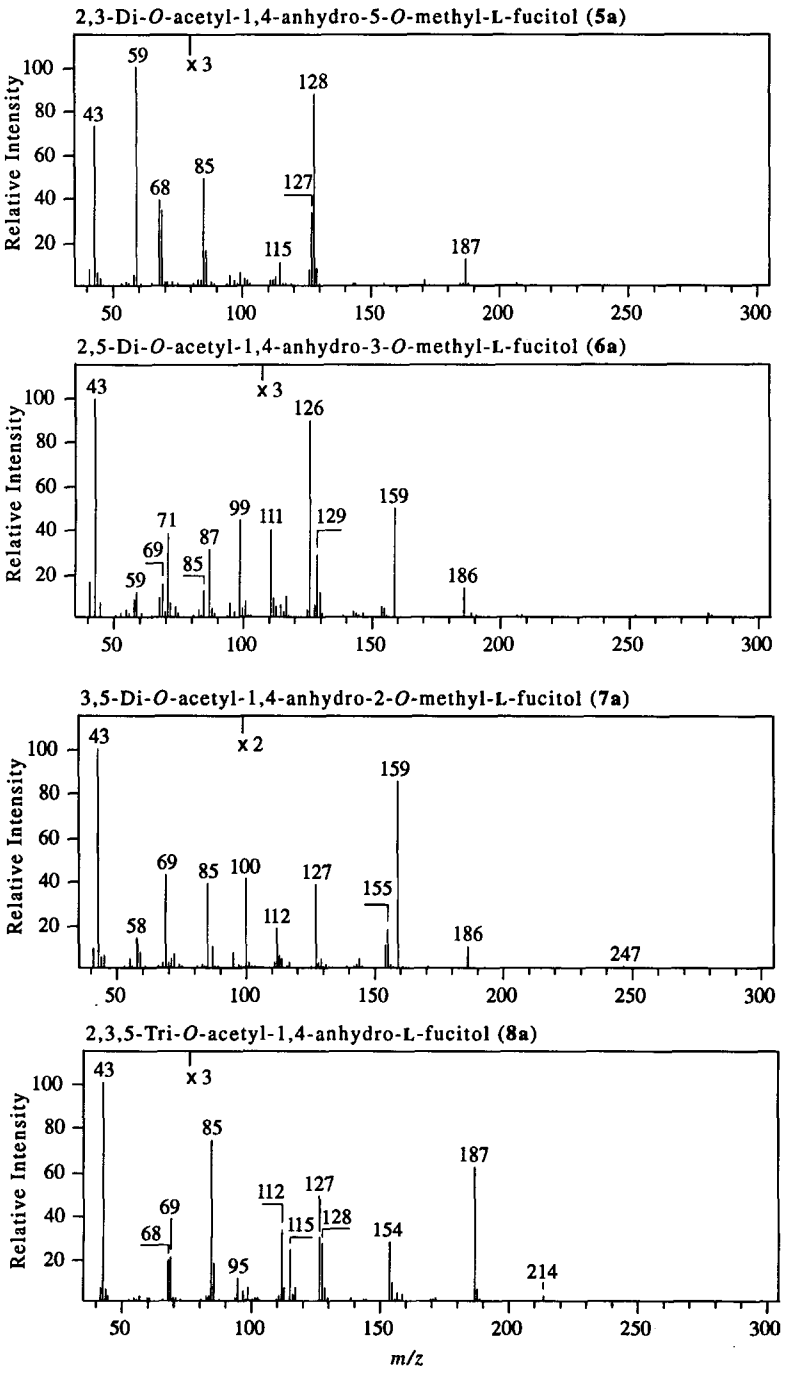
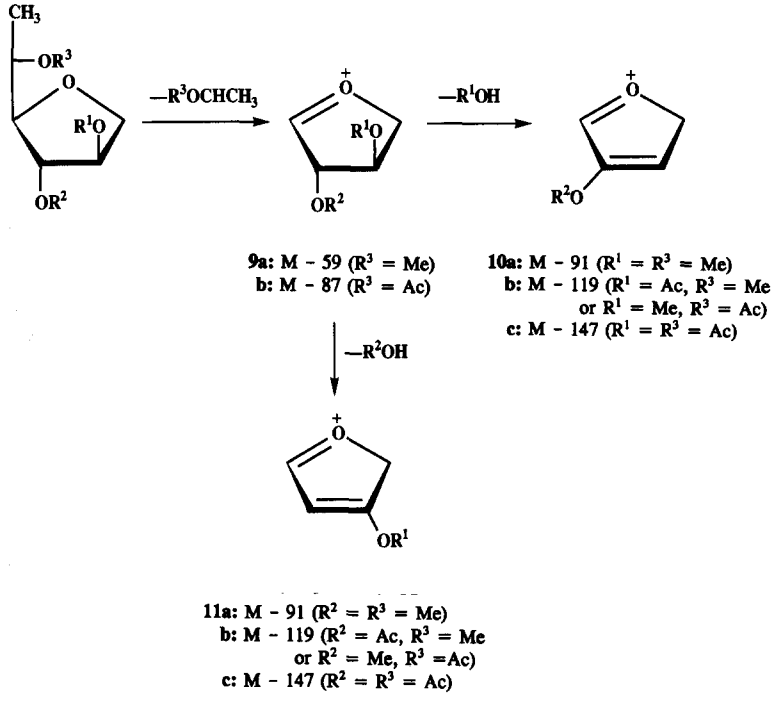


Fig. 2 (continued).



Scheme 1.

routinely analyze carbohydrates when the reductive-cleavage method is used. Research in our laboratory has indicated that the use of three different types of capillary columns is necessary in order to generate a unique set of LTPGLCRI values for each authentic standard, and that LTPGLCRI values are a much more accurate and reliable way to report retention data than relative retention time values [14,23–25]. Therefore, the retention data for compounds **1** and **2a–8a** were obtained on DB-5, DB-17, and RTx-200 capillary columns under the conditions reported previously [14], and their LTPGLCRI values were then calculated (Table 4). In all cases, the standard deviations were less than 0.1 units, which corresponds to an absolute retention time difference of less than 0.3 s.

3. Experimental

General.—Reagents and HPLC solvents were purified and stored as previously described [15]. L-Fucose (Pfanstiehl) was used as obtained.

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 semipreparative 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 gel column (4.6 mm × 25 cm). Both systems

Table 3
Intensity (relative to base peak) of selected fragments observed in the electron-ionization mass spectra of compounds **1** and **2a–8a**

Compound [a]	Molecular weight	Relative intensity (%) [b]					
		M−32	M−59	M−60	M−87	M−91	M−119
1 (None)	190	0.8	11.7	1.4	— [d]	65.1	21.0 [e]
2a (2)	218	0.1	5.4	0.6	—	8.0	28.9
3a (3)	218	0.3	30.7	1.0	—	20.2	100
4a (5)	218	0.8	4.3 [c]	39.7	45.3	—	26.2
5a (2,3)	246	—	4.0	0.2	—	—	10.9
6a (2,5)	246	—	—	4.4	16.4	1.4	—
7a (3,5)	246	—	—	4.7	42.4	8.6 [e]	19.3
8a (2,3,5)	274	—	—	0.7	20.9	—	2.8 [f]

[a] Position of *O*-acetyl group is in parenthesis.
[b] Actual intensities may vary depending on the instrument used, but observed values are given to provide an indication of their relative prominence.
[c] Isotope peak of m/z 158.
[d] Not observed.
[e] Derived from a fragmentation pathway other than depicted in Scheme 1.
[f] Isotope peak of m/z 154.

were fitted with a 2.0-μm pore-size stainless steel in-line 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.

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

Compound [b]	Stationary Phase		
	DB-5	DB-17	RTx-200
1 (None)	1229.98 [c]	1429.92	1390.87
2a (2)	1353.56	1573.91	1587.85
3a (3)	1355.88	1589.77	1598.06
4a (5)	1372.13	1611.93	1632.35
5a (2,3)	1484.40	1754.65	1804.62
6a (2,5)	1492.78	1751.63	1831.70
7a (3,5)	1502.49	1772.63	1849.94
8a (2,3,5)	1617.58	1915.85	2036.58

[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] Position of *O*-acetyl group is in parenthesis.
[c] Values are listed according to increasing retention index on the DB-5 column.

Gas–liquid chromatograms were obtained using a Hewlett–Packard 5890 gas–liquid chromatograph using the same columns and conditions as previously described [14]. 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 [14].

^{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-L-fucitol benzoates (**2b–8b**).—1,4-Anhydro-L-fucitol was prepared from L-fucose by the method of Heard et al. [26] and dried under high vacuum before use.

I. Partial methylation, followed by in situ benzoylation [14].—1,4-Anhydro-L-fucitol (212 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 0.75, 1.5, and 2.5 equiv, respectively, of lithium methylsulfinylmethanide, then, after stirring for 60 min, iodomethane (0.2 mL) was added to each reaction. After stirring for an additional 4 h, 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 for 45 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 and processed as previously described [15].

II. Partial benzoylation followed by methylation [15].—After drying under high vacuum in a flame-dried 50-mL conical flask for 6 days, 50.1 mg of 1,4-anhydro-L-fucitol was dissolved in 2 mL of dry pyridine (stored over CaH_2), and then 58.9 μL (1.5 equiv) of benzoyl chloride and 40 μL of *N*-methylimidazole were added. After stirring for 85 min, cold satd aq $NaHCO_3$ (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 which was partitioned between dichloromethane and water (15 mL each). The organic layer was extracted three times with deionized 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 104.4 mg of $BF_4 \cdot OMe_3$ (2 equiv) was then added. After stirring vigorously overnight, 2 mL of MeOH was added, the reaction mixture was stirred for 15 min, and was then evaporated under vacuum to give a mixture of partially methylated 1,4-anhydro-L-fucitol benzoates as a clear syrup.

Before HPLC separation the mixtures were dissolved in acetonitrile, passed through a Waters Sep-Pak® Vac RC (500 mg) C_{18} cartridge, and then filtered through a 0.2-μm pore-size Acrodisc into a 4-mL screwcap vial fitted with a Teflon liner. Separation of the 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 16 or more applications

were collected, combined and, after removal of solvent by evaporation under vacuum, were dissolved in $CDCl_3$ and identified by ^1H NMR spectroscopy.

*Methylated 1,4-anhydro-*L*-fucitol 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 [14]. The pure standards so obtained and the tri-*O*-methyl derivative (**1**), which was synthesized independently, were then chromatographed individually on the three aforementioned GLC columns under the conditions already described [14,15]. In this way, the relative order of elution of the standards on each column was determined.

*Determination of LTPGLCRI values of methylated 1,4-anhydro-*L*-fucitol 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 [14]. The methylated anhydroalditol acetate standard solution was 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. The LTPGLCRI values were determined in triplicate on each of the columns using the equation depicted in Table 4.

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