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Xanthone *O*-glycosides from *Polygala tenuifolia*

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Abstract

Four xanthone *O*-glycosides, polygalaxanthones IV–VII were isolated from the roots of *Polygala tenuifolia* Willd., together with eight known compounds. The structures of the four xanthone *O*-glycosides were established as 6-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-1-hydroxy-3,7-dimethoxyxanthone (polygalaxanthone IV), 6-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-1,3-dihydroxy-7-methoxyxanthone (polygalaxanthone V), 6-*O*-(β-D-glucopyranosyl)-1,2,3,7-tetramethoxyxanthone (polygalaxanthone VI), and 3-*O*-[α-D-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-1,6-dihydroxy-2,7-dimethoxyxanthone (polygalaxanthone VII), respectively, on the basis of analysis of spectroscopic evidence.

Keywords: *Polygala tenuifolia*; Polygalaceae; Xanthone *O*-glycosides; Polygalaxanthones

1. Introduction

The roots of *Polygala tenuifolia* Willd., ''yuanzhi'', is a well-known traditional Chinese medicine used as an expectorant, tonic, sedative and for preventing dementia (Jiangshu New Medicinal College, 1997). Various xanthones, saponins and oligosaccharide esters have been isolated from this plant (Ito et al., 1977; Sakuma and Shoji, 1981a,b; Miyase et al., 1991, 1992; Ikeya et al., 1991a,b; Fujita et al., 1992). However, the precise mechanism of the therapeutic effects of *P. tenuifolia*, especially on the prevention of dementia, is not completely understood. In this paper, the structural elucidation of four xanthone *O*-glycosides named polygalaxanthones IV–VII (**1**–**4**) is reported.

2. Results and discussion

The *n*-BuOH soluble parts of the 95% aq. EtOH extract of *P. tenuifolia* were subjected to macroporous resin D101 column chromatography, eluted with an EtOH–H_2O gradient. The 50% EtOH aq. eluate was applied to a silica gel column, elution of which afforded xanthone glycosides **1**–**4** along with eight known compounds.

Compound **1** was obtained as a yellow powder and its molecular formula was deduced as $C_{27}H_{32}O_{15}$ from HR SI–MS. Its UV spectrum in MeOH (λ_{max} 237, 256, 308 and 362 nm) was similar to that of 1,6-dihydroxy-3,7-dimethoxyxanthone (λ_{max} 234, 255, 312 and 364 nm) (Ikeya et al., 1991a), suggesting that **1** was a 1,3,6,7-tetraoxygenated xanthone. The IR spectrum of **1** showed the presence of hydroxyl groups (3407 cm^{-1}), a hydrogen bonded ketone (1653 cm^{-1}), and aromatic carbons (1612, 1580, 1478 cm^{-1}). The ^1H NMR spectrum showed a hydrogen bonded hydroxyl signal at δ 13.00 (C-1-OH), two singlet aromatic proton signals at δ 7.45 and 7.29, two meta-coupled aromatic protons at δ 6.60 (1H, *d*, $J=2.1$ Hz), 6.38 (1H, *d*, $J=2.1$ Hz), two anomeric proton signals at δ 5.42 (1H, *d*, $J=7.80$ Hz), 5.25 (1H, *s*), and three methyl signals at δ 3.87 (3H, *s*), 3.86 (3H, *s*) and δ 1.13 (3H, *d*, $J=5.7$ Hz). The ^{13}C NMR spectrum of **1** exhibited eleven aliphatic carbon signals due to two sugar moieties. On acid hydrolysis, **1** afforded glucose and rhamnose. So, **1** must be a 1,3,6,7-tetraoxygenated xanthone glycoside. The positions of the substituents were confirmed through NOESY experiments. In the NOESY spectrum of **1**, cross peaks were found between a methoxyl signal at δ 3.86 and two aromatic protons at δ 6.38 (H-2) and δ 6.60 (H-4), so this methoxyl should be located at C-3 position; the downfield aromatic proton (H-8) at δ 7.45 showed a correlation with the other methoxyl signal at δ 3.87, which was attributed to the C-7 methoxyl signal and the

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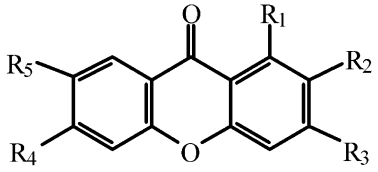
anomeric proton of the glucosyl group at δ 5.42 correlated with H-5 proton at δ 7.29. This indicated that the glucosyl moiety is linked to C-6 of the xanthone, which could be proved further by HMBC spectrum analysis. In the HMBC spectrum, the rhamnose anomeric proton signal at δ 5.25 was correlated to C-2 (δ 75.1) of the glucosyl residue, and the glucosyl anomeric proton signal at δ 5.42 was correlated to the C-6 signal of the aglycone (δ 152.8). The configuration of the glucosyl residue was deduced to be β from the *J* value (7.8 Hz) of the anomeric proton, and of rhamnosyl residue to be α by comparison of the ^{13} C NMR spectroscopic data (Miyase et al., 1999). Thus, **1** was determined to be 6-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-1-hydroxy-3,7-dimethoxyxanthone (polygalaxanthone IV).

Compound **2** was obtained as a yellow powder. Its UV and IR spectra were very similar to **1**, but when adding NaOAC, the UV spectrum of **2** showed a bathochromic shift, indicating the presence of a hydroxyl group at the 3 or 6 position (Fujita et al., 1992). The HR SI–MS data of **2** were consistent with a formula of $C_{26}H_{30}O_{15}$, 14 mass units lower than that of **1**. Comparing the NMR spectral data with those of **1**, this suggested that a hydroxyl group in **2** replaced a methoxyl group in **1**. In the NOESY spectrum of **2**, the hydroxyl signal at δ 10.97 had a correlation with the H-2 (δ 6.36) and H-4 (δ 6.18) signals, suggesting that the hydroxyl group replaced the methoxyl at C-3 position. Thus, **2** was identified as 6-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl - 1,3 - dihydroxy - 7 - methoxyxanthone (polygalaxanthone V).

Compound **3** was obtained as a yellow powder and its molecular formula was elucidated to be $C_{23}H_{26}O_{12}$ from HR SI–MS. Its UV spectrum exhibited characteristic absorption bands of 6-hydroxy-1,2,3,7-tetramethoxyxanthone at λ_{max} 245, 273 and 312 nm (Ito et al., 1977). The ^{1}H NMR spectrum showed three singlet aromatic proton signals at δ 7.50, 7.19 and 7.01, one anomeric proton signal at δ 5.09 (1H, *d*, *J* = 7.0 Hz), and four methyl signals at δ 3.94, 3.87, 3.83 and 3.76 (each 3H, *s*). In the ^{13}C NMR spectrum, signals at δ 61.1 and 60.8 were indicative of two di-*ortho*-substituted methoxyl groups (Frahm and Hambloch, 1982). The C-8b, C-1, C-2, C-3, C-4 and C-4a shifts in the ^{13}C NMR spectrum of **3** were essentially the same as those of onjixanthone I (Ikeya et al., 1991a), indicating the presence of 1,2,3-trimethoxyl moiety in **3**. The NOESY spectrum of 3 showed cross peaks between the aromatic proton (H-4) signal at δ 7.01 and the methoxyl signal at δ 3.94 (3-OMe); the downfield aromatic proton (H-8) signal at δ 7.50 and the methoxyl signal at δ 3.87 (7-OMe) and the anomeric proton signal at δ 5.09 and the H-6 signal at δ 7.16. In the HMBC spectrum, the glucosyl anomeric proton signal at δ 5.09 was correlated to C-6 (δ 152.1) of the aglycone, which further supported the above elucidation. The configuration of the glucosyl residue was deduced to be β from the *J* value (7.0 Hz) of the anomeric proton. Thus, **3** was determined to be 6-*O*-(β-D-glucopyranosyl)-1,2,3,7-tetramethoxyxanthone (polygalaxanthone VI).

Compound **4** was obtained as a pale yellow powder and its maximum absorption in the UV spectrum suggested that **4** had a hydroxyxanthone skeleton (Miyase et al., 1999). The HR SI–MS spectrum gave an $[M-H]^-$ peak at *m*/*z* 611.1619. The ^{1}H NMR spectrum of **4** showed two hydroxyl signals at δ 13.12 (C-1-OH) and 10.99, three isolated aromatic proton signals at δ 7.44, 6.93 and 6.83, two anomeric protons at δ 5.30 (1H, *d*, *J* = 6.5 Hz) and 5.27 (1H, *br s*). The data of the sugar moiety were almost the same with the data of compound **1**, and the data of A ring of the aglycone were identical to those of polyxanthone III (Miyase et al., 1999), which indicated a hydroxyl and a methoxyl residue present at positions 6 and 7 of A ring, respectively. In the HMBC spectrum, the hydrogen-bonded hydroxyl signal at δ 13.1 was correlated to δ 103.4 C-8b, 131.8 (C-2), 153.5 (C-1), and the signal at δ 131.8 (C-2) was also correlated with the methoxyl signal. The methoxyl should thus be located in position 2 of B ring. Moreover, the signal of the isolated aromatic proton at δ 6.83 was correlated to δ 103.4 (C-8b), 131.8 (C-2), 152.1 (C-4a) and 156.4 (C-3), and the anomeric proton of glucose residue was also correlated to δ 156.4 (C-3). Thus **4** was identified as 3-*O*-[α-D-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-1,6-dihydroxy-2,7-dimethoxyxanthone.

Eight other compounds **5**–**12** were identified by comparing their physical and spectral data with the literature values: tenuifoliside A (Ikeya et al., 1991b), 3,6′-disinapoyl sucrose (Ikeya et al., 1991b), polygalitol (Mao et al., 1996), polygalaxanthone III (Miyase et al., 1999), tenuifoliose H (Miyase et al., 1991), tenuifoliose I (Miyase et al., 1991), tenuifoliose L (Miyase et al., 1991), and polygalasaponin XXVIII (Zhang et al., 1996).



1 R_1=OH, R_2=H, R_3=R_5=OMe, R_4=OGlc(2-1)Rha
2 R_1=R_3=OH, R_2=H, R_5=OMe, R_4=OGlc(2-1)Rha
3 R_1=R_2=R_3=R_5=OMe, R_4=OGlc
4 R_1=R_4=OH, R_2=R_5=OMe, R_3=OGlc(2-1)Rha

3. Experimental

3.1. General

Uncorrected mps. were obtained using a XT4A melting point apparatus. Optical rotations were measured on a

Table 1
^{1}H NMR and ^{13}C NMR spectral data of **1–4** (in DMSO-d_6)[a,b]

Position	Compound **1**		Compound **2**		Compound **3**		Compound **4**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		162.4		162.6		152.6		153.5
2	6.38 (*d*, *J* = 2.1)	97.0	6.36 (*d*, *J* = 1.8)	98.0		139.1		131.8
3		165.9		165.1		158.2		156.4
4	6.60 (*d*, *J* = 2.1)	92.5	6.18 (*d*, *J* = 1.8)	93.7	7.01 (*s*)	96.7	6.83 (*s*)	93.8
4a		157.3		157.4		154.1		152.1
4b		151.3		151.2		150.1		152.3
5	7.29 (*s*)	102.9	7.31 (*s*)	103.0	7.19 (*s*)	102.7	6.93 (*s*)	102.7
6		152.8		152.6		152.1		155.1
7		146.9		146.8		146.5		146.3
8	7.45 (*s*)	104.3	7.44 (*s*)	104.3	7.50 (*s*)	105.7	7.44 (*s*)	104.6
8a		113.2		113.2		115.4		111.2
8b		102.6		101.8		109.2		103.4
9		179.1		178.8		173.0		179.5
1-OMe					3.83 (*s*)	61.8		
2-OMe					3.76 (*s*)	61.1	3.75 (*s*)	60.3
3-OMe	3.87 (*s*)	56.1			3.94 (*s*)	56.5		
7-OMe	3.86 (*s*)	55.8	3.85 (*s*)	55.8	3.87 (*s*)	55.9	3.88 (*s*)	55.9
1-OH	13.00 (*s*)		13.01 (*s*)				13.12 (*s*)	
3-OH			10.97(*s*)					
6-OH							10.99 (*s*)	
Glc-1	5.42 (*d*, *J* = 7.8)	97.4	5.40 (*d*, *J* = 7.8)	97.4	5.09 (*d*, *J* = 7.0)	99.7	5.30 (*d*, *J* = 6.5)	97.7
2	3.62 (*t*, *J* = 8.1)	75.1	3.61 (*t*, *J* = 8.4)	75.2	3.44 (*m*)	73.1	3.60 (*t*, *J* = 8)	75.8
3	3.49 (*m*)	77.0	3.48 (*m*)	77.0	3.44 (*m*)	76.7	3.45 (*m*)	77.1
4	3.21 (*m*)	69.7	3.20 (*m*)	69.6	3.17 (*m*)	69.6	3.20 (*m*)	69.7
5	3.49 (*m*)	77.6	3.49 (*m*)	77.6	3.46 (*m*)	77.2	3.50 (*m*)	77.5
6	3.70 (*m*)	60.5	3.69 (*m*)	60.5	3.70 (*m*)	60.7	3.68 (*m*)	60.5
	3.46 (*m*)		3.45 (*m*)		3.47 (*m*)		3.46 (*m*)	
Rham-1	5.25 (*s*)	99.8	5.24 (*s*)	99.9			5.27 (1H, *br s*)	100.0
2	3.30 (*m*)	70.3	3.29 (*m*)	70.3			3.31 (overlapped)	70.3
3	3.68 (*m*)	70.5	3.66 (*m*)	70.5			3.67 (*m*)	70.5
4	3.16 (*m*)	71.8	3.16 (*m*)	71.8			3.18 (*m*)	72.0
5	3.84 (*m*)	68.4	3.82 (*m*)	68.4			3.74 (*m*)	68.6
6	1.13 (*d*, *J* = 5.7)	18.1	1.12 (*d*, *J* = 5.7)	18.1			1.10 (*d*, *J* = 5.5)	18.2

[a] 1D NMR of **1**–**3** were recorded on 300 MHz instrument and 1D NMR of **4** was obtained on 500 MHz instrument; 2D NMR were all recorded on 500 MHz instrument.
[b] Signal assignments were aided by COSY, HMQC, HMBC and NOESY spectra.

Polartronic D polarimeter. UV spectra were recorded on a TU-1901 spectrophotometer, whereas IR spectra were obtained on an AVATER-360 spectrophotometer. MALDI-TOF MS spectra were performed at a LDI 1700 spectrometer, using DHB (dihydroxybenzoic acid) or CCA (α-cyano-4-hydroxycinnamic acid) as matrix, while FAB MS were obtained on a KYKY-ZHP-5# mass spectrometer. ^{1}H NMR, ^{13}C NMR, COSY, HMQC and HMBC spectra were measured on a Jeol JNM-A300 or Bruker AM-500 spectrometer. D101 resin (Tianjin Chemical Co.). CC: silica gel (200–300 mesh, Qingdao Marine Chemical Factory).

3.2. Plant material

The roots of *P. tenuifolia* were collected from Shanxi Province, PR China, in October 2000. The plant was identified by Professor Peng-Fei Tu, School of Pharmaceutical Sciences, Peking University Health Science Center. A voucher specimen (No. 001020) was deposited in the herbarium of School of Pharmaceutical Sciences, Peking University, Beijing, PR China.

3.3. Extraction and isolation

The air-dried roots of *P. tenuifolia* (11 kg) were ground and refluxed with 95% EtOH for 3 h three times. The extract was combined and evaporated in vacuo to yield 4.9 kg of residue, a portion (2 kg) of which was suspended in water and extracted successively with petroleum, $CHCl_3$ and *n*-BuOH. Parts of the *n*-BuOH extract (325 g) were subjected to a macroporous resin D101 column (11.5×85.5 cm). The adsorbed material was eluted successively with H_2O, then 20, 50, 70, and 95%

EtOH. The 50% EtOH eluate (78 g) was applied to a silica gel (1.6 kg), eluted with $CHCl_3$–MeOH in a gradient manner. Fractions 11–14, 23–24 and 26–37 eluted with $CHCl_3$–MeOH (9:1) gave **3** (18 mg), **5** (110.4 mg) and **6** (3.27g), respectively. Fractions 54–60 was further isolated by reduced pressure column chromatography eluted with $CHCl_3$:MeOH:H_2O (6:1:0.1) to give **7** (23.6 mg). Fractions 61–69, eluted with $CHCl_3$–MeOH (8:2) afforded **1** (305.6 mg). Fractions 70–71 were recrystallized from MeOH three times to furnish **2** (70.9 mg) and the mother liquor was subjected to a silica gel with $CHCl_3$:MeOH:H_2O (80:20:5 lower phase) as eluent to afford **4** (12.2 mg) and **8** (17.6 mg). Fractions 79–114 was first subjected to ODS CC, then purified by HPLC with MeOH:H_2O (5:5) as mobile phase to furnish **9** (139.2 mg), **10** (45.6 mg) and **11** (72.1 mg). Fractions 133–148 were also subjected to ODS CC, then purified on a Sephadex LH-20 column, using MeOH as eluent to give **12** (20.5 mg).

3.4. Polygalaxanthone IV (*1*)

Yellow powder, mp 273–275 °C. $[\alpha]_D^{23}$ −60° (*c* 0.70, MeOH). UV λ_{max}^{MeOH} nm (log ε): 237 (4.35), 256 (4.38), 309 (4.02), 361 (4.02). IR ν_{max}^{KBr} cm^{-1}: 3407 (OH), 1653 (C=O), 1612, 1580, 1478 (aromatic ring). HR SI–MS (positive mode): *m/z* 597.1818 ($C_{27}H_{33}O_{15}$ $[M+H]^+$, requires 597.1814). TOF–MS (CCA as matrix) *m/z*: 597 $[M+H]^+$, 619 $[M+Na]^+$. For ^1H NMR and ^{13}C NMR spectral data: see Table 1.

3.5. Polygalaxanthone V (*2*)

Yellow powder, mp 232–235 °C. $[\alpha]_D^{23}$ −81.7° (*c* 0.71, MeOH) UV λ_{max}^{MeOH} nm (log ε): 238 (4.34), 256 (4.38), 309 (4.03), 360 (4.00). $\lambda_{max}^{MeOH+NaOAC}$ nm: 236, 369. IR ν_{max}^{KBr} cm^{-1}: 3389 (OH), 1652 (C=O), 1613, 1580, 1484 (aromatic ring). HR SI–MS (negative mode): *m/z* 581.1514 ($C_{26}H_{29}O_{15}$ $[M-H]^-$, requires 581.1512). TOF–MS (DHB as matrix) *m/z*: 583 $[M+H]^+$, 605 $[M+Na]^+$, 621$[M+K]^+$. For ^1H NMR and ^{13}C NMR spectal data: see Table 1.

3.6. Polygalaxanthone VI (*3*)

Yellow powder, mp 245–247 °C. UV λ_{max}^{MeOH} nm (log ε): 245 (4.62), 273 (4.13), 312 (4.34). IR ν_{max}^{KBr} cm^{-1}: 3396 (OH), 1657 (C=O), 1619, 1472 (aromatic ring). HR SI–MS (positive mode): *m/z* 495.1496 ($C_{23}H_{27}O_{12}$ $[M+H]^+$, requires 495.1497). FAB–MS *m/z*: 495 $[M+H]^+$, 333 $[M+H\text{-Glu}]^+$. ^1H NMR and ^{13}C NMR data: see Table 1.

3.7. Polygalaxanthone VII (*4*)

Pale yellow powder, mp 182–184 °C. UV λ_{max}^{MeOH} nm (log ε): 233 (4.46), 257 (4.40), 319 (4.30), 362 (4.10). IR ν_{max}^{KBr} cm^{-1}: 3396 (OH), 1657 (C=O), 1619, 1472 (aromatic ring). HR SI–MS (negative mode): *m/z* 611.1619 ($C_{27}H_{31}O_{16}$ $[M-H]^-$, requires 611.1617). FAB–MS *m/z*: 612 $[M]^+$, for ^1H NMR and ^{13}C NMR spectral data: see Table 1.

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