Xanthone *O*-Glycosides and Benzophenone *O*-Glycosides from the Roots of *Polygala tricornis*

Jun Li, Yong Jiang, and Peng-Fei Tu*

Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center, No. 38 Xueyuan Road, Haidian District, Beijing 100083, People's Republic of China

Received September 14, 2005

A new benzophenone *O*-glycoside, tricornoside A (**1**), and five new xanthone *O*-glycosides, tricornosides B−F (**4**−**8**), were isolated from the roots of *Polygala tricornis* together with three known glycosides (**2**, **3**, and **9**). The structures of new compounds were elucidated on the basis of chemical and spectroscopic evidence.

We previously reported the isolation of 12 oligosaccharides, called tricornoses A−L, and eight known sucrose esters from the roots of *Polygala tricornis* Gagnep.[1] Herein we report the isolation and structural elucidation of a new benzophenone *O*-glycoside named tricornoside A (**1**) and five new xanthone *O*-glycosides named tricornosides B−F (**4**−**8**). Three known compounds also isolated from this plant were identified by comparison with reported data as garcimangosone D (**2**),[2] arillanin G (**3**),[3] and polygalaxanthone V (**9**).[4]

Tricornoside A (**1**) was obtained as an amorphous powder. Its molecular formula $C_{24}H_{28}O_{13}$ was deduced from the HRESIMS. On acid hydrolysis, it gave glucose and apiose. The IR spectrum showed bands at 3358 and 1614 cm^{-1}, suggesting the presence of hydroxyl and carbonyl groups. The ^1H NMR spectrum of **1** showed the presence of two aromatic protons appearing as broad singlets at δ 6.02 and 6.06, five protons due to a phenyl group at δ 7.42 (2 H, t, J = 7.5 Hz), 7.55 (1 H, t, J = 7.5 Hz), and 7.68 (2 H, d, J = 8.5 Hz), and two anomeric protons at δ 4.89 (1 H, d, J = 7.5 Hz) and 5.11 (1 H, brs). The NMR data of **1** were similar to that of garcimangosone D (6-*O*-β-D-glucopyranosyl-2,4-dihydroxybenzophenone, **2**), [2] except for the presence of one set of apiose moiety signals. The apiose linkage in **1** was established at C-2 of the glucosyl residue by an HMBC experiment, which showed cross-peaks between the signals at δ 5.11 (H-1 of Api) and 75.9 (C-2 of Glc). The anomeric configuration of the apiosyl residue was deduced to be β by comparison of the ^{13}C NMR data of the apiosyl residue,[5] and that of the glucosyl residue to be β from the $^3J_{H1-H2}$ of the anomeric proton signal. Thus, tricornoside A was determined to be 6-*O*-(2-*O*-β-D-apiofuranosyl)-β-D-glucopyranosyl-2,4-dihydroxybenzophenone (**1**).

Tricornoside B (**4**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{27}H_{32}O_{14}$ on the basis of HRESIMS. The IR spectrum of **4** showed the presence of hydroxyl (3411 cm^{-1}), carbonyl (1648 cm^{-1}), and aromatic (1609 cm^{-1}) groups. The UV spectrum in MeOH was similar to that of 2-hydroxy-3,4-dimethoxyxanthone.[3] Acid hydrolysis of **4** yielded glucose and rhamnose, suggesting that **4** was a 2,3,4-trioxygenated xanthone glycoside. The NMR data of **4** (Table 1 and Table 2) were similar to those of arillanin D[3] except for the presence of a rhamnosyl residue in **4** instead of the arabinosyl residue in arillanin D. The linkages of sugar and aglycon residues were determined mainly by an HMBC experiment. In this experiment, long-range correlations were observed between H-1 (δ 5.08) of Glc and C-2 (δ 158.2) of the aglycon and between H-1 (δ 4.69) of Rha and C-6 (δ 67.7) of Glc. This indicated that the glucosyl moiety was linked to C-2 of the aglycon, and the rhamnosyl moiety was linked to C-6 of Glc. The anomeric configuration of the rhamnosyl residue was determined to be α from the ^{13}C NMR chemical shifts of C-3 and C-5,[6] and that of the glucosyl residue to be β from the $^3J_{H1-H2}$ of the anomeric proton signal. Thus, tricornoside B was elucidated as 2-*O*-(6-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranosyl-3,4-dimethoxyxanthone (**4**).

Tricornoside C (**5**) was obtained as a pale yellow powder ($C_{24}H_{26}O_{13}$). The IR and UV spectra were typical of a hydroxylated xanthone. On acid hydrolysis, **5** gave glucose and apiose, suggesting that **5** was a xanthone glycoside. The ^1H NMR spectrum of **5** revealed the presence of two characteristic pairs of *meta*-coupled aromatic protons at δ 6.47 and 6.65, four aromatic protons of a 1,2-disubstituted benzene group at δ 7.38, 7.50, 7.76, and 8.13, and two anomeric protons at δ 4.98 and 5.01. Eleven aliphatic signals among the total of 24 signals in the ^{13}C NMR spectrum could be assigned to two sugar moieties, including two anomeric carbon signals at δ 101.5 and 111.1. The remaining signals were attributable to a xanthone. The substitution pattern of **5** was that of a 1,3-dioxygenated xanthone, based on the HMBC correlations: H-2/C-1, C-3, C-4, C-8b and H-4/C-2, C-3, C-4a, C-8b. In the HMBC spectrum, long-range correlations between H-1 (δ 5.01) of Glc and C-3 (δ 165.9) of the aglycon and between H-1 (δ 4.98) of Api and C-6 (δ 68.9) of Glc indicated that the glucosyl residue was linked to C-3 of the aglycon, and the apiosyl residue was linked to the glucosyl moiety by a (1→6) linkage. Thus, tricornoside C was determined to be 3-*O*-(6-*O*-β-D-apiofuranosyl)-β-D-glucopyranosyl-1-hydroxyxanthone (**5**).

Tricornoside D (**6**) ($C_{25}H_{28}O_{13}$) was obtained as a pale yellow powder. The IR and UV spectra were similar to those of **5**. Comparison of the NMR data of **6** and **5** indicated that the apiosyl residue in **5** was replaced by a rhamnosyl residue in **6**. The rhamnosyl residue was attached at C-6 of Glc, as deduced from HMBC correlations between H-1 (δ 4.71) of Rha and C-6 (δ 67.7) of Glc. Therefore, tricornoside D was determined to be 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranosyl-1-hydroxyxanthone (**6**).

Tricornoside E (**7**) was obtained as a yellow powder ($C_{20}H_{20}O_{11}$). The IR and UV spectra were characteristic of

* Corresponding author. Tel/fax: +86-10-82802750. E-mail: pengfeitu@bjmu.edu.cn.

10.1021/np050347l CCC: $30.25
Published on Web 12/06/2005

Table 1. ^{1}H NMR (500 MHz) Spectroscopic Data of Compounds **4**−**8**[a,b]

no.	4	5	6	7	8
1	7.15 s				
2		6.47 d (2.0)	6.44 d (2.0)	6.78 d (8.0)	6.82 d (8.5)
3				7.68 t (8.0)	7.74 t (8.5)
4		6.65 d (2.0)	6.66 d (2.0)	7.12 d (9.0)	7.08 d (8.5)
5	7.73 d (8.0)	7.50 d (8.5)	7.62 d (8.5)		7.69 d (9.0)
6	7.75 t (8.0)	7.76 t (8.5)	7.75 t (8.5)		7.76 d (8.5)
7	7.39 t (8.0)	7.38 t (8.0)	7.38 t (7.0)		
8	8.18 d (8.0)	8.13 d (8.5)	8.12 d (8.0)	7.35 s	7.71 d (3.0)
OCH_3	3.91 s			3.91 s	
OCH_3	3.97 s				
1-OH				12.85 s	12.58 s
6-OH				10.37 brs	
Glc-1	5.08 d (7.5)	5.01 d (8.0)	5.00 d (8.0)	4.85 d (8.0)	4.92 d (7.5)
2	3.58 t (7.5)	3.49 m	3.58 m	3.43 t (8.0)	3.31 m
3	3.51 t (9.3)	3.51 m	3.50 m	3.28 m	3.33 m
4	3.38 t (9.3)	3.36 m	3.37 m	3.26 m	3.20 m
5	3.70 m	3.70 m	3.67 m	3.24 m	3.58 m
6	4.08 m	4.07 m	4.06 m	3.66 m	3.97 m
	3.62 m	3.61 dd (11.0/7.0)	3.64 m	3.49 dd (11.5/5.5)	3.56 dd (11.0/6.5)
	Rha	Api	Rha		Ara
1	4.69 d (1.5)	4.98 d (1.5)	4.71 d (1.5)		4.17 d (6.5)
2	3.96 m	3.96 d (2.5)	3.97 m		3.40 t (8.0)
3	3.79 dd (9.3/3.3)		3.78 dd (9.0/3.0)		3.33 m
4	3.34 m	4.03 d (9.5)	3.35 m		3.61 m
		3.79 d (9.5)			
5	3.67 m	3.62 brs	3.65 m		3.68 dd (12.0/4.0)
		3.62 brs			3.35 m
6	1.17 d (6.0)		1.20 d (6.0)		

[a] Assignments were based on ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] Compounds **4**, **5**, and **6** were recorded in CD_3OD; **7** and **8** in DMSO-d_6.

Table 2. ^{13}C NMR (125 MHz) Spectroscopic Data of Compounds **4**−**8**[a,b]

no.	4	5	6	7	8
1	101.5	164.3	164.3	160.7	160.9
2	158.2	100.2	100.3	109.8	110.0
3	141.2	165.9	165.9	136.4	137.4
4	154.7	96.2	96.2	107.4	107.2
4a	156.9	158.9	158.9	155.6	155.8
4b	155.7	157.5	157.5	148.2	151.0
5	119.2	118.9	119.2	132.5	119.7
6	135.9	136.7	136.7	145.9	126.4
7	125.2	125.4	125.4	146.4	153.9
8	127.0	126.5	126.4	100.2	110.6
8a	123.1	121.5	121.5	111.2	120.3
8b	112.5	105.6	105.6	107.7	107.9
9	177.4	182.2	182.2	180.3	181.5
OCH_3	62.3			56.0	
OCH_3	62.6				
Glc-1	102.1	101.5	102.2	105.5	101.3
2	74.8	74.7	74.7	73.9	73.2
3	78.2	77.9	77.9	76.0	76.2
4	71.4	71.6	71.5	69.6	69.8
5	77.4	77.2	77.3	77.4	75.7
6	67.7	68.9	67.7	60.8	68.1
	Rha	Api	Rha		Ara
1	102.2	111.1	101.7		103.5
2	72.1	78.2	72.1		70.6
3	72.5	80.5	72.4		72.4
4	74.1	75.1	74.2		67.3
5	69.9	65.9	69.8		64.9
6	17.9		17.9		

[a] Assigned by HSQC and HMBC experiments. [b] Compounds **4**, **5**, and **6** were recorded in CD_3OD; **7** and **8** in DMSO-d_6.

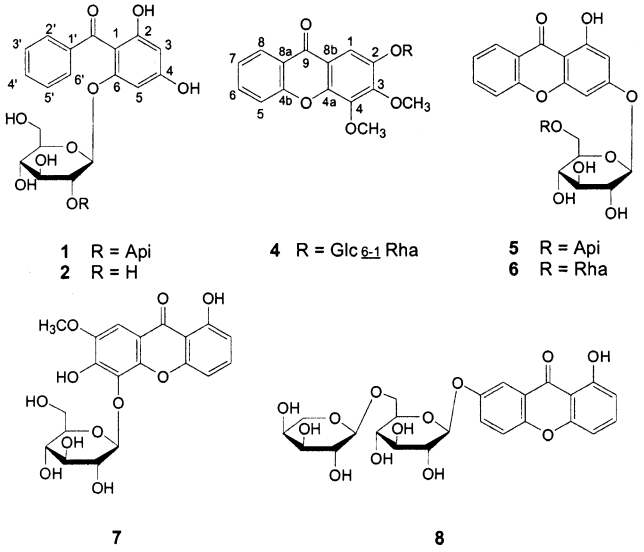

Figure 1. Structures of compounds **1**−**2** and **4**−**8** from the roots of *Polygala tricornis*.

a 1,5,6,7-tetraoxygenated xanthone.[7] Acid hydrolysis of **7** yielded glucose. The ^{1}H NMR spectrum of **7** showed the presence of a hydrogen-bonded hydroxyl singlet at δ 12.85 (C-1-OH), an isolated aromatic proton signal at δ 7.35, ABM-type aromatic proton signals at δ 7.68 (t, J = 8.5 Hz), 7.12 (d, J = 9.0 Hz), and 6.78 (d, J = 8.0 Hz), one methoxyl signal at δ 3.91, and an anomeric proton signal at δ 4.85 (d, J = 8.0 Hz). Six signals in the ^{13}C NMR spectrum were assigned to a glucosyl moiety, and the remaining signals were attributable to a xanthone. The isolated aromatic proton signal at δ 7.35 was assigned as H-8 on the basis of correlations between the signals at δ 7.35 and 180.3 (C-9), 148.2 (C-4b), and 145.9 (C-6). The 7-methoxyl moiety was confirmed by a NOESY experiment, which showed cross-peaks between the methoxyl signal at δ 3.91 and the singlet aromatic proton signal at δ 7.35 (H-8). The glucose linkage in **7** was established at C-5 by an HMBC experiment. The anomeric configuration of the glucosyl residue was deduced to be β. Thus, tricornoside E was established as 5-*O*-β-D-glucopyranosyl-1,6-dihydroxy-7-methoxyxanthone (**7**).

The HRESIMS of tricornoside F (**8**) established the molecular formula of $C_{24}H_{26}O_{13}$. The IR and UV spectra absorption bands suggested a 1,7-dioxygenated xanthone.[8] Acid hydrolysis yielded glucose and arabinose. The NMR

data of **8** were similar to those of wubangziside A[8] except for an arabinosyl residue in **8** instead of the apiosyl residue in wugangziside A. The arabinosyl residue was linked to C-6 of Glc, on the basis of the HMBC correlations between the arabinosyl anomeric proton signal at δ 4.17 and C-6 (δ 68.1) of the Glc. Thus, tricornoside F was determined to be 7-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranosyl-1-hydroxyxanthone (**8**).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Polartronic D polarimeter. UV spectra were recorded on a UV-2401 spectrophotometer. IR spectra (KBr disks) were recorded on an Avater-360 spectrophotometer. ^{1}H, ^{13}C NMR, NOESY, COSY, HMQC, and HMBC spectra were recorded on Bruker AM-500 or JEOL JNM-A300 spectrometers. HRESIMS were measured on a Bruker APEX II mass spectrometer. Column chromatography (CC): D101 (Tianjin Chemical Co.), silica gel (200−300 mesh, Qingdao Marine Chemical Factory). Semipreparative HPLC: Waters 600 controller, Waters column (Prep Nova-Pak HR C_{18} 7.8 × 300 mm), Waters 2487 dual λ absorbance detector, detection wavelength 228, 310 nm. GC analysis was carried out on an Agilent 6890N gas chromatogragh using a HP-5 capillary column (28 m × 0.32 mm, id); detection, FID; detector temperature, 260 °C; column temperature, 180 °C; carrier gas, N_2.

Plant Material. The roots of *P. tricornis* were collected in December 2003, in Yunnan Province, China. The plant was identified by one of the authors (P.-F.T). A voucher specimen (No. 031220) is deposited in the Herbarium of Modern Research Center for TCM, Peking University, Beijing, People's Republic of China.

Extraction and Isolation. The dried roots of *P. tricornis* (3.0 kg) were extracted twice with 95% EtOH under reflux. After evaporation of the solvent under reduced pressure, the 95% EtOH extract (800 g) was suspended in H_2O and extracted with petroleum ether, $CHCl_3$, and *n*-BuOH, respectively. The *n*-BuOH layer (250 g) was adsorbed on a porous polymer gel D101 column (9.5 × 50 cm). The adsorbed material was eluted with 10%, 30%, and 50% aqueous MeOH and MeOH successively, after washing with H_2O. The 10% aqueous MeOH eluate (10.5 g) was chromatographed on a silica gel (200−300 mesh, 300 g) column using $CHCl_3$−MeOH−H_2O (70:10:1) as an eluent to afford fractions A−L. Fraction E (0.7 g) was subjected to semipreparative HPLC (MeOH−H_2O, 28:72) to afford **1** (18 mg, t_R = 8.0 min), **2** (12 mg, t_R = 13.2 min), and **3** (45 mg, t_R = 15.5 min). The 30% aqueous MeOH eluate (15.6 g) was chromatographed on a silica gel (200−300 mesh, 400 g) column using $CHCl_3$−MeOH−H_2O (70:10:1 → 80:20:2) as eluent to afford fractions A−P. Fraction D (0.4 g) was subjected to semipreparative HPLC (MeOH−H_2O, 40:60) to afford **4** (15 mg, t_R = 14.8 min), **5** (25 mg, t_R = 26.5 min), and **6** (22 mg, t_R = 29.2 min). The 50% aqueous MeOH eluate (18.5 g) was chromatographed on a silica gel (200−300 mesh, 500 g) column using $CHCl_3$−MeOH−H_2O (80:20:2 → 70:30:3) as eluent to afford fractions A−M. Fraction D (0.5 g) was subjected to semipreparative HPLC (MeOH−H_2O, 49:51) to afford **7** (35 mg, t_R = 19.8 min), **8** (16 mg, t_R = 21.5 min), and **9** (28 mg, t_R = 24.2 min).

Tricornoside A (1): amorphous powder, $[\alpha]_D^{25}$ −68.2 (*c* 0.75 MeOH); UV (MeOH) λ_{max} 304, 250, 208 nm; IR (KBr) ν_{max} 3358, 2923, 1614, 1452, 1072 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.68 (2H, d, J = 8.5 Hz, H-2′, 6′), 7.55 (1H, t, J = 7.5 Hz, H-4′), 7.42 (2H, t, J = 7.5 Hz, H-3′, 5′), 6.06 (1H, brs, H-5), 6.02 (1H, brs, H-3), 5.11 (1H, brs, Api-1), 4.89 (1H, d, J = 7.5 Hz, Glc-1), 3.64 (1H, brs, Api-2), 3.62 (1H, m, Glc-6a), 3.42 (1H, m, Glc-6b), 3.40 (1H, m, Api-4a), 3.34 (1H, m, Glc-3), 3.24 (1H, m, Glc-5), 3.21 (1H, m, Api-4b), 3.19 (2H, brs, Api-5a, 5b), 3.06 (2H, t, J = 8.5 Hz, Glc-2, 4); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 194.3 (C=O), 160.6 (C-4), 157.3 (C-2), 156.3 (C-6), 138.6 (C-1′), 132.3 (C-4′), 129.0 (C-2′, 6′), 128.1 (C-3′, 5′), 108.4 (Api-1), 108.0 (C-1), 97.6 (Glc-1), 96.3 (C-3), 93.6 (C-5), 78.9 (Api-3), 77.0 (Glc-3), 76.8 (Glc-5), 76.2 (Api-2), 75.9 (Glc-2), 73.6 (Api-4), 69.6 (Glc-4), 64.1 (Api-5), 60.5 (Glc-6); HRESIMS *m/z* 525.1609 [M + H]$^+$ (calcd for $C_{24}H_{29}O_{13}$, 525.1608).

Tricornoside B (4): amorphous powder, $[\alpha]_D^{25}$ −72.5 (*c* 0.86 MeOH); UV (MeOH) λ_{max} 337, 297, 277, 242, 208 nm; IR (KBr) ν_{max} 3411, 2927, 1648, 1609, 1467, 1067 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 581.1866 [M + H]$^+$ (calcd for $C_{27}H_{33}O_{14}$, 581.1870).

Tricornoside C (5): pale yellow powder, $[\alpha]_D^{25}$ −84.5 (*c* 0.92 MeOH); UV (MeOH) λ_{max} 344, 301, 252, 235, 209 nm; IR (KBr) ν_{max} 3410, 2925, 1650, 1609, 1572, 1468, 1072 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 523.1446 [M + H]$^+$ (calcd for $C_{24}H_{27}O_{13}$, 523.1452).

Tricornoside D (6): pale yellow powder, $[\alpha]_D^{25}$ −61.3 (*c* 0.70 MeOH); UV (MeOH) λ_{max} 349, 300, 253, 235, 210 nm; IR (KBr) ν_{max} 3410, 2922, 1651, 1609, 1572, 1468, 1067 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 537.1603 [M + H]$^+$ (calcd for $C_{25}H_{29}O_{13}$, 537.1608).

Tricornoside E (7): yellow powder, $[\alpha]_D^{25}$ −58.5 (*c* 0.75 MeOH); UV (MeOH) λ_{max} 373, 312, 252, 231, 201 nm; IR (KBr) ν_{max} 3300, 2922, 1646, 1601, 1480, 1070 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 437.1075 [M + H]$^+$ (calcd for $C_{20}H_{21}O_{13}$, 437.1084).

Tricornoside F (8): pale yellow powder, $[\alpha]_D^{25}$ −86.4 (*c* 0.81 MeOH); UV (MeOH) λ_{max} 383, 288, 257, 233, 204 nm; IR (KBr) ν_{max} 3371, 2915, 1645, 1608, 1478, 1064 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 523.1448 [M + H]$^+$ (calcd for $C_{24}H_{27}O_{13}$, 523.1452).

Acid Hydrolysis of 1 and 4−8. Each compound (3 mg) was hydrolyzed with 2 M aqueous CF_3COOH (5 mL) at 110 °C for 2 h in a sealed tube. After this period, the reaction mixture was diluted with H_2O (15 mL) and extracted with $CHCl_3$ (3 × 5 mL). After repeated evaporation to dryness of the aqueous layer with MeOH until neutral, the residue was dissolved in pyridine (0.06 mL), then hexamethyldisilazine (0.06 mL) and trimethylsilyl chloride (0.02 mL) were added, and the reaction mixture was stirred at 60 °C for 30 min. The supernatant was subjected to GC. D-Glucose (12.51 min) was detected from **1** and **4**-**8**, D-apiose (5.12 min) was detected from **1** and **5**, L-rhamnose (5.42 min) was detected from **4** and **6**, and L-arabinose (5.36 min) was detected from **8**.

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NP050347L