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Synthesis, separation and NMR spectral analysis of methyl apiofuranosides

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Abstract

Methyl apiofuranosides were prepared by methanolysis of D-apiose. Four methyl apiofuranosides were separated by ion-exclusion and normal phase chromatography and their structures were characterized by ^{1}H and ^{13}C NMR spectroscopy. The composition of methyl apiofuranosides was almost the same as that of D-apiose in D_2O.

Keywords: Apiose; 3-*C*-(Hydroxymethyl)-D-*glycero*-aldotetrose; Methyl apiofuranosides

1. Introduction

The branched-chain aldopentose, D-apiose [3-*C*-(hydroxymethyl)-D-*glycero*-aldotetrose], plays an integral role in the biochemistry of plants [1]. It is one of the components of rhamnogalacturonan II (RG-II) in plant cell walls [2]. Recent studies have revealed that RG-II combines with boron (B) to form a borate diol di-ester (RG-II–borate complex) [3–7]. Glycosyl-linkage analysis indicated that the apiosyl residues of RG-II were the sites of borate esterification [5,7,8]. However, determination of the borate-binding apiosyl residues in the RG-II–B complex is complicated by the fact that the RG-II–B complex contains four apiosyl residues. The borate–apiose (1:2) esters can exist in either of two diastereoisometers [bis(β-D-apiofuranosyl)-(*R*)-2,3:2,3 and -(*S*)-2,3:2,3-borate] since they contain a chiral boron [5]. Since the chirally induced chemical shift differences of ^{11}B NMR spectra are smaller than the linewidth of the ^{11}B signals, it is difficult to distinguish diastereoisomers by ^{11}B NMR spectroscopy. The ^{1}H and ^{13}C spectra of the RG-II–B complex are too complicated to assign.

Snyder and Serianni [9] synthesized stable isotope-labeled DL-apioses and characterized the tautomeric mixture in water solution by NMR spectroscopy. We prepared methyl apiofuranosides as model compounds to study the stereochemistry of the borate–apiose complex. We now report the separation of four methyl apiofuranosides by ion-exclusion and normal phase chromatography and their characterization by ^{1}H and ^{13}C NMR spectroscopy.

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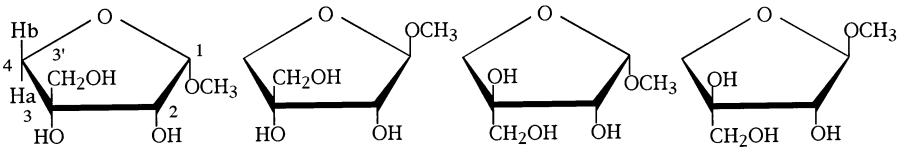

1 2 3 4

Fig. 1. Structures of compounds **1**–**4**.

2. Experimental

General methods.—1,2;3,3′-Di-*O*-isopropylidene-3-*C*-(hydroxymethyl)-α-D-erythrofuranose was purchased from Sigma Chemicals. Methanol–hydrogen chloride solution (5% w/w) and silver carbonate were purchased from Wako Pure Chemicals. ^{1}H and ^{13}C NMR spectra were recorded at 30 °C with a JEOL ALPHA 500 FT MNR (500 MHz), using D_2O as solvent.

Preparation of methyl apiofuranosides.—1,2;3,3′-Di-*O*-isopropylidene-3-*C*-(hydroxymethyl)-α-D-erythrofuranose (100 mg) was hydrolyzed with 2 M CF_3CO_2H (10 mL) for 1 h at 100 °C. CF_3CO_2H was removed by evaporation under dry air at 40 °C. *Iso*propanol (200 μL) was then added and the residual acid was co-evaporated at 40 °C under dry air.

D-Apiose (about 50 mg) was subjected to methanolysis with 5% HCl in MeOH (2 mL) at 100 °C for 16 h. Hydrogen chloride was subsequently neutralized by adding silver carbonate and the resulting silver chloride precipitate was removed by centrifugation. The supernatant was concentrated to a syrup.

Separation of methyl apiofuranosides.—Methyl apiosides were separated using a Shimadzu LC-6A HPLC equipped with a refractive index detector. The initial separation was carried out with a Shim-pack SCR-101P (lead form) (300 × 7.9 mm) column equilibrated at 0.7 mL/min in H_2O at 80 °C, yielding three fractions (Fr. 1, 12.8 min; Fr. 2, 14.9 min; Fr. 3, 31.6 min). Fraction 2 was further separated with a Asahi-pack NH_2P50 column (250 × 4.6 mm) eluted with 97.5 (v/v)% CH_3CN at 0.5 mL/min at room temperature, yielding two additional fractions (Fr. 2-1, 21.0 min; Fr. 2-2, 24.2 min).

3. Results and discussion

Separation.—The treatment of D-apiose with methanolic hydrogen chloride generated the four methyl glycosides, which were separated by ion-exclusion and normal phase chromatography. The four glycosides were initially separated into three fractions by ion-exclusion chromatography. Based on the evidence given below, the components of Fractions 1 and 3 were identified as methyl 3-*C*-(hydroxymethyl)-α-L-threofuranoside (**4**) and methyl 3-*C*-(hydroxymethyl)-α-D-erythrofuranoside (**1**), respectively. Fraction 2 contained a mixture of methyl 3-*C*-(hydroxymethyl)-β-D-erythrofuranoside (**2**) and 3-*C*-(hydroxymethyl)-β-L-threofuranoside (**3**). These two methyl glycosides were separated by normal phase chromatography, yielding two fractions (2-1 and 2-2), whose constituents were identified as methyl 3-*C*-(hydroxymethyl)-β-L-threofuranoside (**3**) and methyl 3-*C*-(hydroxymethyl)-β-D-erythrofuranoside (**2**), respectively (Fig. 1).

^{1}H and ^{13}C NMR spectroscopy.—The proton chemical shifts of each methyl glycoside were assigned by 2D HOHAHA (Table 1). The protons of the hydroxymethyl group in **1** and **2** are equivalent, while those of **3** and **4** are nonequivalent. This may be due to constrained rotation of the exocyclic hydroxymethyl group in **3** and **4** due to intramolecular hydrogen bonding between OH-2 and the hydroxymethyl group. Apiofuranoside ring configuration of **1**, **2**, **3** and **4** was confirmed by comparing single, vicinal $^{1}H–^{1}H$ scalar coupling constants ($^{3}J_{1,2}$) with those reported for methyl apiosides and DL-apioses [9,10] and NOE. The values of $^{3}J_{1,2}$ for **1** (4.9 Hz) and **3** (4.3 Hz) indicated that H-1 and H-2 are cis. When H-1 and H-2 are trans, $^{3}J_{1,2}$ is usually close to 1 Hz, indicating that both oxygen atoms take up *quasi*-

Table 1
^{1}H chemical shifts of methyl apiofuranosides in D_2O

Compound	Chemical shift (ppm)[a]						
	H-1	H-2	H-3′a[b]	H-3′b[b]	H-4a[b]	H-4b[b]	OCH_3
1	5.01 (4.9)[c]	4.02	3.62	3.62	3.91 (10.4)	4.06	3.45
2	4.99 (3.7)	3.95	3.66	3.66	3.91 (10.4)	4.06	3.46
3	5.20 (4.3)	4.13	3.71 (12.2)	3.77	3.77 (9.8)	4.00	3.48
4	4.97 (1.2)	4.08 (1.2)	3.70 (12.2)	3.83	3.9. (9.8)	4.03	3.43

[a] Relative to external acetone (δ 2.23) in D_2O.
[b] Protons of the hydroxymethyl group and attached to C-4 (Fig. 1) were numbered as H-3′a and H-3′b, and H-4a and H-4b, respectively.
[c] Values in parenthesis are J_{HH} observed at the indicated sites.

Table 2
^{13}C chemical shifts of methyl apiofuranosides in D_2O

Compound	Chemical shift (ppm)[a]					
	C-1	C-2	C-3	C-3′	C-4	OCH_3
1	103.96	72.53	77.65	65.15	74.38	56.21
2	110.21	77.31	80.09	64.25	74.32	56.78
3	105.38	77.66	81.89	63.56	73.70	56.78
4	111.15	80.68	81.85	63.12	75.34	56.10

[a] Values are referenced to the carbon signal of external d_4-methanol (49.7 ppm).

axial positions. Compound **4** has a $^3J_{1,2}$ value of 1.2 Hz, confirming its α-configuration. However, $^3J_{1,2}$ for **2** is 3.7 Hz, and Angyal et al. [10] reported that $^3J_{1,2}$ for methyl 3-*C*-(hydroxymethyl)-β-D-erythrofuranoside is 3.5 Hz. The 2D NOESY spectrum of **2** contains cross peaks between H-2 and the protons of the hydroxymethyl group, and H-2 and H-4b, indicating that H-2, the hydroxymethyl group and H-4b are found on the same face of the ring for this compound and confirming its structure to be methyl 3-*C*-(hydroxymethyl)-β-D-erythrofuranoside (**2**). Similar NOE cross peaks were observed between H-2 and the protons of the hydroxymethyl group in the spectrum of **1**, indicating that H-2 and the hydroxymethyl group are on the same side, confirming the structure of **1** to be methyl 3-*C*-(hydroxymethyl)-α-D-erythrofuranoside (**1**). The ring structures of **3** and **4** were confirmed by 2D NOESY spectroscopy in the same way as described above. The ^{13}C signals were assigned by HMQC (Table 2). The signals at δ 103.96, 110.21, 105.38 and 111.15 are assigned to the anomeric carbons of **1**, **2**, **3** and **4**, respectively. These assignments confirmed the structure of methyl 3-*C*-(hydroxymethyl)-α- and β-D-erythrofuranosides and methyl 3-*C*-(hydroxymethyl)-α- and β-L-threofuranosides.

The proportions of the methyl apiofuranosides were 20% **1**, 53% **2**, 7% **3** and 20% **4** as determined by ^{1}H NMR spectroscopy. These proportions are almost the same as that of D-apiose in D_2O at equilibrium, as determined by ^{1}H NMR spectroscopy [9].

The separation of the four methyl apiofuranosides described here is simple and useful for preparing apiose derivatives.

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