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Saponin and Sapogenol. XLIX.[1] On the Constituents of the Roots of *Glycyrrhiza inflata* BATALIN from Xinjiang, China. Characterization of Two Sweet Oleanane-Type Triterpene Oligoglycosides, Apioglycyrrhizin and Araboglycyrrhizin

Isao KITAGAWA,*[,a] Kazuyuki HORI,[a] Masahiro SAKAGAMI,[a] Fumi HASHIUCHI,[a]
Masayuki YOSHIKAWA,[a] and Jiali REN[b]

Faculty of Pharmaceutical Sciences, Osaka University,[a] 1–6, Yamada-oka, Suita, Osaka 565, Japan and Xinjiang Institute of Chemistry, Academia Sinica,[b] Wulumuqi, P.R. China. Received January 16, 1993

Two sweet oleanane-type triterpene oligoglycosides named apioglycyrrhizin and araboglycyrrhizin were isolated from the air-dried roots of *Glycyrrhiza inflata* BATALIN, collected in Xinjiang province (Shinkyo-Kanzo in Japanese), together with glycyrrhizin (3), licorice-saponins A3 (8), G2 (10), and H2 (11) and known flavonoid glycosides. On the basis of chemical and physicochemical evidence, the structures of apioglycyrrhizin and araboglycyrrhizin have been determined to be expressed as 3-*O*-[*β*-D-apiofuranosyl(1→2)-*β*-D-glucuronopyranosyl]glycyrrhetic acid (1) and 3-*O*-[*α*-L-arabinopyranosyl(1→2)-*β*-D-glucuronopyranosyl]glycyrrhetic acid (2), respectively.

During the course of these studies, it has been found that the hydroxyl groups in the oligosaccharide moiety of the glucuronide saponins may be partially methylated by prolonged treatment with diazomethane in methanol. The sweetness of the saponins hitherto isolated from various Glycyrrhizae Radix has been examined and a structure–sweetness relationship, as compared with glycyrrhizin, has been found.

Keywords *Glycyrrhiza inflata*; Glycyrrhizae Radix; apioglycyrrhizin; araboglycyrrhizin; oleanane-type triterpene oligoglycoside; diazomethane methylation unusual

As a part of our chemical studies on the constituents of various botanically identified Chinese licorice-roots, we have reported the isolation of ten oleanane-type triterpene oligoglycosides, designated licorice-saponins A3 (**8**), B2, C2, D3, E2, F3, G2 (**10**), H2 (**11**), J2, and K2, from the air-dried roots of *Glycyrrhiza uralensis* FISCHER collected in the northeastern part of China ("Tohoku-Kanzo," 東北甘草) and have elucidated their chemical structures.[1,2] As a continuation of that work, we have compared the chemical constituents of various Chinese licorice-roots collected from Xinjiang province (so-called "Shinkyo-Kanzo," 新疆甘草). From one of those Shinkyo-Kanzo, the botanical origin of which was identified as *Glycyrrhiza inflata* BATALIN (Leguminosae),[3] we have isolated two sweet oleanane-type triterpene oligoglycosides, named apioglycyrrhizin (**1**) and araboglycyrrhizin (**2**), together with glycyrrhizin (**3**), licorice-saponins A3 (**8**),[2] G2 (**10**),[1] and H2 (**11**)[1] and several known flavonoid glycosides such as liquiritin,[2,4] liquiritin apioside[2,4] and isoliquiritin apioside.[2,5]

In this paper, we present a full account of the structure elucidation of apioglycyrrhizin (**1**) and araboglycyrrhizin (**2**) and also describe a rather unusual methylation with diazomethane of hydroxyl groups in the sugar moiety of the saponins.[6] In addition, the structure–sweetness relationship, in relation to the sweet taste of glycyrrhizin (**3**), is discussed.

The methanol extract of the licorice-root was subjected to solvent fractionations and chromatographic purifications as shown in Fig. 1. Thus, the methanol extract was first partitioned into an ethyl acetate–water mixture and the

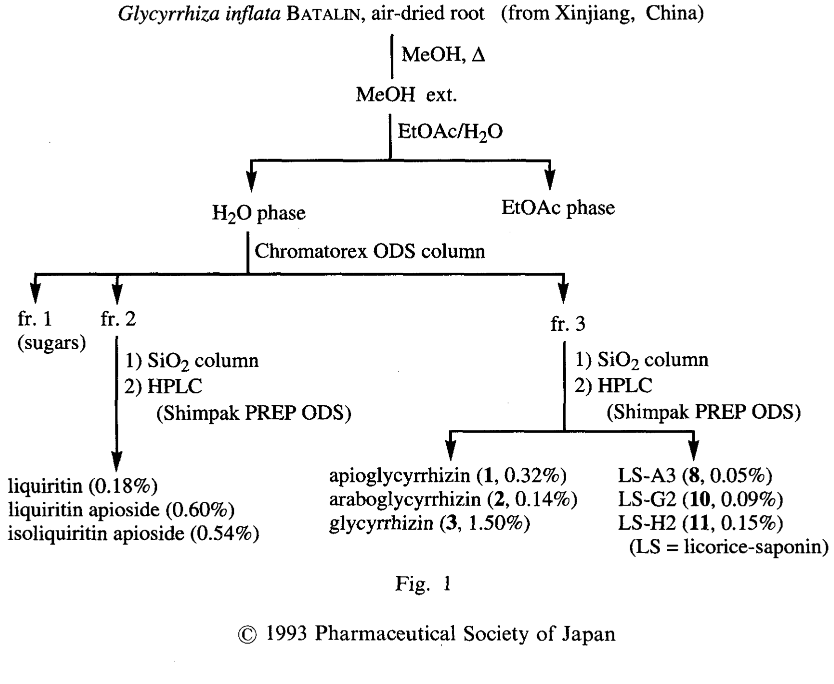

Fig. 1

water-soluble portion was then subjected to reversed-phase silica gel column chromatography to provide the oligoglycoside fractions (frs. 2 and 3). Repeated separation of the oligoglycoside fractions by means of ordinary-phase silica gel column chromatography and subsequent HPLC furnished apioglycyrrhizin (**1**), araboglycyrrhizin (**2**), glycyrrhizin (**3**), licorice-saponins A3 (**8**), G2 (**10**), and H2 (**11**) from fr. 3 and liquiritin, liquiritin apioside and isoliquiritin apioside from fr. 2.

Apioglycyrrhizin (1) Apioglycyrrhizin (**1**) was obtained as colorless needles of mp 193—195 °C. The ultraviolet (UV) spectrum of **1** showed the absorption maximum at 249 nm ($\varepsilon = 9300$) ascribable to a conjugated enone chromophore, whereas the infrared (IR) spectrum showed strong absorption bands due to carboxyl and enone moieties (1716, 1650 cm^{-1}) and a group of broad absorption bands (3500—3100, 1028 cm^{-1}) indicative of the glycosidic structure of **1**. The proton and carbon signals due to the sapogenol moiety observed in the proton and carbon-13 nuclear magnetic resonance (^1H- and ^{13}C-NMR) spectra of **1**, were found to be superimposable on those of glycyrrhizin (**3**). Detailed ^1H-NMR decoupling experiments on **1** have resulted in the following assignments; δ 4.18 (dd, $J = 7.7$, 9.0 Hz) to 2′-H, 4.25 (br s) to 5″-H_2, 4.28 (dd, $J = 9.0$, 9.0 Hz) to 3′-H, 4.37, 4.69 (both d, $J = 9.4$ Hz) to 4″-H_2, 4.44 (dd, $J = 9.0$, 9.8 Hz) to 4′-H, 4.51 (d, $J = 9.8$ Hz) to 5′-H, 4.90 (br s) to 2″-H, 4.96 (d, $J = 7.7$ Hz) to 1′-H, and 6.38 (br s) to 1″-H, thus suggesting the oligosaccharide part of **1** to be an apiofuranosyl(1→2)-β-glucuronopyranosyl structure.

Methylation of apioglycyrrhizin (**1**) with diazomethane afforded the dimethyl ester **1a** which was subjected to sodium borohydride ($NaBH_4$) reduction to yield **1b**. Methanolysis of **1b** with 9% hydrogen chloride in methanol provided methyl glycyrrhetate (**4a**) together with methyl D-glucopyranoside and methyl D-apiofuranoside, and the latter products were further subjected to acidic hydrolysis to furnish D-glucose ($[\alpha]_D$ +47°) and D-apiose ($[\alpha]_D$ +9.1°), respectively. Complete methylation of **1b** with methyl iodide and dimsyl carbanion,[7] and subsequent methanolysis, liberated methyl 2,3,5-tri-*O*-methylapiofuranoside and methyl 3,4,6-tri-*O*-methylglucopyranoside.

In order to determine the β-anomeric configuration of the D-apiofuranosyl moiety in apioglycyrrhizin (**1**), Klyne's rule[8] has been applied to **1** and glycyrrhetic acid monoglucuronide (**5**)[9] which was prepared from **1**. Thus, the partial methanolysis of **1** with 9% hydrogen chloride in methanol and subsequent alkaline hydrolysis provided the prosapogenol **5**. The $[M]_D$ (**1**) $-$ $[M]_D$ (**5**) value is $-150°$, while the $[M]_D$ of methyl α-D-apiofuranoside (**6a**) is +190°[10] and the $[M]_D$ of methyl β-D-apiofuranoside (**6b**) is $-156°$.[10] Furthermore, it has been found that the anomeric orientation of the D-apiofuranoside moiety may be corroborated by comparing the ^{13}C-NMR data for **1**, **1a**, and **1b** with those for methyl α-D- and β-D-apiofuranosides (**6a**, **6b**) which are shown in Table II. Consequently, the β-D-apiofuranoside structure was concluded to be present in **1**.

The accumulated evidence described above has led us to express apioglycyrrhizin as 3-*O*-[β-D-apiofuranosyl(1→2)-β-D-glucuronopyranosyl]glycyrrhetic acid (**1**).

During the course of above structure elucidation studies, we have found that prolonged treatment of apioglycyrrhizin (**1**) with excess diazomethane in methanol–ether gave 2″-*O*-methylapioglycyrrhizin dimethyl ester (**1c**, 30%) and the 3″-*O*-methyl counterpart **1d** (15%) in addition to the ordinary dimethyl ester **1a** (45%). Treatment of **1c** with

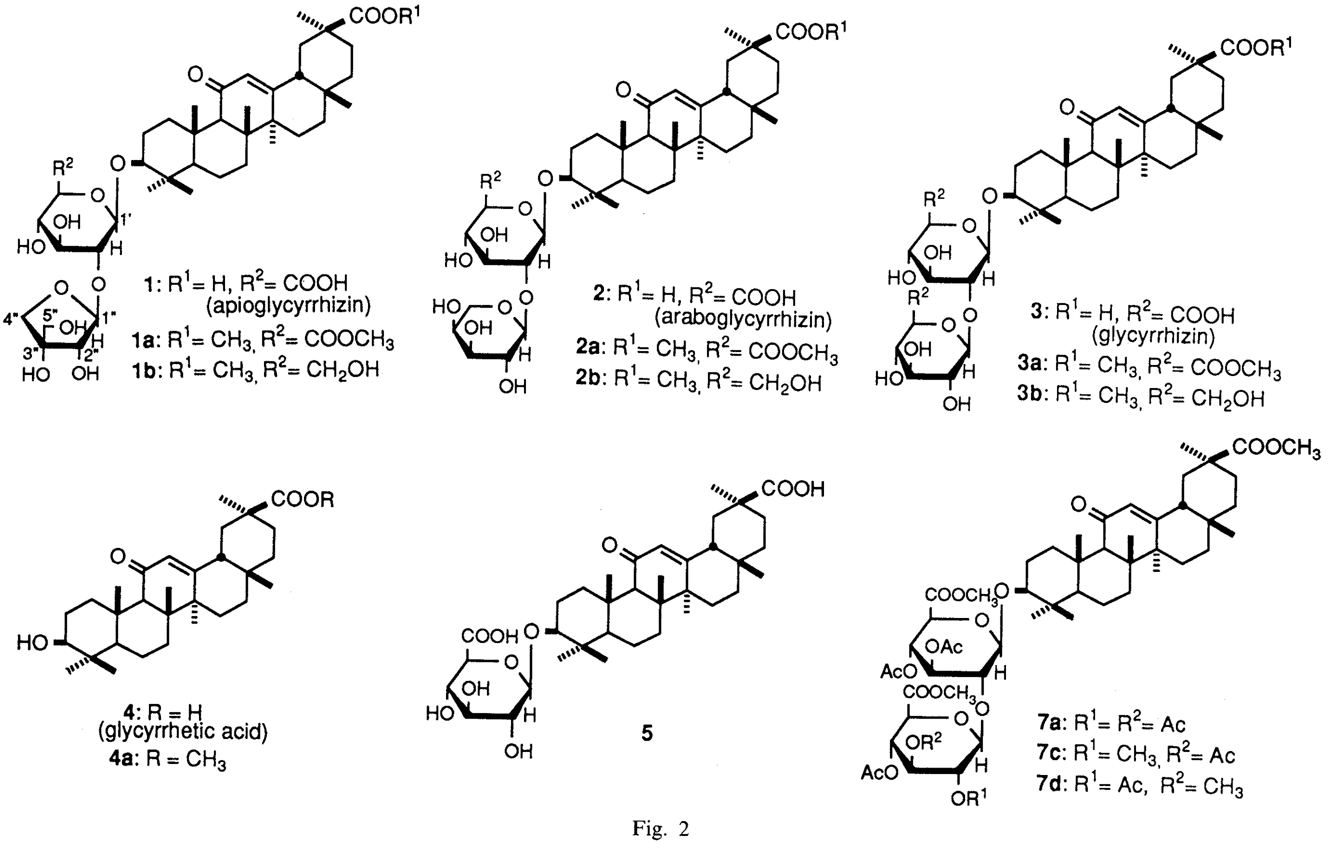

Fig. 2

TABLE I. ^{13}C-NMR Data for **1**, **1a**, **1b**, **1c**, **1d**, **2**, **2a**, **3**, **3a**, **3c**, and **3d** (125 MHz, Pyridine-d_5, δ_C)

	1	**1a**	**1b**	**1c**	**1d**	**2**	**2a**	**3**	**3a**	**3c**	**3d**
C-3	88.8	89.1	88.7	89.2	89.2	88.9	89.0	88.9	89.2	89.5	89.2
C-11	199.1	199.4	199.5	199.4	199.4	199.4	199.3	199.3	199.0	199.2	199.3
C-12	128.4	128.7	128.7	128.8	128.7	128.6	128.6	128.8	128.7	128.5	128.6
C-13	169.7	168.9	169.0	169.0	169.0	169.5	168.9	169.4	168.7	168.8	169.7
C-30	178.8	176.8	176.8	176.8	176.8	179.1	176.7	178.8	176.5	176.7	176.8
30-OMe		51.6$^{a)}$	51.6	51.6$^{a)}$	51.6$^{a)}$		51.5$^{a)}$		51.5$^{a)}$	51.5$^{a)}$	51.5$^{a)}$
C-1′	105.4	105.7	105.5	105.6	105.7	105.2	105.1	104.6	104.5	104.7	104.8
C-2′	79.4$^{a)}$	79.2$^{b)}$	79.5	80.8	79.3	83.4	83.1	83.9	84.0	85.0	84.1
C-3′	79.0$^{a)}$	78.1$^{c)}$	78.5$^{a)}$	79.3	78.3	77.4	76.6$^{b)}$	76.2	76.2$^{b)}$	76.6	77.2
C-4′	73.0	73.0	71.9	73.1	73.0	73.0	72.6	72.7	72.2	72.6$^{b)}$	72.3$^{b)}$
C-5′	77.8$^{b)}$	76.8	78.0$^{a)}$	77.9	77.5	77.4	77.2$^{b)}$	77.1	77.1	78.0$^{c)}$	77.2$^{c)}$
C-6′	172.0	170.3	62.8	170.4	170.3	172.3	170.2	172.1$^{a)}$	169.5$^{c)}$	170.0	170.0
6′-OMe		51.9$^{a)}$		52.0$^{a)}$	51.9$^{a)}$		51.9$^{a)}$		51.8$^{a)}$	51.8$^{a)}$	51.8$^{a)}$
C-1″	110.9	111.2	111.1	109.0	111.5	106.6	106.6	106.2	106.3	104.0	106.2
C-2″	77.6$^{b)}$	77.7$^{c)}$	78.0$^{a)}$	87.2	76.8	73.6	73.5	76.2	75.9$^{b)}$	80.6	75.7
C-3″	80.1$^{a)}$	80.4$^{b)}$	80.4	76.8	85.3	74.1	74.1	77.1	77.1	75.7	86.9
C-4″	75.2	75.5	75.5	75.6	71.7	69.2	68.9	72.7	72.4	72.4$^{b)}$	71.9$^{b)}$
C-5″	65.3	65.9	66.0	66.0	63.0	66.9	66.8	77.1	77.1	77.8$^{c)}$	76.4$^{c)}$
C-6″								171.7$^{a)}$	169.7$^{c)}$	169.6	169.7
2″-OMe				58.7						55.0	
3″-OMe					52.8$^{a)}$						60.8
6″-OMe									51.8$^{a)}$	51.8$^{a)}$	52.0$^{a)}$

a—c) Assignments may be interchanged within the same column.

TABLE II. ^{13}C-NMR Data for Methyl α-D-Apiofuranoside (**6a**) and Methyl β-D-Apiofuranoside (**6b**) (125 MHz, Pyridine-d_5, δ_C)

	C-1	C-2	C-3	C-4	C-5	C-1-OMe
Methyl α-D-apiofuranoside (**6a**)	104.5	75.2	77.7	73.4	65.5	55.0
Methyl β-D-apiofuranoside (**6b**)	111.5	77.7	80.3	74.9	65.5	55.3

$NaBH_4$ and subsequent methanolysis, liberated methyl 2-*O*-methylapiofuranoside and methyl glucopyranoside, while methyl 3-*O*-methylapiofuranoside and methyl glucopyranoside were obtained through the same treatment of **1d**. These findings and a comparison of the ^{13}C-NMR data for **1c** and **1d** with those for **1** and **1a**, have led to the formulation of the structures of **1c** and **1d** as shown, having a 2″-*O*-methyl- and a 3″-*O*-methylapiofuranosyl moiety, respectively.

In order to find a further example of the unusual methylation with diazomethane of hydroxyl groups in the sugar part of glucuronide-saponin,[11] glycyrrhizin (**3**) was treated with excess diazomethane for 24 h and, in addition to the ordinary trimethyl ester **3a** (38%), the 2″-*O*-methyl (**3c**, 25%) and the 3″-*O*-methyl (**3d**, 29%) derivatives of **3a** were obtained. Acetylation of **3a**, **3c**, and **3d** with acetic anhydride in pyridine provided the pentaacetate **7a**, and tetraacetates **7c** and **7d**, respectively. Treatment of **3c** and **3d** with $NaBH_4$ followed by methanolysis of the products, yielded methyl 2-*O*-methylglucopyranoside from **3c** and methyl 3-*O*-methylglucopyranoside from **3d**, in addition to methyl glucopyranoside as the common product. By comparing in detail the ^{1}H-NMR data for **7a**, **7c**, and **7d**, and by comparing the ^{13}C-NMR data for **3c** and **3d** with those for **3** and **3a** (Table I), the structures of **3c** and **3d** were confirmed to be as shown. In 1970, Aritomi and Kawasaki reported that the hydroxyl groups in the sugar moiety of C- and O-D-glucopyranosides were partially methylated with diazomethane in methanol and the reactions were considerably favored in the presence of a small amount of stannous chloride. They presumed that a contaminant in aged methanol stored in cans may be responsible for this unusual methylation.[12] In our experiments, however, freshly distilled methanol was used and methyl D-glucopyranoside and **1b**, which lacks the glucuronic acid moiety, were not methylated under these reaction conditions. So, it has been presumed that the present unusual methylation with excess diazomethane may be a characteristic property of glucuronidesaponin.

Araboglycyrrhizin (2) Araboglycyrrhizin (**2**), obtained as fine crystals of mp 237—238 °C, showed a UV absorption maximum at 249 nm (ε = 9300) and IR absorption bands due to hydroxyl, ester, and enone functions at 3500—3200 (br), 1711, 1658, and 1033 cm^{-1}, which were similar to those observed for apioglycyrrhizin (**1**). Treatment of **2** with 9% hydrogen chloride in methanol provided glycyrrhetic acid (**4**), methyl D-glucuronide and methyl L-arabinoside. Ordinary methylation of **2** with diazomethane yielded the dimethyl ester **2a**. The ^{1}H- and ^{13}C-NMR spectra (Table I) of **2a** showed signals at δ 4.96 (d, J = 7.6 Hz, 1′-H) and 5.16 (d, J = 6.7 Hz, 1″-H) and δ_C 105.1 (1′-C) and 106.6 (1″-C), which suggested the presence of the β-D-glucuronide moiety and the α-L-arabinoside moiety in **2**.

Treatment of araboglycyrrhizin dimethyl ester (**2a**) with $NaBH_4$ provided the monomethyl ester **2b**, which was then subjected to methanolysis to furnish methyl glycyrrhetate (**4a**), methyl D-glucopyranoside and methyl L-arabinoside. Acidic hydrolysis of the latter methyl glycosides gave D-glucose ([α]$_D$ +47°) and L-arabinose ([α]$_D$ +97°), respectively. Furthermore, complete methylation of **2b** and subsequent methanolysis liberated methyl 2,3,4-tri-*O*-methylarabinopyranoside and methyl 3,4,6-tri-*O*-methylglucopyranoside. Consequently, the structure of araboglycyrrhizin has been concluded to be 3-*O*-[α-L-arabinopy-

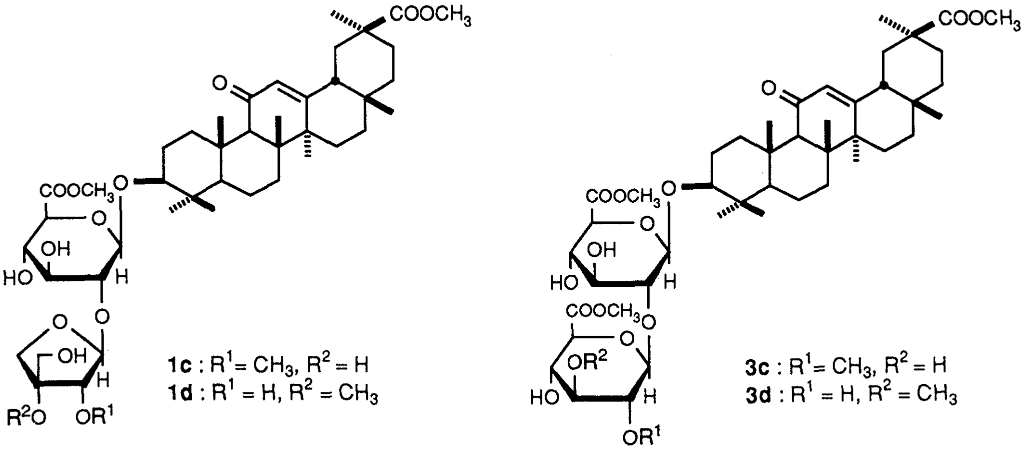

Fig. 3

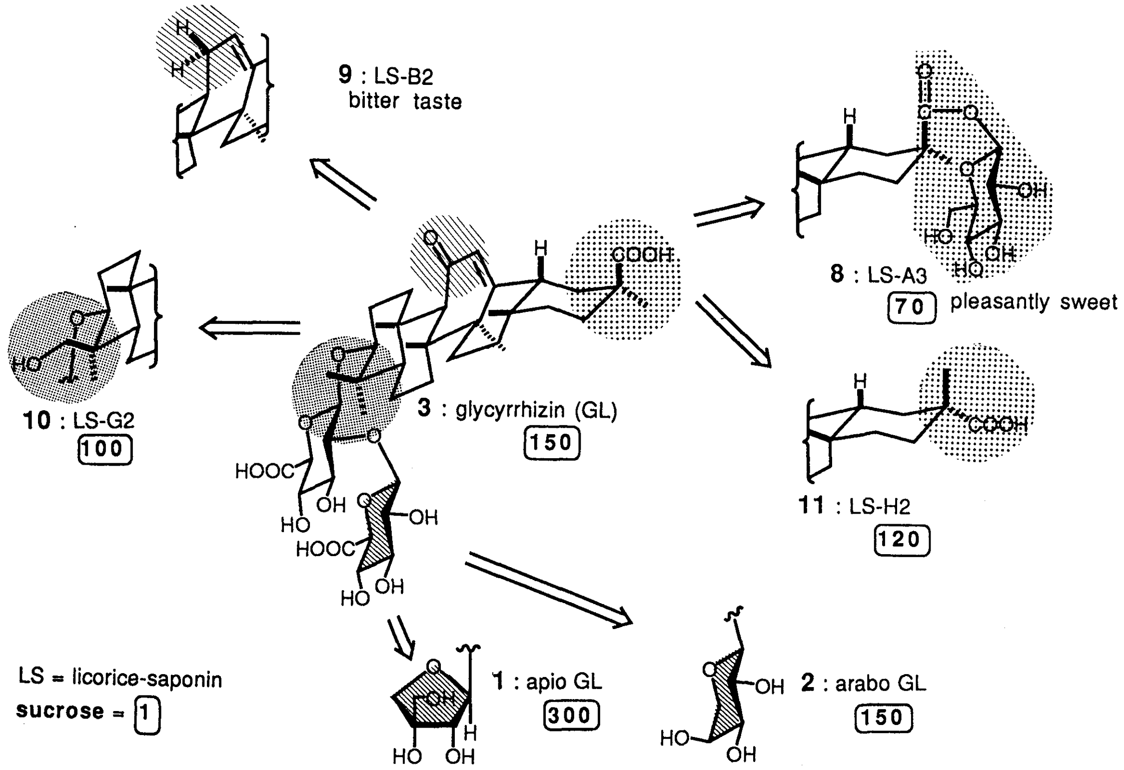

Fig. 4

ranosyl(1→2)-β-D-glucuronopyranosyl]glycyrrhetic acid (**2**) as shown.

Glycyrrhizin (**3**) is an efficient sweetening agent. After detailed chemical analyses of the triterpene oligoglycoside constituents of Chinese licorice-roots, twelve new glucuronide-saponins have so far been reported (this and previous papers[1,2]). We preliminarily examined the sweetness of those saponins as compared with glycyrrhizin (**3**).[13] The results are depicted in Fig. 4, where the intensities of sweetness are shown in boxes, taking the sweetness of sucrose as 1. It has been found that apioglycyrrhizin (**1**) is about 2 times sweeter than **3** which is 150 times as sweet as sucrose, while the sweetness of araboglycyrrhizin (**2**), licorice-saponins G2 (**10**) or H2 (**11**) is comparable to that of **3**. On the other hand, it is interesting that licorice-saponin A3 (**8**) shows slightly reduced but more pleasant sweetness as compared to **3**, whereas licorice-saponin B2 (**9**) lacks the sweet taste but exhibits a bitter taste and causes vomiting.

Experimental

Instruments for obtaining physical data and experimental conditions for chromatography were the same as described in our previous papers[1,2] except in the following respects. High-resolution secondary ion mass spectrometry (SIMS) (positive) was taken with a Hitachi M-2000A double-focusing spectrometer connected with a Hitachi M-0201 data processing system for using the peak matching program. The primary ion was Xe^+ and the accelerating voltages of primary and secondary ions were 10 and 2 kV, respectively. For the accurate mass measurements, Ultramark 1621 (GL Sciences) was used as the internal standard mass marker.

Isolation of Apioglycyrrhizin (1), Araboglycyrrhizin (2), Glycyrrhizin (3), Licorice-saponins A3 (8), G2 (10), and H2 (11) and Flavonoid Glycosides The air-dried roots of *Glycyrrhiza inflata* (from Xinjiang province, China, cut, 1 kg) was extracted with MeOH (2 l each) under reflux three times. Evaporation of the solvent under reduced pressure from the combined extract gave the MeOH extract (300 g), which was partitioned into an ethyl acetate–water (1 : 1) mixture (2 l). Removal of the solvent from the water phase and the ethyl acetate phase under reduced pressure below 40 °C, yielded the aqueous extract (200 g) and the ethyl acetate extract (100 g). The aqueous extract (200 g) was subjected to reversed-phase silica gel column chromatography [Chromatorex ODS 1.0 kg, gradient elution with H_2O–MeOH (9 : 1—1 : 9)] to furnish three fractions: fr. 1 [eluted with H_2O–MeOH (9 : 1—7 : 1); mainly sugar constituent, 95 g], fr. 2 [H_2O–MeOH (7 : 1—6 : 1); mainly sugar and flavonoid glycosides, 45 g], fr. 3 [H_2O–MeOH (6 : 1—4 : 1); mainly saponins, 45 g], and later eluates [H_2O–MeOH (2 : 1—1 : 9); others 20 g].

Silica gel column chromatography [SiO_2 1.4 kg, $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] of fr. 2 (45 g) and subsequent preparative HPLC [Shimpak PREP ODS, 20 mm × 25 cm, MeOH–H_2O (5 : 1)] provided liquiritin, liquiritin apioside, and isoliquiritin apioside in yields of 0.18, 0.60, and 0.54% from the air-dried root, respectively. Silica gel column chromatography [SiO_2 720 g, gradient elution with $CHCl_3$–MeOH–H_2O (10 : 3 : 1 lower phase—6 : 4 : 1)] of fr. 3 (20 g) followed by preparative HPLC [Shimpak PREP ODS, 20 mm × 25 cm, CH_3CN–1% AcOH (65 : 35)], afforded apioglycyrrhizin (**1**), araboglycyrrhizin (**2**), glycyrrhizin (**3**), and licorice-saponins A3 (**8**), G2 (**10**), and H2 (**11**) in yields of 0.32, 0.14, 1.50, 0.05, 0.09, and 0.15% from the root, respectively. Flavonoid glycosides and **3**, **8**, **10**, and **11** were shown to be identical with authentic samples by comparing the ^{1}H-NMR, IR, and $[\alpha]_D$ data and by mixed melting point determination.

Apioglycyrrhizin (**1**), mp 193—195 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +43° (c=0.29, MeOH). *Anal.* Calcd for $C_{41}H_{64}O_{14} \cdot H_2O$: C, 61.44; H, 8.33. Found: C, 61.69; H, 8.30. UV λ_{max}^{MeOH} nm (ε): 249 (9300). IR ν_{max}^{KBr} cm^{-1}: 3500—3100 (br), 2913, 1716, 1650, 1410, 1028. ^{1}H-NMR (500 MHz, pyridine-d_5, 25 °C, δ): 0.89, 1.09, 1.12, 1.23, 1.43 (all 3H, s), 1.34 (3H × 2, s), 3.03 (1H, br d, J=*ca.* 14 Hz, 18-H), 3.38 (1H, dd, J=4.2, 11.0 Hz, 3α-H), 4.18 (1H, dd, J=7.7, 9.0 Hz, 2′-H), 4.25 (2H, br s, 5″-H_2), 4.28 (1H, dd, J=9.0, 9.0 Hz, 3′-H), 4.37, 4.69 (each 1H, ABq, J=9.4 Hz, 4″-H_2), 4.44 (1H, dd, J=9.0, 9.8 Hz, 4′-H), 4.51 (1H, d, J=9.8 Hz, 5′-H), 4.90 (1H, br s, 2″-H), 4.96 (1H, d, J=7.7 Hz, 1′-H), 5.95 (1H, s, 12-H), 6.38 (1H, br s, 1″-H). ^{13}C-NMR: as given in Table I.

Araboglycyrrhizin (**2**), mp 237—238 °C (colorless fine needles from MeOH–H_2O), $[\alpha]_D^{23}$ +31° (c=0.25, MeOH). *Anal.* Calcd for $C_{41}H_{62}O_{14} \cdot H_2O$: C, 61.79; H, 8.09. Found: C, 61.44; H, 8.17. UV λ_{max}^{MeOH} nm (ε): 249 (9300). IR ν_{max}^{KBr} cm^{-1}: 3500—3200 (br), 2924, 1711, 1658, 1396, 1033. ^{1}H-NMR (500 MHz, pyridine-d_5, 25 °C, δ): 1.10, 1.15, 1.26, 1.28, 1.35, 1.36, 1.42 (all 3H, s), 3.05 (br d, J=*ca.* 13 Hz, 18-H), 3.40 (1H, dd, J=5.0, 11.4 Hz, 3α-H), 3.82 (1H, dd, J=1.5, 12.2 Hz, 5″-H), 4.18 (1H, dd, J=7.6, 9.0 Hz, 2′-H), 4.25 (1H, dd, J=3.4, 8.5 Hz, 3″-H), 4.30—4.40 (3H, m, 3′-H, 4′-H, 4″-H), 4.44 (1H, dd, J=3.1, 12.2 Hz, 5″-H), 4.54 (1H, d, J=9.0 Hz, 5′-H), 4.61 (1H, dd, J=6.7, 8.5 Hz, 2″-H), 5.03 (1H, d, J=7.6 Hz, 1′-H), 5.99 (1H, s, 12-H) (The 1″-H signal was not assignable due to overlap of the H_2O signal in the measurement at 25 °C); (40 °C, δ): 0.80, 1.10, 1.14, 1.27, 1.34, 1.35, 1.46 (all 3H, s), 3.03 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.42 (1H, dd, J=4.8, 11.4 Hz, 3α-H), 3.80 (1H, dd, J=1.8, 12.2 Hz, 5″-H), 4.17 (1H, dd, J=8.0, 9.0 Hz, 2′-H), 4.22 (1H, dd, J=3.4, 7.6 Hz, 3″-H), 4.26—4.34 (3H, m, 3′-H, 4′-H, 4″-H), 4.40 (1H, dd, J=3.4, 12.2 Hz, 5″-H), 4.51 (1H, d, J=9.0 Hz, 5′-H), 4.57 (1H, dd, J=6.4, 7.6 Hz, 2″-H), 5.19 (1H, d, J=6.4 Hz, 1″-H), 5.97 (1H, s, 12-H) (In this case, the 1′-H signal was not assignable due to overlap of the H_2O signal). ^{13}C-NMR: as given in Table I.

Diazomethane Methylation of Apioglycyrrhizin (1) Giving the Dimethyl Ester (1a) An ice-cooled solution of **1** (20 mg) in MeOH (2 ml) was treated with ethereal diazomethane (*ca.* 20 ml) until the yellow color persisted. The solution was left standing for 1 h, then the solvent was removed under reduced pressure to furnish the dimethyl ester (**1a**, 21 mg).

1a: mp 219—220 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ +37° (c=0.76, MeOH). *Anal.* Calcd for $C_{43}H_{66}O_{14} \cdot 3H_2O$: C, 59.98; H, 8.42. Found: C, 60.08; H, 8.48. UV λ_{max}^{MeOH} nm (ε): 249 (9600). IR ν_{max}^{KBr} cm^{-1}: 3500—3150 (br), 2910, 1727, 1651, 1405, 1044. ^{1}H-NMR (500 MHz. pyridine-d_5, δ): 0.79, 1.08, 1.11, 1.21, 1.23, 1.36, 1.43 (all 3H, s), 3.06 (1H, br d, J=*ca.* 14 Hz, 18-H), 3.34 (1H, dd, J=4.2, 10.8 Hz, 3α-H), 3.76, 3.77 (both 3H, s, OCH_3 × 2), 4.17 (1H, dd, J=7.6, 9.0 Hz, 2′-H), 4.28 (2H, br s, 5″-H), 4.30 (1H, dd, J=9.0, 9.0 Hz, 3′-H), 4.35 (1H, dd, J=9.0, 9.6 Hz, 4′-H), 4.44, 4.74 (both 1H, ABq, J=9.5 Hz, 4″-H_2), 4.48 (1H, d, J=9.6 Hz, 5′-H), 4.92 (1H, d, J=2.1 Hz, 2″-H), 4.95 (1H, d, J=7.6 Hz, 1′-H), 5.88 (1H, s, 12-H), 6.41 (1H, d, J=2.1 Hz, 1″-H). ^{13}C-NMR: as given in Table I.

$NaBH_4$ Reduction of 1a A solution of **1a** (15 mg) in MeOH (3 ml) was treated with $NaBH_4$ (10 mg) and the whole mixture was stirred at room temperature (24 °C) for 2 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The product, obtained by evaporation of the solvent from the filtrate under reduced pressure, was subjected to silica gel column chromatography [SiO_2 2 g, $CHCl_3$–MeOH (8 : 1)] to afford **1b** (10 mg).

1b: mp 179—182 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ +41° (c=0.35, MeOH). *Anal.* Calcd for $C_{42}H_{66}O_{13} \cdot 2H_2O$: C, 61.90; H, 8.66. Found: C, 61.83; H, 8.60. UV λ_{max}^{MeOH} nm (ε): 249 (9300). IR ν_{max}^{KBr} cm^{-1}: 3500—3100 (br), 2940, 1725, 1651, 1380, 1073. ^{1}H-NMR (500 MHz, pyridine-d_5, δ): 0.79, 1.09, 1.13, 1.20, 1.24, 1.35, 1.41 (all 3H, s), 3.06 (1H, br d, J=*ca.* 14 Hz, 18-H), 3.39 (1H, dd, J=4.0, 10.6 Hz, 3α-H), 3.73 (3H, s, methoxyl), 3.98 (1H, m, 5′-H), 4.08—4.18 (2H, m, 2′-H, 3′-H), 4.28 (1H, br s, 5″-H), 4.30 (1H, dd, J=5.4, 10.5 Hz, 6′-H), 4.42, 4.73 (both 1H, ABq, J=9.5 Hz, 4″-H_2), 4.47 (1H, dd, J=2.1, 10.5 Hz, 6′-H), 4.88 (1H, d, J=7.6 Hz, 1′-H), 4.94 (1H, d, J=2.8 Hz, 2″-H), 5.88 (1H, s, 12-H), 6.41 (1H, d, J=2.8 Hz, 1″-H). ^{13}C-NMR: as given in Table I.

Methanolysis of 1b A solution of **1b** (200 mg) in 9% HCl–MeOH (3 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the inorganic materials were removed by filtration. The product, obtained by evaporation of the solvent from the filtrate under reduced pressure, was subjected to silica gel column chromatography [SiO_2 5 g, $CHCl_3$–MeOH (10 : 1)] to afford methyl glycyrrhetate (**4a**, 90 mg), methyl D-glucopyranoside (an α,β-anomeric mixture, 31 mg) and methyl D-apiofuranoside (an α,β-anomeric mixture, 20 mg). Methyl D-glucopyranoside and methyl D-apiofuranoside were respectively identified by TLC [developing with $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] comparison with authentic samples which were prepared by methanolysis of D-glucose and D-apiose. Methyl glycyrrhetate (**4a**) was shown to be identical with an authentic sample by comparisons of $[\alpha]_D$, IR, and ^{1}H-NMR data, and by mixed melting point determination.

Methyl D-glucopyranoside (10 mg), obtained from **1b**, was dissolved in 3% H_2SO_4 and the whole mixture was heated under reflux for 2 h. The reaction mixture was neutralized with Amberlite IRA-2 (OH^- form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate provided D-glucose [$[\alpha]_D^{23}$ +47° (c=1.20, 24 h after dissolving in H_2O)]. A solution of methyl D-apiofuranoside (10 mg), obtained from **1b**, was also heated under reflux for 2 h. The reaction mixture was worked up as described above to yield D-apiose [$[\alpha]_D^{23}$ +9.1° (c=0.50), 24 h after dissolving in H_2O].

Complete Methylation of 1b Followed by Methanolysis A solution of **1b** (3 mg) in DMSO (0.5 ml) was treated with a dimsyl carbanion solution (1 ml, prepared with NaH 100 mg and DMSO 2 ml) and the whole mixture was stirred in the dark at room temperature (24 °C) for 1 h. The reaction mixture was then treated with methyl iodide (1 ml) and the whole was stirred for a further 2 h. The reaction mixture was poured into ice-water and the whole was extracted with ethyl acetate. The ethyl acetate extract was washed with saturated saline and dried over $MgSO_4$ powder, then filtered. Evaporation of the solvent from the filtrate under reduced pressure afforded a product (4.7 mg). Without further purification, the product was dissolved in 9% HCl–MeOH (0.5 ml) and the solution was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the products were identified by TLC [benzene–acetone (2 : 1)] and GLC with methyl 2,3,5-tri-*O*-methylapiofuranoside and methyl 3,4,6-tri-*O*-methylglucopyranoside. GLC conditions: 5% BDS on Uniport B (80—100 mesh); 3 mm (i.d.) × 2 m glass column; column temperature 160 °C; N_2 flow rate 35 ml/min; t_R: methyl 2,3,5-tri-*O*-methylapiofuranoside, 1 min 14 s, 2 min 27 s and methyl 3,4,6-tri-*O*-methylglucopyranoside, 5 min 27 s, 7 min 43 s.

Partial Methanolysis of Apioglycyrrhizin (1) Followed by Alkaline Hydrolysis A solution of **1** (40 mg) in 9% HCl–MeOH (1 ml) was stirred at room temperature (24 °C) for 2 h and then neutralized with Dowex 1 × 2 (OH^- form). After removal of the resin by filtration, the solvent was evaporated from the filtrate under reduced pressure to furnish a product (45 mg). The product, without further purification, was dissolved in 10% aqueous K_2CO_3–MeOH (1 : 1, 1 ml) and the whole mixture was stirred at room temperature (23 °C) for 30 min. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure afforded a product, which was purified by silica gel column chromatography [SiO_2 2 g, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase)] to furnish **5** (10 mg). The product was identified by comparing the $[\alpha]_D$ and ^{1}H-NMR data (in pyridine-d_5) with reported values.[9]

5: An amorphous powder, $[\alpha]_D^{23}$ +75° (c=0.24, MeOH); +72° (c=0.33, 10% MeOH–$CHCl_3$). *Anal.* Calcd for $C_{36}H_{54}O_{10} \cdot H_2O$: C, 65.04; H, 8.49. Found: C, 64.93, H, 8.40. UV λ_{max}^{MeOH} nm (ε): 249 (10200). IR ν_{max}^{KBr} cm^{-1}: 3400—3100 (br), 2940, 1729, 1648, 1405, 1071. ^{1}H-NMR (500 MHz, pyridine-d_5, δ): 0.79, 1.03, 1.09, 1.25, 1.31, 1.32, 1.45 (all 3H, s), 3.08 (1H, br d, J=*ca.* 14 Hz, 18-H), 3.44 (1H, dd, J=4.0, 11.0 Hz, 3α-H), 4.16 (1H, dd, J=7.9, 8.0 Hz, 2′-H), 4.32 (1H, dd, J=8.0, 8.2 Hz, 3′-H), 4.59 (1H, dd, J=8.2, 8.2 Hz, 4′-H), 4.66 (1H, d, J=8.2 Hz, 5′-H), 5.03 (1H, d, J=7.9 Hz, 1′-H), 5.98 (1H, s, 12-H).

Preparation of Methyl α- and β-D-Apiofuranoside (6a, 6b) from Liquiritin Apioside A solution of liquiritin apioside (1.0 g) in 9% HCl–MeOH (10 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Dowex 1×2 (OH^- form) and the resin was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the product was purified by silica gel column chromatography [SiO_2 25 g, $CHCl_3$–MeOH (6 : 1)] to afford a mixture of methyl α- and β-D-apiofuranosides (160 mg). Since an attempt at direct separation of two anomers was unsuccessful, the methyl D-apiofuranoside mixture was derived to the silylated derivatives and then subjected to chromatographic separation. Thus, a solution of methyl D-apiofuranosides (70 mg) in dimethylformamide (DMF) (0.5 ml) was treated with *tert*-butyldimethylsilyl chloride (170 mg) and imidazole (35 mg) and the whole was stirred at room temperature (22 °C) for 1 h. The reaction mixture was poured into ice-water and the whole was extracted with ethyl acetate. After work-up of the ethyl acetate extract in the usual manner, the product (150 mg) was subjected to silica gel column chromatography [SiO_2 15 g, *n*-hexane–acetone (40 : 1)] to afford methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-α- and -β-D-apiofuranosides. A solution of the silylated derivative of methyl α-D-apiofuranoside (22 mg) in tetrahydrofuran (THF) (1 ml) was treated with tetra-*n*-butylammonium fluoride. The reaction mixture was poured into ice-water and the whole was extracted with ethyl acetate. Evaporation of the solvent under reduced pressure from the ethyl acetate extract gave a residue, which was purified by silica gel column chromatography [SiO_2 3 g, benzene–acetone (1 : 1)] to afford methyl α-D-apiofuranoside (**6a**, 12 mg). The silylated derivative of methyl β-D-apiofuranoside (55 mg) was dissolved in THF (2 ml) and the solution was treated with tetra-*n*-butylammonium chloride as described above to afford methyl β-D-apiofuranoside (**6b**, 35 mg). Compounds **6a** and **6b** were identified by comparing the $[\alpha]_D$ and ^1H-NMR data (in D_2O) with reported values.[10)]

6a: Colorless syrup. *Anal.* Calcd for $C_6H_{12}O_5 \cdot 2H_2O$: C, 39.56; H, 7.75. Found: C, 39.41; H, 7.55. $[\alpha]_D^{23}$ +116° (c=0.11, MeOH): +133° (c=0.20, H_2O). ^1H-NMR (90 MHz, pyridine-d_5, δ): 3.47 (3H, s, methoxyl), 4.07 (2H, s, 5-H_2), 4.21, 4.57 (both 1H, ABq, J=9.5 Hz, 4-H_2), 4.57 (1H, d, J=4.6 Hz, 2-H), 5.15 (1H, d, J=4.6 Hz, 1-H); (90 MHz, D_2O, δ): 3.42 (3H, s, methoxyl), 3.59 (2H, s, 5-H_2), 3.87, 4.05 (both 1H, ABq, J=9.5 Hz, 4-H_2), 4.00 (1H, d, J=4.6 Hz, 2-H), 4.98 (1H, d, J=4.6 Hz, 1-H). ^{13}C-NMR: as given in Table II.

6b: Colorless syrup. *Anal.* Calcd for $C_6H_{12}O_5 \cdot 2H_2O$: C, 39.56; H, 7.75. Found: C, 39.33; H, 7.51. $[\alpha]_D^{23}$ −95° (c=0.16, MeOH): −98° (c=0.22, H_2O). ^1H-NMR (500 MHz, pyridine-d_5, δ): 3.41 (3H, s, methoxyl), 4.08 (2H, s, 5-H_2), 4.29, 4.45 (both 1H, ABq, J=9.5 Hz, 4-H_2), 4.60 (1H, d, J=2.4 Hz, 2-H), 5.35 (1H, d, J=2.4 Hz, 1-H); (90 MHz, D_2O, δ): 3.43 (3H, s, methoxyl), 3.63 (2H, s, 5-H_2), 3.86, 4.15 (both 1H, ABq, J=10.0 Hz, 4-H_2), 3.93 (1H, d, J=3.5 Hz, 2-H), 4.96 (1H, d, J=3.5 Hz, 1-H). ^{13}C-NMR: as given in Table II.

Diazomethane Methylation of Apioglycyrrhizin (1) Giving 1a, 1c, and 1d A solution of **1** (50 mg) in MeOH (3 ml) was treated with ethereal diazomethane (excess amount, *ca.* 300 ml). The mixture was left standing for 24 h, then the solvent was removed under reduced pressure to furnish a product. The product was purified by silica gel column chromatography [SiO_2 5 g, $CHCl_3$–MeOH (8 : 1)] to afford **1a** (23 mg, 45%), **1c** (16 mg, 30%), and **1d** (8 mg, 15%).

1c: mp 138—139 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +41° (c=0.40, $CHCl_3$). *Anal.* Calcd for $C_{44}H_{68}O_{14} \cdot 3H_2O$: C, 60.39; H, 8.52. Found: C, 60.34; H, 8.35. FAB-MS (positive, %): 821 [$(M+H)^+$, 3], 467 [$(M-\text{sugars}+H)^+$, 100]. UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 249 (9300). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3500—3100 (br), 2945, 1727, 1651, 1380, 1044. ^1H-NMR (500 MHz, pyridine-d_5, δ): 0.78, 1.10, 1.14, 1.20, 1.26, 1.38 1.42 (all 3H, s), 3.08 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.34 (1H, dd, J=4.4, 10.6 Hz, 3α-H), 3.69, 3.71, 3.74 (each 3H, s, OCH_3×3), 4.12 (1H, dd, J=9.0, 9.0 Hz, 3′-H), 4.17 (1H, dd, J=7.6, 9.0 Hz, 2′-H), 4.27 (2H, br s, 5″-H_2), 4.33, 4.69 (both 1H, ABq, J=9.2 Hz, 4″-H_2), 4.38 (1H, dd, J=9.0, 9.8 Hz, 4′-H), 4.44 (1H, d, J=2.0 Hz, 2″-H), 4.52 (1H, d, J=9.8 Hz, 5′-H), 4.99 (1H, d, J=7.6 Hz, 1′-H), 5.87 (1H, s, 12-H), 6.38 (1H, d, J=2.0 Hz, 1″-H). ^{13}C-NMR: as given in Table I.

1d: mp 133—135 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +25° (c=0.40, $CHCl_3$). *Anal.* Calcd for $C_{44}H_{68}O_{14} \cdot 3H_2O$: C, 60.39; H, 8.52. Found: C, 60.22; H, 8.47. FAB-MS (positive, %): 821 [$(M+H)^+$, 2], 467 [$(M-\text{sugars}+H)^+$, 100]. UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 249 (9300). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3550—3100 (br), 2940, 1727, 1651, 1400, 1044. ^1H-NMR (500 MHz, pyridine-d_5, δ): 0.78, 1.09, 1.10, 1.18, 1.24, 1.32, 1.41 (all 3H, s), 3.03 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.35 (1H, dd, J=4.2, 11.6 Hz, 3α-H), 3.61, 3.71, 3.72 (each 3H, s, OCH_3×3), 4.10 (1H, dd, J=9.0, 9.0 Hz, 3′-H), 4.20 (1H, dd, J=7.7, 9.0 Hz, 2′-H), 4.24 (2H, br s, 5″-H_2), 4.41, 4.55 (both 1H, ABq, J=9.8 Hz, 4″-H_2), 4.47 (1H, d, J=9.8 Hz, 5′-H), 4.85 (1H, d, J=2.0 Hz, 2″-H), 4.90 (1H, d, J=7.7 Hz, 1′-H), 5.85 (1H, s, 12-H), 6.20 (1H, d, J=2.0 Hz, 1″-H). ^{13}C-NMR: as given in Table I.

$NaBH_4$ Reduction of 1c Followed by Methanolysis A solution of **1c** (4 mg) in MeOH (3 ml) was treated with $NaBH_4$ (5 mg) and the whole was stirred at room temperature (24 °C) for 2 h. The reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and the resin was removed by filtration. After evaporation of the solvent from the filtrate under reduced pressure, the product was dissolved in 9% HCl–MeOH (0.5 ml) and the whole solution was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the inorganic materials were removed by filtration. Removal of the solvent from the filtrate under reduced pressure gave a product, which was dissolved in pyridine (0.3 ml) and the solution was treated with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA, 0.3 ml) to afford the trimethylsilyl (TMS) derivatives of methyl 2-*O*-methylapiopyranoside and methyl glucopyranoside; these were identical with authentic samples by GLC comparison. GLC conditions: 2% OV-17 on Chromosorb WAWDMCS (80—100 mesh); 3 mm (i.d.) × 2 m glass column; column temperature 160 °C; N_2 flow rate 40 ml/min; t_R, methyl 2-*O*-methyl-3,5-di-*O*-trimethylsilylapiofuranoside, 3 min 42 s, 4 min 57 s and methyl 2,3,4,6-tetra-*O*-trimethylsilylglucopyranoside, 7 min 30 s, 11 min 10 s.

$NaBH_4$ Reduction of 1d Followed by Methanolysis A solution of **1d** (4 mg) in MeOH (3 ml) was treated with $NaBH_4$ (5 mg) and the whole was stirred at room temperature (24 °C) for 2 h. The reaction mixture was worked up and the product was subjected to methanolysis with 9% HCl–MeOH (0.5 ml) as described above for **1c**. Silylation of the methanolysis product with BSTFA–pyridine (1 : 1, 0.5 ml) gave the trimethylsilyl derivatives of methyl 3-*O*-methylapiofuranoside and methyl glucopyranoside, which were identical with authentic samples on GLC (under the same conditions as described above for **1c**): methyl 3-*O*-methyl-2,5-di-*O*-trimethylsilylapiofuranoside, 4 min 10 s, 5 min 34 s and methyl 2,3,4,6-tetra-*O*-trimethylsilylglucopyranoside, 7 min 30 s, 11 min 10 s.

Diazomethane Methylation of Glycyrrhizin (3) Giving 3a, 3c, and 3d A solution of **3** (100 mg) in MeOH (5 ml) was treated with an excess of ethereal diazomethane (*ca.* 500 ml) and the whole mixture was left standing for 24 h. Evaporation of the solvent from the reaction mixture under reduced pressure gave a product, which was purified by silica gel column chromatography [SiO_5 5 g, $CHCl_3$–MeOH (8 : 1)] to furnish **3a** (39 mg, 38%), **3c** (26 mg, 25%), and **3d** (30 mg, 29%).

3c: mp 243—245 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +40° (c=0.60, $CHCl_3$). *Anal.* Calcd for $C_{46}H_{70}O_{16} \cdot 2H_2O$: C, 60.37; H, 8.15. Found: C, 60.27; H, 8.08. UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 249 (11800). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3550—3100 (br), 2955, 1724, 1655, 1440, 1030. ^1H-NMR (500 MHz, pyridine-d_5, δ): 0.79, 1.10, 1.17, 1.19, 1.27, 1.37, 1.39 (all 3H, s), 3.00 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.36 (1H, dd, J=4.2, 11.0 Hz, 3α-H), 3.70, 3.73, 3.79, 3.87 (each 3H, s, OCH_3×4), 4.10—4.55 (8H, m, sugar protons), 4.97 (1H, d, J=8.0 Hz, 1′-H), 5.66 (1H, d, J=7.8 Hz, 1″-H), 5.86 (1H, s, 12-H). ^{13}C-NMR: as given in Table I.

3d: mp 238—240 °C (colorless fine needles from MeOH), $[\alpha]_D^{23}$ +46° (c=0.83, $CHCl_3$). *Anal.* Calcd for $C_{46}H_{70}O_{16} \cdot 2H_2O$: C, 60.37; H, 8.15. Found: C, 60.39; H, 8.10. UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε): 249 (11200). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3500—3100 (br), 2940, 1726, 1660, 1440, 1040. ^1H-NMR (500 MHz, pyridine-d_5, δ): 0.78, 1.10, 1.13, 1.18, 1.28, 1.31, 1.39 (all 3H, s), 3.01 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.35 (1H, dd, J=4.2, 11.0 Hz, 3α-H), 3.70, 3.72, 3.83, 3.87 (each 3H, s, OCH_3×4), 4.09—4.50 (8H, m, sugar protons), 4.94 (1H, d, J=7.6 Hz, 1′-H), 5.32 (1H, d, J=7.7 Hz, 1″-H), 5.86 (1H, s, 12-H). ^{13}C-NMR: as given in Table I.

$NaBH_4$ Reduction of 3c and 3d and Subsequent Methanolysis A solution of **3c** (5 mg) in MeOH (3 ml) was treated with $NaBH_4$ (8 mg) and the whole was stirred at room temperature (24 °C) for 2 h. The reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure gave a product, which was dissolved in 9% HCl–MeOH (0.5 ml) and the solution was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the inorganic materials were filtered off. Removal of the solvent from the filtrate under reduced pressure provided a product, which was subjected to trimethylsilylation with BSTFA–pyridine (1 : 1, 0.5 ml) as described above for **1c**. The products were identified by GLC with the trimethylsilyl derivatives of methyl 2-*O*-methylglucopyranoside and methyl glucopyranoside.

Compound **3d** (5 mg) was subjected to the same treatment as described above for **3c** and the products were identified by GLC with the trimethylsilyl derivatives of methyl 3-*O*-methylglucopyranoside and methyl glucopyranoside. GLC conditions: 2% OV-17 on Chromosorb WAWDMCS (80—100 mesh); 3 mm (i.d.) × 2 m glass column; column temperature 160 °C; N_2 flow rate 40 ml/min; t_R, methyl 2-*O*-methyl-3,4,6-tri-*O*-trimethylsilylglucopyranoside, 9 min 35 s, 14 min 22 s and methyl 2,3,4,6-tetra-*O*-trimethylsilylglucopyranoside, 7 min 30 s, 11 min 10 s for **3c**; methyl 3-*O*-methyl-2,4,6-tri-*O*-trimethylsilylglucopyranoside, 10 min 2 s, 14 min 45 s and methyl 2,3,4,6-tetra-*O*-trimethylsilylglucopyranoside, 7 min 30 s, 11 min 10 s for **3d**.

Acetylation of 3a Giving 7a A solution of **3a** (5 mg) in pyridine (2 ml) was treated with acetic anhydride (1 ml) and the mixture was left standing at room temperature (25 °C) for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with ethyl acetate. The ethyl acetate extract was worked up in the usual manner to furnish **7a** (5 mg).

7a: Colorless syrup, $[\alpha]_D^{23}$ +38° (c=0.20, $CHCl_3$). High-resolution SIMS (positive): Calcd for $C_{55}H_{78}O_{21}Na$ $(M+Na)^+$: 1097.4933; Found: 1097.4992. IR ν_{max}^{KBr} cm^{-1}: 2945, 1726, 1660, 1625, 1420, 1060. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 0.81, 0.82, 1.03, 1.12, 1.13, 1.15, 1.36 (all 3H, s), 1.99, 2.00, 2.03, 2.04, 2.11 (all 3H, s, acetyl methyl × 5), 2.82 (1H, d, J=*ca.* 13 Hz, 18-H), 3.26 (1H, dd, J=4.4, 11.0 Hz, 3α-H), 3.69, 3.71, 3.71 (all 3H, s, $OCH_3 \times 3$), 3.85 (1H, dd, J=7.6, 9.2 Hz, 2′-H), 3.99 (1H, d, J=10.1 Hz), 4.00 (1H, d, J=10.1 Hz) [5′-H, 5″-H], 4.50 (1H, d, J=7.6 Hz, 1′-H), 4.73 (1H, d, J=8.3 Hz, 1″-H), 4.92 (1H, dd, J=8.3, 9.2 Hz, 2″-H), 5.10—5.23 (4H, m, other sugar protons), 5.66 (1H, s, 12-H).

Acetylation of 3c Giving 7c A solution of **3c** (3 mg) in pyridine (2 ml) was treated with acetic anhydride (1 ml) and the mixture was left standing at room temperature (25 °C) for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with ethyl acetate. The ethyl acetate extract was worked up in the usual manner to give **7c** (3 mg).

7c: Colorless syrup, $[\alpha]_D^{23}$ +47° (c=0.18, $CHCl_3$). High-resolution SIMS (positive): Calcd for $C_{54}H_{79}O_{20}$ $(M+H)^+$: 1047.5164; Found: 1047.5200. IR ν_{max}^{KBr} cm^{-1}: 2940, 1728, 1665, 1620, 1433, 1080. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 0.80, 0.81, 1.02, 1.12, 1.13, 1.15, 1.36 (all 3H, s), 1.99, 2.01, 2.06, 2.07 (all 3H, s, acetyl methyl × 4), 2.84 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.22 (1H, dd, J=4.2, 10.8 Hz, 3α-H), 3.39, 3.69, 3.70, 3.75 (all, 3H, s, $OCH_3 \times 4$), 3.83 (1H, dd, J=7.6, 9.4 Hz, 2′-H), 3.94 (1H, d, J=9.8 Hz), 4.04 (1H, d, J=9.8 Hz) [5′-H, 5″-H], 4.53 (1H, d, J=7.6 Hz), 4.55 (1H, d, J=7.6 Hz) [1′-H, 1″-H], 5.04—5.28 (5H, m, other sugar protons), 5.67 (1H, s, 12-H).

Acetylation of 3d Giving 7d A solution of **3d** (3 mg) in pyridine (2 ml) was treated with acetic anhydride (1 ml). The reaction mixture was treated as described above for **3a** to give **7d** (3 mg).

7d: Colorless syrup, $[\alpha]_D^{23}$ +41° (c=0.18, $CHCl_3$). High-resolution SIMS (positive): Calcd for $C_{54}H_{78}O_{20}Na$ $(M+Na)^+$: 1069.4984; Found: 1069.5033. IR ν_{max}^{KBr} cm^{-1}: 2920, 1728, 1663, 1615, 1430, 1090. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 0.80, 0.82, 1.02, 1.13, 1.14, 1.26, 1.34 (all 3H, s), 2.00, 2.01, 2.04, 2.08 (all 3H, s, acetyl methyl × 4), 2.86 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.24 (1H, dd, J=4.0, 11.0 Hz, 3α-H), 3.47, 3.69, 3.71, 3.72 (all 3H, s, $OCH_3 \times 4$), 3.74 (1H, dd, J=7.6, 9.5 Hz, 2′-H), 3.82 (1H, d, J=10.0 Hz), 3.99 (1H, d, J=9.5 Hz) [5′-H, 5″-H], 4.41 (1H, d, J=7.6 Hz, 1′-H), 4.79 (1H, d, J=7.9 Hz, 1″-H), 5.03 (1H, dd, J=7.9, 9.5 Hz, 2″-H), 5.11—5.28 (4H, m, other sugar protons), 5.66 (1H, s, 12-H).

Diazomethane Methylation of Araboglycyrrhizin (2) Giving Dimethyl Ester (2a) A solution of **2** (30 mg) in MeOH (3 ml) was treated with ethereal diazomethane (*ca.* 30 ml) until the yellow color persisted. The mixture was left standing for 1 h, then the solvent was removed by evaporation under reduced pressure to furnish the dimethyl ester (**2a**, 31 mg).

2a: mp 135—136 °C (colorless fine prism from MeOH), $[\alpha]_D^{23}$ +27° (c=0.60, MeOH). *Anal.* Calcd for $C_{43}H_{66}O_{14} \cdot 2H_2O$; C, 61.26; H, 8.37. Found: C, 61.08; H, 8.35. UV λ_{max}^{MeOH} nm (ε): 249 (11650). IR ν_{max}^{KBr} cm^{-1}: 3500—3200 (br), 2926, 1722, 1641, 1380, 1055. ^{1}H-NMR (500 MHz, pyridine-d_5, δ): 0.79, 1.10, 1.12, 1.18, 1.28, 1.32, 1.41 (all 3H, s), 3.05 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.36 (1H, dd, J=4.4, 10.8 Hz, 3α-H), 3.69, 3.72 (both, 3H, s, $-OCH_3 \times 2$), 3.74 (1H, dd, J=1.6, 12.2 Hz, 5″-Hz), 4.13 (1H, dd, J=7.6, 9.0 Hz, 2′-H), 4.22 (1H, dd, J=3.4, 8.2 Hz, 3″-H), 4.26—4.38 (3H, m, 3′-H, 4′-H, 5′-H), 4.44 (1H, dd, J=3.1, 12.2 Hz, 5″-H), 4.54 (1H, dd, J=6.7, 8.2 Hz, 2″-H), 4.96 (1H, d, J=7.6 Hz, 1′-H), 5.16 (1H, d, J=6.7 Hz, 1″-H), 5.86 (1H, s, 12-H). ^{13}C-NMR: as given in Table I.

Methanolysis of Araboglycyrrhizin (2) A solution of **2** (3 mg) in 9% HCl–MeOH (1 ml) was heated under reflux for 3 h. The reaction mixture was neutralized with Dowex 1 × 2 (OH^- form) and the resin was removed by filtration. The reaction products in the filtrate were shown by TLC [$CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase), *n*-BuOH–AcOEt–H_2O (4 : 1 : 5, upper phase)] to be identical with authentic glycyrrhetic acid (**4**), methyl glucuronide and methyl arabinoside.

$NaBH_4$ Reduction of 2a Giving 2b A solution of **2a** (25 mg) in MeOH (5 ml) was treated with $NaBH_4$ (15 mg) and the mixture was stirred at room temperature (24 °C) for 2 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was filtered off. Evaporation of the solvent from the filtrate under reduced pressure gave a product, which was purified by silica gel column chromatography [SiO_2 2 g, $CHCl_3$–MeOH (5 : 1)] to provide **2b** (14 mg).

2b: mp 174—175 °C (colorless fine prisms from MeOH), $[\alpha]_D^{23}$ +65° (c=0.40, MeOH). *Anal.* Calcd for $C_{43}H_{68}O_{13} \cdot 3H_2O$: C, 60.97; H, 8.80. Found: C, 61.00; H, 8.80. UV λ_{max}^{MeOH} nm (ε): 249 (9300). IR ν_{max}^{KBr} cm^{-1}: 3500—3210 (br), 2940, 1728, 1662, 1387, 1050. ^{1}H-NMR (500 MHz, pyridine-d_5, δ): 0.79, 1.10, 1.15, 1.20, 1.28, 1.34, 1.42 (all 3H, s), 3.04 (1H, br d, J=*ca.* 13 Hz, 18-H), 3.40 (1H, dd, J=4.0, 11.0 Hz, 3α-H), 3.72 (3H, s, OCH_3), 3.80 (1H, dd, J=2.0, 12.2 Hz, 5″-H), 4.18 (1H, dd, J=7.6, 8.8 Hz, 2′-H), 4.26 (1H, dd, J=3.4, 8.5 Hz, 3″-H), 4.30—4.38 (5H, m, 3′-H, 4′-H, 5′-H, 6′-H, 4″-H), 4.42 (1H, dd, J=3.5, 12.2 Hz, 5″-H), 4.51 (1H, dd, J=2.2, 12.0 Hz, 6′-H), 4.60 (1H, dd, J=6.7, 8.5 Hz, 2″-H), 4.92 (1H, d, J=7.6 Hz, 1′-H), 5.20 (1H, d, J=6.7 Hz, 1″-H), 5.89 (1H, s, 12-H). ^{13}C-NMR (125 MHz, pyridine-d_5, δ_C): 88.7 (C-3), 199.4 (C-11), 128.7 (C-12), 168.8 (C-13), 176.7 (C-30), 51.5 (30-OCH_3), 104.9 (C-1′), 83.7 (C-2″), 78.1 (C-3′), 71.6 (C-4′), 78.5 (C-5′), 62.7 (C-6′), 106.5 (C-1″), 73.6 (C-2″), 74.1 (C-3″), 68.9 (C-4″), 66.8 (C-5″).

Methanolysis of 2b A solution of **2b** (100 mg) in 9% HCl–MeOH (3 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 1 × 2 (OH^- form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure provided a product, which was subjected to silica gel column chromatography [SiO_2 5 g, $CHCl_3$–MeOH (10 : 1)] to afford methyl glycyrrhetate (**4a**, 45 mg), methyl D-glucopyranoside (14 mg) and methyl L-arabinoside (10 mg). Methyl L-arabinoside (8 mg), obtained from **2b**, was dissolved in 3% H_2SO_4 (2 ml) and the mixture was heated under reflux for 2 h. The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate provided L-arabinose [$[\alpha]_D^{23}$ +97° (c=0.50, 24 h after dissolving in H_2O)]. A solution of methyl D-glucopyranoside (8 mg, obtained from **2b**) in 3% H_2SO_4 (2 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was worked up as described above to furnish D-glucose [$[\alpha]_D^{23}$ +47° (c=0.50, 24 h after dissolving in H_2O)].

Complete Methylation of 2b Followed by Methanolysis A solution of **2b** (5 mg) in DMSO (0.2 ml) was treated with a dimsyl carbanion solution (1 ml, prepared from NaH 100 mg and DMSO 2 ml) and the mixture was stirred in the dark at room temperature (24 °C) for 1 h. The reaction mixture was then treated with methyl iodide (1 ml) and the whole was stirred for a further 2 h. The reaction mixture was poured into ice-water and the whole was extracted with ethyl acetate. The ethyl acetate extract was worked up in the usual manner to afford a product (5.1 mg). Without further purification, the product was dissolved in 9% HCl–MeOH (1 ml) and the solution was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the products were shown by TLC [benzene–acetone (2 : 1)] and GLC to be identical with methyl 2,3,4-tri-*O*-methylarabinopyranoside and methyl 3,4,6-tri-*O*-methylglucopyranoside. GLC conditions: 5% DEGS on Uniport B (80—100 mesh); 3 min (i.d.) × 2 m glass column; column temperature 160 °C; N_2 flow rate 40 ml/min; t_R: methyl 2,3,4-tri-*O*-methylarabinopyranoside, 1 min 50 s and methyl 3,4,6-tri-*O*-methylglucopyranoside, 5 min 30 s, 8 min 10 s.

Sensory Evaluation for Relative Sweetness Sweetness relative to sucrose was evaluated by a human sensory panel. All samples were dissolved in water to make 0.01% (w/v) solutions and sucrose solutions were prepared at graduated concentrations from 0.5 to 5.0% (w/v) with an interval of 0.5% (w/v). All samples were evaluated by twelve panelists. The panelists were asked to taste a sample solution and to estimate its sweetness intensity relative to that of the sucrose solution of appropriate concentration. Panelists were asked to taste each sample in this way four times.

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