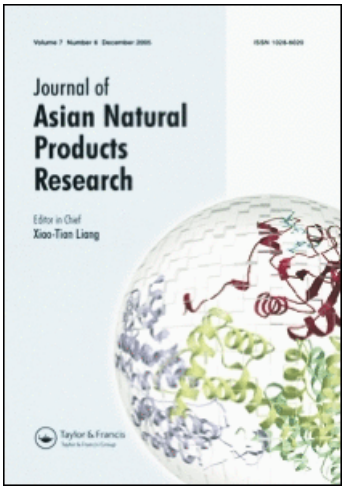

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Li, J. , Jiang, Y. and Tu, P. -F.(2006) 'New acylated triterpene saponins from *Polygala tenuifolia* willd', Journal of Asian Natural Products Research, 8: 6, 499 — 503
To link to this Article: DOI: 10.1080/10286020500173358
URL: http://dx.doi.org/10.1080/10286020500173358

Journal of Asian Natural Products Research, Vol. 8, No. 6, September 2006, 499–503

New acylated triterpene saponins from *Polygala tenuifolia* willd

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(Received 16 December 2004; revised 4 February 2005; in final form 25 February 2005)

Two new acylated presenegenin glycosides *E*-onjisaponin H (**5**) and *Z*-onjisaponin (**6**) together with seven known saponins were isolated from the roots of *Polygala tenuifolia* Willd. Compounds **5** and **6** were obtained as a pair of isomers due to *trans* and *cis*-*p*-methoxycinnamoyl. Their structures were elucidated mainly by 2D-NMR techniques including $^{1}H-^{1}HCOSY$, TOCSY, HSQC, HMBC as 3-*O*-(β-D-glucopyranosyl) presenegenin 28-{*O*-β-D-apiofuranosyl-(1 → 3)-*O*-[β-D-xylopyranosyl-(1 → 4)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-4-*O*-[(*E*)-*p*-methoxycinnamoyl]-β-D-fucopyranosyl} ester (**5**) and its (*Z*)-isomer (**6**).

Keywords: *Polygala tenuifolia*; Triterpene saponin; *E*-Onjisaponin H; *Z*-Onjisaponin H

1. Introduction

The roots of *Polygala tenuifolia* Willd., 'Yuanzhi', are a well-known traditional Chinese medicine used as an expectorant, tonic, sedative and for preventing dementia. Various xanthones, saponins and oligosaccharide esters have been isolated from this plant [1–7]. We previously reported xanthone *O*-glycosides and oligosaccharide esters from this plant [8–9]. Herein we report the isolation and structure elucidation of two new triterpene glycosides named *E*-onjisaponin H (**5**) and *Z*-onjisaponin H (**6**) (figure 1). Seven known triterpene saponins (**1**–**4** and **7**–**9**) isolated from this plant were identified by comparison of the spectral data with reported in literature, as desaylsenegasaponin b (**1**) [10], desaylsenegasaponin c (**2**) [11], polygalasaponin XXVIII (**3**) [12], desaylsenegasaponin III (**4**) [11], polygalasaponin XXXII (**7**) [12], onjisaponin A (**8**) [11] and *Z*-onjisaponin A (**9**) [11].

2. Results and discussion

Compound **5** was obtained as an amorphous powder. Its ESI-MS exhibited a quasi-molecular ion peak at *m/z* 1560 $[M + NH_4]^+$, and in conjunction with the analysis of the ^{13}C NMR spectrum, its molecular formula was deduced to be $C_{74}H_{110}O_{34}$. On acid hydrolysis,

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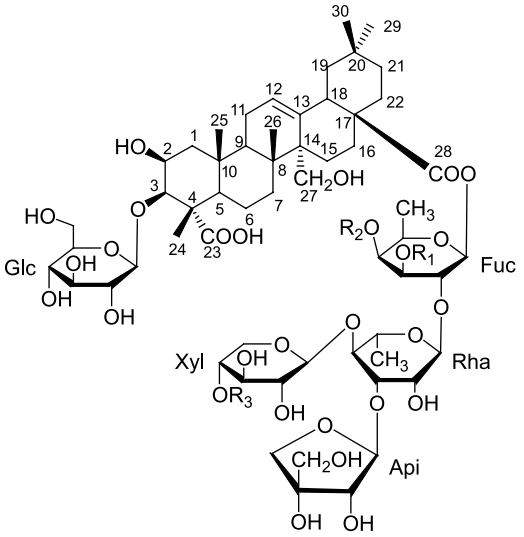
Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500173358



	R_1	R_2	R_3	
5	Rha	A	H	A = *E*-*p*-methoxycinnamoyl
6	Rha	B	H	B = *Z*-*p*-methoxycinnamoyl
7	Rha	A	Ara	

Figure 1. Structures of compounds **5**–**7**.

compound **5** afforded glucose, fucose, rhamnose, xylose and apiose. While on alkaline hydrolysis, it gave (*E*)-*p*-methoxycinnamic acid and tenuifolin (3-*O*-*β*-D-glucopyranosyl presengenin). Comparison of the NMR spectral data of **5** with that of **7** indicated the lack of one set of arabinosyl signals in **5**. This suggested both compounds **5** and **7** have the same triterpene skeleton and sugar moieties except for the lack of an arabinosyl moiety in **5**. Six anomeric proton signals [δ 5.04 (d, $J = 7.0$ Hz), 5.28 (d, $J = 7.0$ Hz), 5.54 (brs), 5.82 (brs), 6.07 (d, $J = 7.5$ Hz) and 6.13(d, $J = 3.5$ Hz), which correlated in the HSQC spectrum with δ (C) 105.2, 105.0, 104.8, 102.2, 94.9 and 111.7, respectively] were observed in the ^{1}H NMR spectrum of **5**, affirming six sugar moieties in the structure. Sugar proton and carbon signals in the NMR spectra (see Experimental and table 1) were assigned by ^{1}H–^{1}HCOSY, TOCSY, HMBC and HSQC spectra. The sugar linkages were further confirmed to be the same sequence with that of **7** by the HMBC correlations. In the HMBC spectrum, long-range correlations were observed between H-1 of Glc (δ 5.04) and C-3 of aglycone (δ 85.9), H-1 of Fuc (δ 6.07) and C-28 of aglycone (δ 176.4), H-1 of Xyl (δ 5.28) and C-4 of Rha (δ 77.9), H-1 of Rha′ (δ 5.54) and C-3 of Fuc (δ 79.8), H-1 of Rha (δ 5.82) and C-2 of Fuc (δ 76.5), H-1 of Api (δ 6.13) and C-3 of Rha (δ 82.6), H-4 of Fuc (δ 5.88) and (*E*)-*p*-methoxycinnamoyl carbonyl carbon (δ 167.1). From these data, the structure of compound **5** was elucidated as 3-*O*-(*β*-D-glucopyranosyl) presenegenin 28-{*O*-*β*-D-apiofuranosyl-(1 → 3)-*O*-[*β*-D-xylopyranosyl-(1 → 4)]-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*O*-[*α*-L-rhamnopyranosyl-(1 → 3)]-4-*O*-[(*E*)-*p*-methoxycinnamoyl]-β-D-fucopyranosyl} ester.

Compound **6** was obtained as an amorphous powder. Its ESI-MS exhibited the same quasi-molecular ion peak at *m/z* 1560 $[M + NH_4]^+$ with **5**. The ^{1}H and ^{13}C NMR chemical shifts of **6** were similar to those of **5** except for the appearance of a (*Z*)-*p*-methoxycinnamoyl group in **6** instead of the (*E*)-*p*-methoxycinnamoyl group in **5**. This indicated both compounds **6**

Table 1. ^{13}C NMR spectral data of compounds **5**–**7** (125 MHz, in pyridine-d_5).

No.	***5***	***6***	***7***	*No.*	***5***	***6***	***7***
Aglycone				C-3 sugar			
1	44.2	44.4	44.2	Glc-1	105.2	105.3	105.3
2	70.2	70.3	70.3	2	75.1	75.2	75.3
3	85.9	85.9	86.1	3	78.3	78.3	78.4
4	52.8	52.8	52.7	4	71.4	71.5	71.6
5	52.5	52.5	52.5	5	78.3	78.3	78.4
6	21.2	21.2	21.3	6	62.6	62.6	62.7
7	33.8	33.9	34.0	C-28 sugar			
8	41.1	41.1	41.3	Fuc-1	94.9	95.0	95.0
9	49.2	49.3	49.4	2	76.5	76.5	76.8
10	37.0	37.0	37.1	3	79.8	79.8	80.1
11	23.5	23.5	23.6	4	73.6	73.5	73.3
12	127.6	127.7	127.8	5	70.7	70.5	70.8
13	138.9	138.9	139.0	6	16.9	16.8	17.0
14	47.9	47.9	48.0	Rha-1 (F-2)	102.2	102.2	102.3
15	24.5	24.5	24.6	2	71.6	71.7	71.8
16	24.0	24.0	24.1	3	82.6	82.7	82.7
17	46.9	46.9	47.1	4	77.9	77.9	78.1
18	42.0	42.0	42.2	5	68.8	68.9	68.9
19	45.5	45.6	45.6	6	18.6	18.7	18.8
20	30.8	30.8	30.9	Rha-1′ (F-3)	104.8	105.0	105.0
21	33.8	33.9	34.0	2	72.3	72.4	72.3
22	32.1	32.2	32.3	3	73.2	73.1	72.8
23	180.8	180.8	180.9	4	73.6	73.5	73.7
24	14.2	14.2	14.4	5	71.1	71.2	71.0
25	17.6	17.5	17.7	6	18.6	18.7	18.8
26	19.1	19.2	19.2	Xyl-1	105.0	105.0	104.8
27	64.4	64.4	64.5	2	75.6	75.6	74.7
28	176.4	176.5	176.6	3	78.4	78.4	85.9
29	33.0	33.1	33.2	4	70.8	70.8	69.4
30	24.0	24.0	24.2	5	67.1	67.2	66.6
Cinn.				Api-1	111.7	111.8	111.9
1	127.3	127.7	127.5	2	77.9	77.9	77.8
2	130.4	133.2	130.6	3	79.9	79.8	80.1
3	114.7	114.0	114.8	4	74.3	74.3	74.7
4	161.9	161.1	162.1	5	63.9	63.9	64.1
5	114.7	114.0	114.8	Ara			
6	130.4	133.2	130.6	1			105.6
7	145.6	144.8	145.7	2			72.5
8	115.6	116.4	115.8	3			74.3
9	167.1	166.3	167.3	4			69.4
OMe	55.3	55.1	55.5	5			67.2

and **5** have the same triterpene skeleton and sugar moieties except for the presence of the (*Z*)-*p*-methoxycinnamoyl moiety in **6** instead of the (*E*)-*p*-methoxycinnamoyl moiety in **5**. The site of linkage of (*Z*)-*p*-methoxycinnamoyl group was determined by the HMBC correlations. In the HMBC spectrum, long-range correlations were observed between the (*Z*)-*p*-methoxycinnamoyl carbonyl carbon signal at δ 166.3 and H-4 of Fuc at δ 5.79. Thus, the structure of compound **6** was elucidated as 3-*O*-(β-D-glucopyranosyl) presenegenin 28-{*O*-β-D-apiofuranosyl-(1 → 3)-*O*-[β-D-xylopyranosyl-(1 → 4)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-4-*O*-[(*Z*)-*p*-methoxycinnamoyl]-β-D-fucopyranosyl} ester.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Polatronic D polarimeter. UV spectra were recorded on a UV-2401 spectrophotometer. ESI-MS spectra were performed on a QSTAR mass spectrometer. ^{1}H, ^{13}C NMR, TOCSY, HSQC and HMBC spectra were measured on a Bruker AM-500 spectrometer. D101 resin (Tianjin Chemical Co.), ODS silica gel (Fuji Co.), the column chromatography silica gel (200–300 mesh) (Qingdao Marine Chemical Factory).

3.2 Plant material

The roots of *Polygala tenuifolia* were collected from Shanxi Province and identified by one of the authors (Tu). A voucher specimen is deposited in the Herbarium of Modern Research Center for TCM, Peking University, Beijing, China.

3.3 Extraction and isolation

The air-dried roots of *P. tenuifolia* (3.0 kg) were extracted with 70% methanol (20 L × 3) under reflux. The extractive solution was combined and evaporated *in vacuo* to yield 1.05 kg of residue, 950 g of which was suspended in water and extracted successively with EtOAc and *n*-BuOH. Parts of *n*-BuOH extract (190 g) were subjected to a macroporous resin D101 column (10 × 65 cm). The adsorbed material was eluted with H_2O, 30%, and 60% EtOH, respectively. The 30% EtOH eluate (34.6 g) was chromatographed on silica gel (800 g), eluting with $CHCl_3$–MeOH–H_2O in a gradient manner (100:1:0 → 65:35:10, organic layer), Fr.76–80 and Fr.124–148 were purified by HPLC with MeOH 0.05% TFA/H_2O (48:52) as mobile phase to furnish **1** (65 mg), **2** (34 mg), **3** (55 mg) and **4** (23 mg), respectively. The 60% EtOH eluate (52.6 g) was chromatographed on silica gel (1200 g), eluting with $CHCl_3$–MeOH–H_2O in a gradient manner (100:1:0 → 65:35:10, organic layer), Fr.38–47 and Fr.64–73 were chromatographed on ODS silica gel column eluted with MeOH–H_2O (65:35), and purified by HPLC with MeOH–0.05% TFA/H_2O (48:52) as mobile phase to yield **5** (35 mg), **6** (28 mg), **7** (55 mg), **8** (124 mg) and **9** (26 mg), respectively.

3.3.1 E-Onjisaponin H (5). Amorphous powder, mp 212–215°C; $[\alpha]_D^{25}$ −5.4 (*c* 0.52, MeOH); ESI-MS (*m/z*): 1560 $[M + NH_4]^+$. UV λ_{max} (MeOH) nm: 228, 311. ^{1}H NMR (500 MHz, in pyridine-d_5) δ: 7.91 (1H, d, $J = 16.0$ Hz, H-γ of *E*-*p*-methoxycinnamoyl), 7.37 (2H, d, $J = 8.0$ Hz, H-2, 6 of *E*-*p*-methoxycinnamoyl), 6.96 (2H, d, $J = 8.0$ Hz, H-3, 5 of *E*-*p*-methoxycinnamoyl), 6.51 (1H, d, $J = 16.0$ Hz, H-β of *E*-*p*-methoxycinnamoyl), 6.13 (1H, d, $J = 3.5$ Hz, Api-H-1), 6.07 (1H, d, $J = 8.0$ Hz, Fuc-H-1), 5.85 (1H, t-like, H-12), 5.82 (1H, brs, Rha-H-1), 5.54 (1H, brs, Rha′-H-1), 5.28 (1H, d, $J = 7.0$ Hz, Xyl-H-1), 5.04 (1H, d, $J = 7.0$ Hz, Glc-H-1), 4.66 (1H, m, H-2), 4.59 (1H, brs, H-3), 4.05, 3.81 (each 1H, d, $J = 12.0$ Hz, CH_2-27), 3.65 (3H, s, OCH_3), 1.94 (3H, s, CH_3-24), 1.73 (3H, d, $J = 6.0$ Hz, Rha′-CH_3), 1.70 (3H, d, $J = 6.0$ Hz, Rha-CH_3), 1.56 (3H, s, CH_3-25), 1.32 (3H, d, $J = 6.0$ Hz, Fuc-CH_3), 1.11 (3H, s, CH_3-26), 1.00 (3H, s, CH_3-30), 0.76 (3H, s, CH_3-29). ^{13}C NMR (125 MHz, in pyridine-d_5) data (see table 1).

3.3.2 Z-Onjisaponin H (6). Amorphous powder, mp 211–215°C; $[\alpha]_D^{25}$ −8.4 (*c* 0.76, MeOH); ESI-MS (*m/z*): 1560 $[M + NH_4]^+$. UV λ_{max} (MeOH) nm: 310. ^{1}H NMR (500 MHz,

in pyridine-d_5) δ: 7.98 (2H, d, $J = 8.5$ Hz, H-2, 6 of *Z*-*p*-methoxycinnamoyl), 7.02 (2H, d, $J = 8.5$ Hz, H-3, 5 of *Z*-*p*-methoxycinnamoyl), 6.86 (1H, d, $J = 13.5$ Hz, H-γ of *Z*-*p*-methoxycinnamoyl), 6.14 (1H, d, $J = 3.0$ Hz, Api-H-1), 6.01 (1H, d, $J = 8.0$ Hz, Fuc-H-1), 5.95 (1H, d, $J = 12.5$ Hz, H-β of *Z*-*p*-methoxycinnamoyl), 5.83 (1H, t-like, H-12), 5.75 (1H, brs, Rha-H-1), 5.52 (1H, brs, Rha′-H-1), 5.28 (1H, d, $J = 7.5$ Hz, Xyl-H-1), 5.07 (1H, d, $J = 7.0$ Hz, Glc-H-1), 4.66 (1H, m, H-2), 4.60 (1H, brs, H-3), 4.06, 3.80 (each 1H, d, $J = 12.0$ Hz, CH_2-27), 3.61 (3H, s, OCH_3), 1.98 (3H, s, CH_3-24), 1.71 (3H, d, $J = 6.0$ Hz, Rha′-CH_3), 1.68 (3H, d, $J = 6.0$ Hz, Rha-CH_3), 1.61 (3H, s, CH_3-25), 1.25 (3H, d, $J = 6.0$ Hz, Fuc-CH_3), 1.11 (3H, s, CH_3-26), 1.02 (3H, s, CH_3-30), 0.77 (3H, s, CH_3-29). ^{13}C NMR (125 MHz, in pyridine-d_5) data (see table 1).

3.4 Hydrolysis of saponins

3.4.1 Alkaline hydrolysis of 5 and 6. Each compound (3 mg) was refluxed with 5% NaOH aq. (1 mL) for 1.5 h. The reaction mixture was adjusted to pH 6 with dilute HCl, and extracted with EtOAc (3 × 2 mL), then the water layer was extracted with H_2O-saturated *n*-BuOH (3 × 2 mL). From the EtOAc layer, (*E*)-*p*-methoxycinnamic acid was detected, and tenuifolin was detected from the *n*-BuOH layer, by means of TLC comparison with authentic sample ($CHCl_3$–MeOH–H_2O 65:35:10, organic layer). The plate was sprayed with 10% H_2SO_4/EtOH reagent by heating at 105°C.

3.4.2 Acid hydrolysis of 5 and 6. Each compound (2 mg) was hydrolyzed with 2M HCl–dioxane (1:1, 1 mL), refluxed for 2 h. After removing the solvent under reduced pressure, the residue was suspended in H_2O and extracted with chloroform (3 × 1 mL). Glucose, rhamnose, xylose, apiose and fucose were detected in the remaining H_2O layer of **5** and **6**, by means of TLC comparison with standard sugars using solvent system $CHCl_3$–MeOH–H_2O (8:5:1, v/v). The plate was sprayed with diphenylaminephosphoric acid reagent by heating at 105°C.

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