Xanthone *C*-Glycoside and Acylated Sugar from *Polygala tenuifolia*

Yukinobu IKEYA,* Kô SUGAMA, and Masao MARUNO

Tsumura Central Research Laboratories, 3586 Yoshiwara Ami-machi, Inashiki-gun, Ibaraki 300-11, Japan.
Received May 30, 1994; accepted August 5, 1994

A new xanthone *C*-glycoside, polygalaxanthone III (1), and a new acylated sugar, tenuifoliside E (2) were isolated from the roots of *Polygala tenuifolia*. Their structures were characterized as 4-*C*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-1,3,6-trihydroxy-7-methoxyxanthone (1) and β-D-(1-*O*-acetyl-3-*O*-feruloyl-6-*O*-sinapoyl)-fructofuranosyl-α-D-(2,4,6-*O*-triacetyl)glucopyranoside (2), respectively, on the basis of chemical and spectral evidence including two dimensional nuclear magnetic resonance (2D-NMR) studies.

Keywords *Polygala tenuifolia*; polygalaxanthone III; tenuifoliside E; Polygalaceae; phenolic glycoside

Crude drug "Yuanzhi (Japanese name: Onji)", the roots of *Polygala tenuifolia* WILLD. (Polygalaceae) is a well-known Chinese medicine used as an expectorant, tonic, sedative and for treatment of amnesia. Various xanthones, saponins and multi-acylated oligosaccharides have been isolated from this material by Ito *et al.*,[1] Sakuma and Shoji,[2] and Miyase *et al.*[3] We previously reported the isolation and structure determination of five phenylpropanoid sugar esters from this plant.[4] This paper deals with the isolation and structural elucidation of a new xanthone *C*-glycoside (**1**) and a new phenylpropanoid sucrose ester (**2**) from this material.

Polygalaxanthone III (**1**) was obtained as a yellow amorphous powder and its molecular formula was determined to be $C_{25}H_{28}O_{15}$ from the high-resolution fast atom bombardment mass spectrum (FAB-MS). The infrared (IR) spectrum of **1** showed the presence of hydroxyl groups (3408 cm^{-1}), a chelated ketone (1640 cm^{-1}) and aromatic rings (1614 cm^{-1}). The ultraviolet (UV) spectrum in MeOH (λ_{max} 241, 258, 316 and 363 nm) is similar to that of 1,6-dihydroxy-3,7-dimethoxyxanthone (**3**; λ_{max} 234, 255, 312 and 364 nm),[5] suggesting that **1** is a 1,3,6,7-tetraoxygenated xanthone. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** (Table I) showed two anomeric proton signals at δ 5.73 (d, J=9.8 Hz) and 5.64 (d, J=2.7 Hz), three singlet aromatic proton signals at δ 6.60, 7.05 and 7.73, and one methoxyl signal at δ 3.91 (3H, s). Further, the ^{1}H-NMR spectrum of in **1** dimethylsulfoxide-d_6 (DMSO-d_6) showed a chelated hydroxyl (C-1–OH) signal at δ 13.81 (not appearing in pyridine-d_5). The carbon nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** (Table I) exhibited eleven

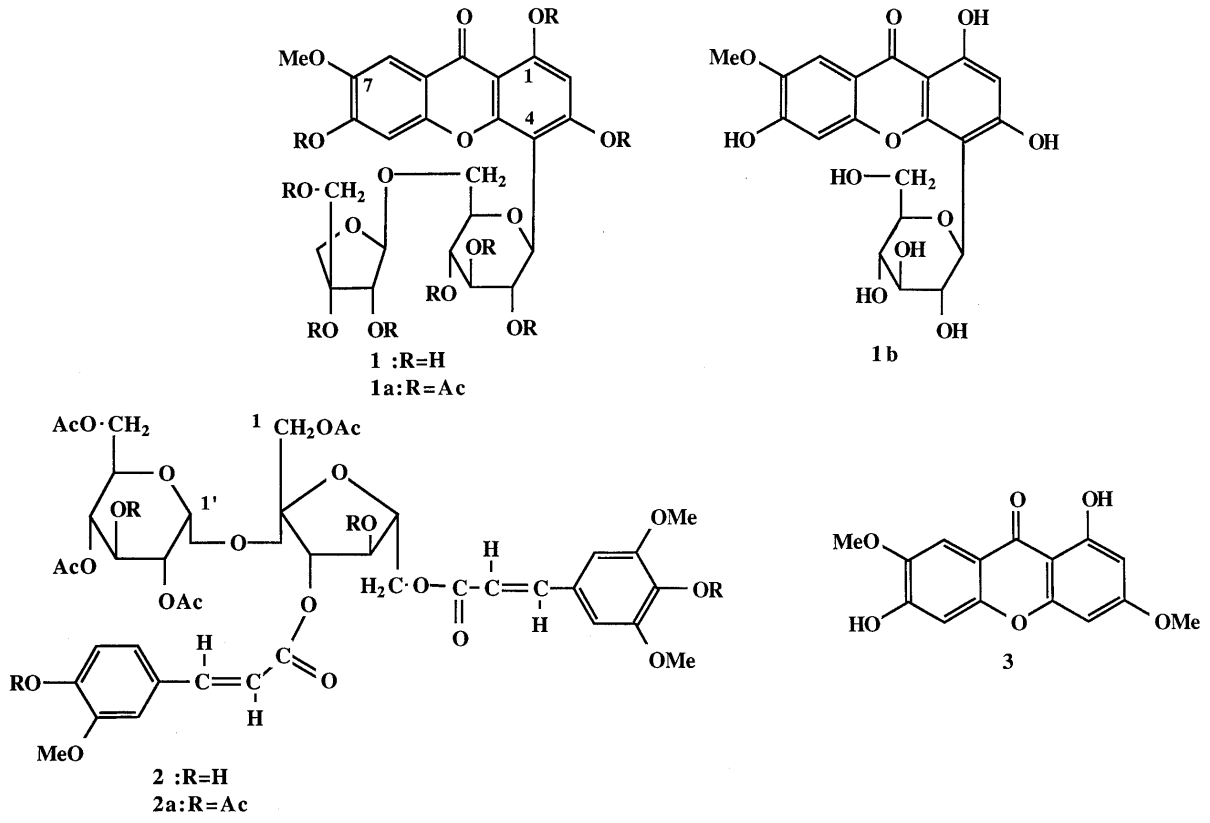

Chart 1

TABLE I. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds **1**, **1a**, **1b** and **3** in Pyridine-d_5

	^{1}H-NMR (500 MHz)			^{13}C-NMR (125 MHz)			
	1	**1a**[a,b]	**1b**	**1**	**1a**[a,c]	**1b**	**3**
Xanthone							
1				165.2[d]	157.4	163.3	163.8
2	6.60, s	7.23, s	6.62, s	94.6	110.6	95.1	97.4
3				165.3[d]	154.1	167.5	166.3
4				108.4	118.1	109.1	92.7
4a				157.4	149.6	157.8	158.4
4b				157.4	149.7	156.7	156.5
5	7.05, s	7.20, s	7.09, s	103.5	112.4	103.7	103.9
6				153.2	146.0	153.2	153.4
7				147.3	149.0	147.0	147.7
8	7.73, s	7.68, s	7.80, s	105.4	106.3	105.6	105.4
8a				111.9	120.4	112.4	112.7
9				179.9	174.0	179.7	180.2
9a				102.6	112.1	102.2	103.8
OMe	3.91, 3H, s	3.91, 3H, s	3.78, 3H, s	56.1	56.5	56.0	55.9, 56.0
Glucose							
1′	5.73, d (9.8)	4.85, d (10.2)	5.87, d (9.7)	75.3	72.3	75.8	
2′	5.17, t (9.8)	5.70, t (9.8)	5.28, t (9.7)	72.3	69.6	72.7	
3′	4.39, t (9.0)	5.31, t (9.8)	4.36, t (9.2)	80.2	74.5	80.8	
4′	4.35, t (9.0)	5.05, t (9.8)	4.53, t (9.2)	71.7	69.3	71.8	
5′	4.18[e]	3.78, ddd (9.8, 5.7, 2.4)	4.16, dt (9.2, 3.6)	81.2	77.8	82.8	
6′	4.23, dd (10.7, 5.5)	3.53, dd (12.1, 5.7)	3.75, 2H, d (3.6)	69.2	66.9	62.5	
	4.63, br d (10.7)	3.73, dd (12.1, 2.4)					
Apiose							
1″	5.64, d (2.7)	4.90, br s		110.7	106.7		
2″	4.65, d (2.7)	5.25, d (0.8)		77.6	76.0		
3″				80.3	83.7		
4″	4.30, d (9.4)	4.11, d (10.5)		74.8	72.4		
	4.53, d (9.4)	4.18, d (10.5)					
5″	4.13, 2H, s	4.50, d (12.3)		65.2	62.9		
		4.65, d (12.3)					

J values in Hz. Abbreviations: br=broad, d=doublet, s=singlet, t=triplet. *a*) This compound was measured in $CDCl_3$. *b*) Other signals: δ 1.79, 1.98, 1.99, 2.02, 2.06, 2.07, 2.35, 2.45, 2.54 (each 3H, s, 9×OAc). *c*) Other signals: δ 20.4, 20.5, 20.6, 20.7×3, 21.1, 21.3, 21.4, 167.8, 168.0, 168.6, 169.0, 169.6, 169.7, 169.8, 170.3, 170.4 (9×OAc). *d*) Assignments within any vertical column may be reversed. e) Overlapping with other signals. Signal assignments of **1** and **1a** were confirmed by the ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY spectra.

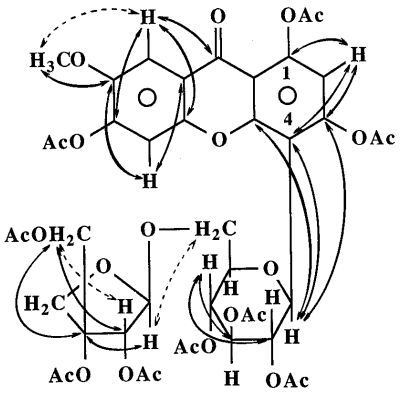

Fig. 1. NOE (←---→) and Long-Range Correlations (←——→) Observed in NOESYPH and COLOC Spectra of **1a** (in $CDCl_3$)

aliphatic carbon signals due to two sugar moieties. The C-5—C-8a signals of **1** are very similar to those of **3**, suggesting that **1** has a hydroxyl group and a methoxyl group at the C-6 and C-7 positions, respectively, like **3**. The correlation spectroscopy *via* long-range coupling (COLOC) spectrum of the peracetate (**1a**) of **1** supported the 1,3,6-triacetoxy-7-methoxyxanthone structure in **1a** as shown in Fig. 1. The phase-sensitive nuclear Overhauser effect spectroscopy (NOESYPH) spectrum of **1a** (Fig. 1) showed appreciable NOE between the methoxyl signal (δ 3.91) and the H-8 signal (δ 7.68). This indicates that the methoxyl group is located at C-7.

The ^{13}C-NMR spectrum of **1** suggested the presence of β-apiofuranosyl moiety.[6] Methanolysis of **1** with 9% HCl in MeOH provided methyl α-apiofuranoside and methyl β-apiofuranoside together with **1b**, $C_{20}H_{20}O_{11}$. On treatment with 2 N HCl, **1** gave **1b** and D-apiose, $[\alpha]_D$ +7.9° (H_2O). These facts indicate the presence of a D-apiofuranosyl moiety in **1**. The β-anomeric configuration of the D-apiofuranosyl moiety in **1** was confirmed by Klyne's rule[7] ($[M]_D$ of **1**-$[M]_D$ of **1b** value is −101°; $[M]_D$ of methyl β-D-apiofuranoside is −156°).[8] In the ^{13}C-NMR spectrum of **1b**, six aliphatic carbon signals due to a sugar moiety were appeared. The appearance of the anomeric carbon signal at higher field (δ 75.3) and the resistance to hydrolysis with HCl suggested that **1b** possessed a *C*-glycoside nature. Ferric chloride oxidation of **1b** afforded D-glucose as major product and arabinose as minor product, indicating that the sugar moiety in **1b** is D-glucose.[9,10] In the ^{13}C-NMR spectra of **1** and **1b**, the C-6 signal (δ 62.5) in the glucopyranosyl moiety of **1b** showed an upfield shift of 6.7 ppm compared with that (δ 69.2) of **1**, showing the oligosaccharide part of **1** to be

TABLE II. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds **2** and **2a** in CD_3OD

	^{1}H-NMR (500 MHz)		^{13}C-NMR (125 MHz)
	2[a]	**2a**[a]	**2**[b]
Fructose			
1	4.09, d (11.5)	4.30, 2H, s	66.7
	4.26, d (11.5)		
2			104.0
3	5.35, d (7.8)	5.60, d (5.4)	79.6
4	4.42, t (7.8)	5.55, t (5.4)	73.8
5	4.22, ddd (7.8, 4.9, 4.2)	4.42, ddd (6.1, 5.4, 4.6)	81.6
6	4.50, dd (11.6, 4.9)	4.48, dd (11.8, 6.1)	64.6
	4.53, dd (11.6, 4.2)	4.57, dd (11.8, 4.6)	
Glucose			
1′	5.68, d (3.7)	5.75, d (3.7)	90.5
2′	4.82, dd (10.1, 3.7)	4.89, dd (10.4, 3.7)	74.0
3′	4.06, dd (10.1, 9.4)	5.42, dd (10.4, 9.5)	69.9
4′	4.84, dd (10.3, 9.4)	4.98, dd (10.3, 9.5)	72.4
5′	4.24, ddd (10.3, 5.7, 2.4)	4.31, dt (10.3, 3.7)	70.1
6′	4.12, dd (11.9, 5.7)	4.19, 2H, d (3.7)	64.2
	4.17, dd (11.9, 2.4)		
Feruloyl			
1″			127.6
2″	7.12, d (1.8)	7.41, d (1.9)	112.0
3″			149.6
4″			151.2
5″	6.93, d (7.5)	7.06, d (8.1)	116.7
6″	7.11, d (7.5, 1.8)	7.24, dd (8.1, 1.9)	124.6
7″	7.65, d (15.8)	7.79, d (16.0)	148.3
8″	6.35, d (15.8)	6.62, d (16.0)	114.8
9″			168.1
OMe	3.95, 3H, s	3.88, 3H, s	56.7
Sinapoyl			
1‴			126.8
2‴, 6‴	6.80, 2H, s	6.99, 2H, s	107.1
3‴, 5‴			149.6
4‴			139.9
7‴	7.70, d (15.9)	7.73, d (16.0)	147.5
8‴	6.37, d (15.9)	6.60, d (16.0)	115.7
9‴			168.8
OMe	3.92, 6H, s	3.85, 6H, s	57.0

J values in Hz. Abbreviations: d=doublet, s=singlet, t=triplet. *a*) Other signals: 2, δ 1.96, 2.05, 2.12, 2.13 (each 3H, s, 4×OAc); **2a**, δ 1.91, 1.93, 1.98, 2.07, 2.12, 2.13 (each 3H, s, 6×OAc), 2.26 (6H, s, 2×OAc). *b*) Other signals: δ 20.7×2, 20.8, 21.0, 171.8, 172.1, 172.2, 172.7 (4×OAc). All assignments were confirmed by the ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY spectra.

an apiofuranosyl-(1→6)-β-glucopyranosyl structure. This was confirmed from the NOE experiment of **1a** (Fig. 1). The position of the glucopyranosyl moiety was determined to link at the C-4 position of xanthone nucleus from the COLOC spectrum as shown in Fig. 1. Consequently, the structure of polygalaxanthone III was determined to be 4-*C*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-1,3,6-trihydroxy-7-methoxyxanthone (**1**).

Tenuifoliside E (**2**) was obtained as a white amorphous powder and its high-resolution FAB-MS gave an $[M+H]^+$ ion peak at *m/z* 893.2705 ($C_{41}H_{49}O_{22}$). The IR spectrum of **2** showed the presence of hydroxyl groups (3448 cm^{-1}), carbonyl groups (1744 and 1724 cm^{-1}), a double bond (1632 cm^{-1}) and an aromatic ring (1604 cm^{-1}). The ^{1}H-NMR spectrum (Table II) showed that **2** possesses four acetyl groups, a feruloyl group and a sinapoyl group. The ^{13}C-NMR spectrum of **2** (Table II) showed twelve carbon signals arising from a disaccharide moiety. The appearance of two anomeric carbon signals at δ 104.0 and 90.5 suggested the disaccharide to be sucrose.[11] On alkaline methanolysis of **2** with 3% sodium methoxide in methanol, **2** afforded methyl ferulate, methyl

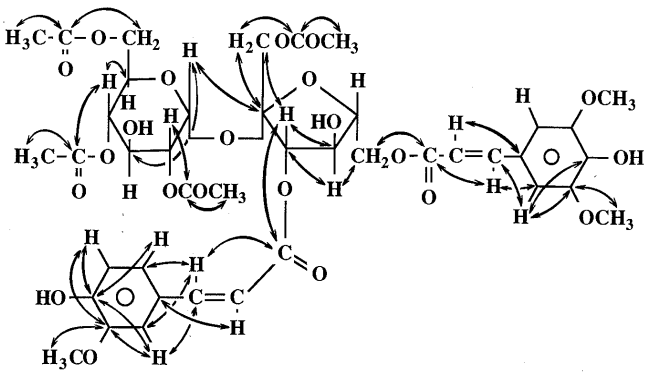

Fig. 2. Long-Range Correlations (⟷) Observed in HMBC Spectrum of **2** (in CD_3OD)

sinapate and sucrose. From these observations, **2** was suggested to be a sucrose ester having three acetyl, one feruloyl and one sinapoyl groups.

The ^{1}H-NMR spectrum of the peracetate (**2a**) of **2** (Table II) showed signals belonging to two aromatic acetyl groups at δ 2.26 (6H, s)and two new aliphatic acetyl groups in addition to four aliphatic acetyl signals in **2**. The H-4 signal of the fructofuranosyl moiety and the H-3′ signal of the glucopyranosyl moiety in the ^{1}H-NMR spectrum of **2a** showed downfield shifts of 1.05 and 1.50 ppm, respectively, compared with those of **2**. This indicates the presence of C-4 and C-3′ free hydroxyl groups in **2**. From the heteronuclear multiple bond connectivity (HMBC) spectrum, the substitutive positions of the acyl groups in **2** were determined as shown in Fig. 2. From the above observations, tenuifoliside E was determined to be β-D-(1-*O*-acetyl-3-*O*-feruloyl-6-*O*-sinapoyl)fructofuranosyl-α-D-(2,4,6-*O*-triacetyl)glucopyranoside (**2**).

Though many xanthones have been isolated from *P. tenuifolia*, this is the first isolation of xanthone *C*-glycoside.

Experimental

The instruments used for obtaining physical data and the conditions for chromatography were the same as described in the previous paper.[4] Gas liquid chromatography (GLC) was carried out on a Shimadzu gas chromatograph GC-14A with hydrogen flame ionization detector.

Isolation 1 and 2 The dried roots (4.8 kg) of *P. tenuifolia* (purchased from Yamamoto Yakuhin Kogyo Co. Ltd., Tokyo) were pulverized and extracted three times with boiling MeOH (20 l, 3 h). The concentrated methanolic extract (1084.8 kg) was dissolved in H_2O and successively extracted with ether and BuOH. The BuOH solution was concentrated to give a dark mass (638.8 g). A part (408 g) of this mass was chromatographed on Sepabeads SP-207 (Mitsubishi Chemical Industries, Ltd.) (2.6 l), developing with H_2O (7 l), 20% MeOH (6.5 l), 40% MeOH (6.5 l) and then MeOH (6.5 l). The MeOH eluate was concentrated to give a residue (182.7 g), which was chromatographed on SiO_2 (1.15 kg) with a mixture of $CHCl_3$–MeOH. The fraction (0.58 g) eluted with $CHCl_3$–MeOH (9:1) was purified by preparative thin layer chromatography (prep. TLC) [hexane–acetone (1:1)] to give **2** (50 mg, yield 0.0016%). A part (13.6 g) of the fraction eluted with $CHCl_3$–MeOH (3:7) and MeOH was rechromatographed on Sephadex LH-20 (5 cm i.d. × 25 cm) developing with MeOH to give crude **1** (2.06 g). This crude **1** was purified by prep. TLC [EtOAc–MeOH–H_2O (6:2:1)] to give **1** (255 mg, yield 0.11%).

Polygalaxanthone III (1) A yellow amorphous powder, $[\alpha]_D^{24}$ −7.8° (*c*=0.602, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3408 (OH), 1640 (C=O), 1614 (aromatic ring). UV λ_{max}^{MeOH} nm (log ε): 207 (4.31), 241 (4.39), 258 (4.37), 316 (4.05), 363 (4.09). Positive ion FAB-MS *m/z*: 569 $[M+H]^+$. High-resolution FAB-MS, Calcd for $C_{25}H_{29}O_{15}$ ($[M+H]^+$): 569.1506. Found: 569.1505.

Tenuifoliside E (2) A white amorphous powder, $[\alpha]_D^{24}$ −45.5° (c=1.12, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3448 (OH), 1744, 1724 (C=O), 1632 (C=C), 1604 (aromatic ring). UV λ_{max}^{MeOH} nm (log ε): 202 (4.48), 220 (4.34), 237 (4.33), 330 (4.45). Negative ion FAB-MS m/z: 891 $[M-H]^-$. Positive ion FAB-MS m/z: 893 $[M+H]^+$. High-resolution FAB-MS, Calcd for $C_{41}H_{49}O_{22}$ ($[M+H]^+$): 893.2716. Found: 893.2705.

Acetylation of 1 A solution of **1** (45 mg) in a mixture of Ac_2O (0.25 ml), 4-(dimethylamino)pyridine (10 mg) and pyridine (0.5 ml) was stirred at 50 °C for 7 h, then diluted with ether. The ethereal solution was washed with 1 N HCl, 5% $NaHCO_3$, then H_2O and concentrated to give a residue. The residue was purified by prep. TLC [hexane–EtOAc (1:2)] to give **1a** (32 mg) as white powder (from ether–hexane), mp 122—125 °C, $[\alpha]_D^{24}$ +2.1° (c=0.960, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1780, 1754 (C=O), 1622 (aromatic ring). Positive ion FAB-MS m/z: 969 $[M+Na]^+$, 947 $[M+H]^+$. High-resolution FAB-MS, Calcd for $C_{43}H_{47}O_{16}$ ($[M+H]^+$): 947.2457. Found: 947.2508.

Methanolysis of 1 A solution of **1** (40 mg) in 9% HCl–MeOH (2 ml) was heated under reflux for 4 h. After cooling, the reaction mixture was neutralized with Amberlite IR-410 (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 prep. TLC [EtOAc–MeOH–H_2O (7:4:1)] to afford **1b** (Rf 0.83, 10.5 mg), crude methyl β-apiofuranoside (Rf 0.79, 5.1 mg) and methyl α-apiofuranoside (Rf 0.69, 2.8 mg) as colorless syrup. Crude methyl β-apiofuranoside was further purified by prep. TLC [acetone–CH_2Cl_2–H_2O (14:6:1)] to give methyl β-apiofuranoside (2.6 mg) as colorless syrup.

Methyl α-apiofuranoside and methyl β-apiofuranoside were identified by comparing the ^{1}H- and ^{13}C-NMR data with reported values.[6]

1b: A light yellow amorphous powder, $[\alpha]_D^{24}$ +12.9° (c=0.557, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 1644 (C=O), 1620 (aromatic ring). Positive ion FAB-MS m/z: 437 $[M+H]^+$. High-resolution FAB-MS, Calcd for $C_{20}H_{21}O_{11}$ ($[M+H]^+$): 437.1084. Found: 437.1081.

Hydrolysis of 1 A solution of **1** (60 mg) in 2 N HCl (2 ml) was stirred at room temperature for 48 h, then neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated and purified by prep. TLC [EtOAc–MeOH–H_2O (7:2:1)] to give a yellow amorphous powder (Rf 0.69, 18.7 mg) and a syrup (Rf 0.43, 10.5 mg), $[\alpha]_D^{23}$ +7.9° (c=0.33, 24 h after dissolving in H_2O), which was identified as D-apiose (an α,β-anomeric mixture) by comparison of TLC and $[\alpha]_D$ with those of D-apiose prepared from apigenin-7-O-apioside. Yellow amorphous powder was identical with **1b** by comparing the ^{1}H-NMR, IR and $[\alpha]_D$.

Oxidation of 1b with $FeCl_3$ A mixture of **1b** (12 mg) and $FeCl_3 \cdot 6H_2O$ (70 mg) in H_2O (2 ml) was refluxed for 8 h. The reaction mixture was passed through an Amberlite MB-3 column and the elute was concentrated to give a residue (8.4 mg). A part (0.5 mg) of the residue was treated with hexamethyldisilazane and trimethylchlorosilane in pyridine to afford trimethylsilyl (TMS) derivatives of glucose and arabinose; these were identical with authentic samples by GLC comparison. GLC conditions: column, HP-1 (0.31 mm i.d. × 25 m); He flow rate, 50 ml/min; column temperature, 100 °C→180 °C (5 °C/min); t_R, 1,2,3,4,6-penta-O-trimethylsilylglucopyranoside, 14.9, 17.0 min and 1,2,3,4-tetra-O-trimethylsilylarabinopyranoside, 9.2, 9.7 min. The residue (7.9 mg) was purified by prep. TLC [$CHCl_3$–MeOH–H_2O (20:10:1), Rf 0.15] to give a syrup (2.1 mg), $[\alpha]_D^{23}$ +44° (c=0.18, H_2O), which was identified as D-glucose by TLC [BuOH–acetone–H_2O (20:10:1), Rf 0.35].

Acetylation of 2 A solution of **2** (15 mg) in a mixture of Ac_2O (0.25 ml) and pyridine (0.5 ml) was allowed to stand at room temperature overnight, then diluted with ether. The ethereal solution was washed with 1 N HCl, 5% $NaHCO_3$ and H_2O, and then concentrated to give a residue. This residue was purified by prep. TLC [hexane–acetone (1:1)] to give **2a** (9.5 mg) as a white amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 1754 (C=O), 1638 (C=C), 1598 (aromatic ring). Positive ion FAB-MS m/z: 1083 $[M+Na]^+$. High-resolution FAB-MS, Calcd for $C_{49}H_{56}O_{26}Na$ ($[M+Na]^+$): 1083.2958. Found: 1083.2962.

Alkaline Methanolysis of 2 Methanolysis of **2** (4.0 mg) was carried out with 3% NaOMe in MeOH (1 ml) at room temperature for 30 min. The reaction mixture was passed through an Amberlite IR-120B column. Methyl ferulate, methyl sinapate and sucrose were detected in the mixture by TLC. Methyl ferulate: Rf 0.30 [hexane–acetone (2:1)]. Methyl sinapate: Rf 0.24 [hexane–acetone (2:1)]. Sucrose: Rf 0.35 [BuOH–acetone–H_2O (4:5:1)]; Rf 0.33 [$CHCl_3$–MeOH–H_2O (6:4:1)].

Acknowledgements The authors thank Mr. K. Kano, Mr. T. Katsuhara, and Miss Y. Imamura, Tsumura Central Research Laboratories for measurements of MS and COLOC spectra.

References

1) H. Ito, H. Taniguchi, T. Kita, Y. Matsuki, E. Tachikawa, T. Fujita, *Phytochemistry*, **16**, 1614 (1977); T. Fujita, D.-Y. Liu, S. Ueda, Y. Takeda, *ibid.*, **31**, 3977 (1992).
2) S. Sakuma, J. Shoji, *Chem. Pharm. Bull.*, **29**, 2431 (1981); *Idem*, *ibid.*, **30**, 810 (1982).
3) T. Miyase, Y.Iwata, A. Ueno, *Chem. Pharm. Bull.*, **39**, 3082 (1991); *Idem*, *ibid.*, **40**, 2741 (1992); T. Miyase, A. Ueno, *Shoyakugaku Zasshi*, **47**, 267 (1993).
4) Y. Ikeya, K. Sugama, M. Okada, H. Mitsuhashi, *Chem. Pharm. Bull.*, **39**, 2600 (1991).
5) Y. Ikeya, K. Sugama, M. Okada, H. Mitsuhashi, *Phytochemistry*, **30**, 2061 (1991).
6) I. Kitagawa, K. Hori, M. Sakagami, F. Hashiuchi, M. Yoshikawa, J. Ren, *Chem. Pharm. Bull.*, **41**, 1350 (1993).
7) W. Klyne, *Biochem. J.*, **47**, xil (1950).
8) S. J. Angyal, C. L. Bodkin, J. A. Mills, P. M. Pojar, *Aust. J. Chem.*, **30**, 1259 (1977).
9) M. Aritomi, T. Kawasaki, *Chem. Pharm. Bull.*, **32**, 2676 (1984).
10) B. H. Koeppen, D. G. Roux, *Biochem. J.*, **97**, 444 (1965).
11) N. Shimazaki, Y. Mimaki, Y. Sashida, *Phytochemistry*, **30**, 1475 (1991).