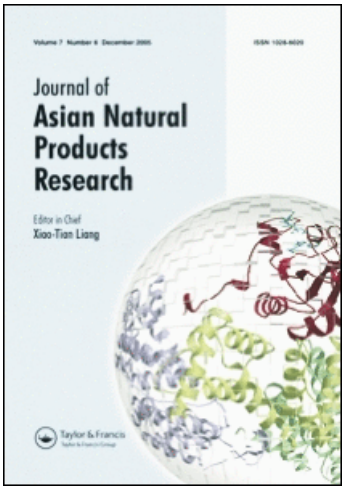

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A novel triterpenoid saponin from *Polygala tenuifolia* Willd.

Tun-Hai Xu[ac], Gang Lv[b], Ya-Juan Xu*[b], Sheng-Xiu Xie[b], Hong-Feng Zhao[b], Dong Han[b], Yun-Shan Si[b], Yu Li[a], Jian-Zhao Niu[a] and Dong-Ming Xu[b]

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A new triterpenoid saponin, tenuifoside A, was isolated together with three known triterpenoid saponins **2**, **3**, and **4** from the roots of *Polygala tenuifolia* Willd. With the help of chemical and spectral analyses (IR, MS, 1D-NMR, and 2D-NMR), the structure of the new saponin was elucidated as 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-[β-D-apiofuranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-[4-*O*-*p*-methoxycinnamoyl]-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-fucopyranosyl ester (**1**). Three known triterpenoid saponins (**2**–**4**) were identified on the basis of spectroscopic data.

Keywords: *Polygala tenuifolia* Willd; radix polygala; triterpenoid saponin; tenuifoside A

1. Introduction

The root of *Polygala tenuifolia* Willd., a Chinese traditional medicine named 'Yuan Zhi,' has been recorded in Chinese Pharmacopeia as a neuroleptics, sedative, and expectorant for the treatment of insomnia, amnesia and cough [1]. In the previous research, complex triterpenoid saponins were isolated and identified [2–4]. In this paper, we report the structure elucidation of a new triterpenoid saponin tenuifoside A (**1**), together with three known triterpenoid saponins **2**, **3**, and **4** by using 1D and 2D NMR techniques, ESIMS analysis as well as chemical methods.

2. Results and discussion

Tenuifoside A (**1**) was obtained as a white powder. The IR spectrum showed absorptions for hydroxyl group (3425 cm^{-1}), carbonyl group (1711 cm^{-1}), and double bond (1632 cm^{-1}). The acidic hydrolysis of **1** with hydrochloric acid afforded glucose, fucose, rhamnose, xylose, and apiose as the sugar components was identified by comparison with authentic samples on TLC. Compound **1** exhibited the molecular formula $C_{79}H_{118}O_{38}$ by its HRMS analysis. The ESIMS of **1** showed a positive ion peak at *m/z* 1697 $[M + Na]^+$ and a negative ion peak at *m/z* 1673 $[M - H]^-$. The ^1H NMR spectrum showed signals ascribable to five tertiary methyl groups at δ 0.66, 0.89, 1.02, 1.44, and 1.83, a trisubstituted olefinic signal at δ 5.76 (1H, s), and seven anomeric proton signals at δ 4.95 (1H, d, $J = 7.5$ Hz, glc-H-1), 5.98 (1H, d, $J = 8.0$ Hz, fuc-H-1), 5.71 (1H, br s, rha-H-1), 5.44 (1H, br s, rha-H-1′), 5.14 (1H, d, $J = 7.0$ Hz, xyl-H-1), 5.05 (1H, d, $J = 7.5$ Hz, xyl-H-1′), 6.00 (1H, br s, api-H-1), and three secondary methyl signals ascribable to 6-deoxyhexose at δ 1.33 (3H, d, $J = 5.6$ Hz,

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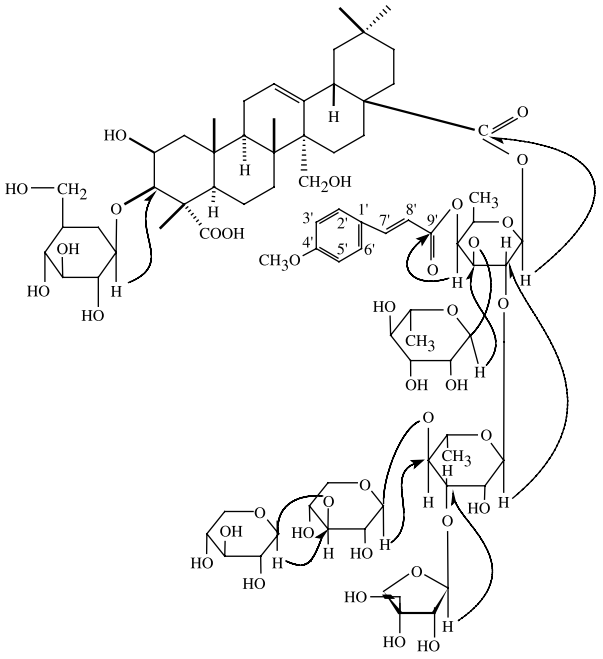
fuc-H-6), 1.58 (3H, d, $J = 5.9$ Hz, rha-H-6), 1.64 (3H, d, $J = 6.0$ Hz, rha-H-6′), and *p*-methoxycinnamoyl signals at δ7.36 (1H, d, $J = 8.5$ Hz, H-2′), 6.95 (1H, d, $J = 8.5$ Hz, H-3′), 6.95 (1H, d, $J = 8.5$ Hz, H-5′), 7.36 (1H, d, $J = 8.5$ Hz, H-6′), 7.88 (1H, d, $J = 16.0$ Hz, H-7′), 6.52 (1H, d, $J = 16.0$ Hz, H-8′)], 3.67 (3H, s). The ^{13}C NMR spectrum of **1** (Table 1) showed two carbonyl carbon signal at δ 176.6 and 181.5, one pair of the olefinic carbons of the aglycone part at δ 127.9, 139.1, and seven anomeric carbon signals at δ95.0, 102.3, 104.9, 105.1, 105.4, 105.8, and 111.9 and *p*-methoxycinnamoyl carbon signals at δ 127.5 (C′-1), 130.7 × 2 (C′-2, C′-6), 114.9 × 2 (C′-3, C′-5), 162.1 (C′-4), 145.8 (C′-7), 115.8 (C′-8),167.3 (C′-9), 55.5 (MeO). These observations suggested that **1** was a triterpenoid saponin, which was acylated with *p*-methoxycinnamoyl esters. In a comparison of the ^{13}C NMR signals for aglycone of **1** with those of known saponin polygalasaponin XXXII [5], all signals due to the aglycone of **1** were almost superimposable with those of polygalasaponin XXXII, indicating the aglycone of **1** was same as that of polygalasaponin XXXII, which was presenegenin and its 3-hydroxy group and 28-carbonyl group carried a sugar moiety, respectively. Based on the ^{1}H and ^{13}C NMR spectral data of **1**, the anomeric configuration of the sugar moieties were determined as β-configurations for glucose, fucose, xylose, and apiose, and α-configurations for rhamnose [6,7]. When the ^{1}H and ^{13}C NMR signals of **1** were compared with those of polygalasaponin XXXII, the ^{1}H and ^{13}C NMR spectral data of *p*-methoxycinamoyl signals of **1** were similar to those of polygalasaponin XXXII (Table 1), and sugar signals were similar to those of polygalasaponin XXXII, except for the appearance of xylopyranosyl signals linked to C-3 of rhamnose instead of arabinopyranosyl signals [5], indicating the sugar moieties of **1** were same as those of polygalasaponin XXXII, except for xylose linked to C-3 of rhamnose, instead of arabinose.

The positions of the sugar residues in **1** were defined unambiguously by the HMBC experiment (Scheme 1). A HMBC correlation between H-1 (δ 4.59) of the glucose and C-3 (δ 86.1) of the aglycone, indicated that a glucopyranosyl was linked to C-3 of the aglycone. Additionally, the HMBC correlations between H′-1 (δ 5.98) of the fucose and C-28 (δ 176.7) of the aglycone, H-1 (δ 5.71) of the rhamnose and C-2 (δ 76.6) of the fucose, H-1′ (δ 5.44) of the rhamnose′ and C-3 (δ 80.1) of the fucose, H-1 (δ 5.14) of the xylose and C-4 (δ 78.5) of the rhamnose, H-1′ (δ 5.05) of the xylose′ and C-3 (δ 86.6) of

Table 1. ^{13}C NMR spectral data of compound **1** (δ/$_{ppm,}$ 400 MHz, C_5D_5N).

C	δ	C	δ	C	δ	C	δ	C	δ
1	44.4	18	42.2	4′	162.1	3	80.1	2	77.6
2	70.4	19	45.6	5′	114.9	4	73.5	3	80.1
3	86.1	20	30.9	6′	130.7	5	71.4	4	74.5
4	53.1	21	33.3	7′	145.8	6	17.1	5	64.2
5	52.7	22	32.4	8′	115.8	Rha 1	102.3	Xyl 1	104.9
6	21.4	23	181.5	9′	167.4	2	71.6	2	74.9
7	34.0	24	14.5	OMe	55.5	3	82.7	3	86.6
8	41.3	25	17.8	C3-		4	78.5	4	69.4
9	49.5	26	19.3	Glc 1	105.4	5	68.9	5	66.7
10	37.1	27	64.6	2	75.4	6	18.9	Xyl 1′	105.8
11	24.2	28	176.6	3	78.2	Rha 1′	105.1	2′	76.6
12	127.9	29	33.3	4	71.1	2′	72.3	3′	78.5
13	139.1	30	23.8	5	78.5	3′	72.6	4′	71.1
14	47.1	Cinnamoyl		6	62.8	4′	73.8	5′	67.0
15	24.7	1′	127.5	C28-		5′	70.9		
16	24.2	2′	130.7	Fuc 1	95.0	6′	18.9		
17	48.1	3′	114.9	2	76.6	Api 1	111.9		



Scheme 1. Key HMBC correlations for compound **1**.

xylose, H-1 (δ 6.00) of the apiose and C-3 (δ 82.7) of the rhamnose, H-4 (δ 5.76) of fucose and C-9′ (δ 167.4) of the *p*-methoxycinnamoyl, showed that the six-saccharide residue *O*-β-D-xylopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-[β-D-apiofuranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-[4-*O*-*p*-methoxycinnamoyl]-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-fucopyranosyl was linked to the presenegenin unit at C-28. Therefore, the structure of **1** was determined as 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-[β-D-apiofuranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-[4-*O*-*p*-methoxycinnamoyl]-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-fucopyranosyl ester.

Three known compounds (**2**–**4**) were identified as presenegenin 3-*O*-β-D-glucopyranside (tenuifolin) (**2**),[2] 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-xylopranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester (polygalasaponin XXVII) (**3**)[5] and 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-xylopranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[β-D-apiofuranosyl-(1 → 3)]-β-D-fucopyranosyl ester (polygalasaponin XXIV) (**4**) [5] by comparison of their physical and spectroscopic data with those reported in the literature, respectively.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Kofler-microscope apparatus and are uncorrected. The optical rotations were determined on WZZ-15 autopolarimeter. The IR spectra were measured on a Y-Zoom scroll Fourier transform infrared spectrometer with a KBr disc. The ESIMS was recorded on LCQ-1700 ESIMS instrument. The NMR spectra were obtained on a Bruker AM-500 instrument, using TMS as internal standard. HPLC was performed using an ODS column (Shim-park PREF-ODS, 250 × 4.6 mm). Column chromatography was performed on silica gel (200–300 mesh, Qingdao Chemical Industry Qingdao, China) and reversed silica gel (25 × 2.5 cm, Nacalai Tesque, Kyoto, Japan). Macroporous resin D_{101}

made in Tianjin gel Co. Spots were detected after spraying with 10% H_2SO_4 in EtOH.

3.2 Plant material

The roots of *P. tenuifolia* Willd. were purchased from the Company of Chinese Medicinal Materials in Changchun, Jilin Province, China, in September 2004, and identified by Prof Minglu Deng, Changchun College of Traditional Chinese Medicine. A voucher specimen (0400908) has been deposited in the Herbarium of Academy of Traditional Chinese Medicine and Material Medica of Jilin Province.

3.3 Extraction and isolation

The dried and powdered roots (7.5 kg) of the plant were extracted three times with H_2O at boiling, and the extract was concentrated under reduced pressure, to obtain a crude residue (215 g), which was chromatographed over a D_{101} macroporous resin column (10 × 80 cm), eluted successively with H_2O, 30% EtOH, and 60% EtOH. The 60% EtOH eluate was concentrated to dryness (13.5 g) and chromatographed over a silica gel column (200–300 mesh) eluted with *n*-BuOH to give fractions 1–5. Fraction 3 was subjected to HPLC (column: 10 × 250 mm, RP-18, 10 μm, flow rate: 3.0 ml/min) with MeOH–H_2O (8:2–7:3) as mobile phase to afford compound **1** (63 mg).

3.3.1 Tenuifoside A (*1*)

White powder, m.p. 242–244°C, $[\alpha]_D^{18} = -39$, (*c* 0.45, MeOH). IR (KBr) (ν_{max}): 3425, 2931, 1711, 1632, 1453, 1255, 1070, and 1036 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) δ 0.66, 0.89, 1.02, 1.44, 1.83 (each 3H, s, CH_3), 5.76 (1H, s, 12-H), 4.95 (1H, d, $J = 7.5$ Hz, glc-H-1), 5.98 (1H, d, $J = 8.0$ Hz, fuc-H-1), 5.71 (1H, br s, rha-H-1), 5.44 (1H, br s, rha-H-1′), 5.14 (1H, d, $J = 7.0$ Hz, xyl-H-1), 5.05 (1H, d, $J = 7.5$ Hz, xyl-H-1′), 6.00 (1H, br s, api-H-1), 1.33 (3H, d, $J = 5.6$ Hz, fuc-H-6), 1.58 (3H, d, $J = 5.9$ Hz, rha-H-6), 1.64 (3H, d, $J = 6.0$ Hz, rha-H-6′), *p*-methoxycinnamoyl signals δ 7.36 (1H, d, $J = 8.5$ Hz, H-2′), 6.95 (1H, d, $J = 8.5$ Hz, H-3′), 6.95 (1H, d, $J = 8.5$ Hz, H-5′), 7.36 (1H, d, $J = 8.5$ Hz, H-6′), 7.88 (1H, d, $J = 16$ Hz, H-7′), 6.52 (1H, d, $J = 16$ Hz, H-8′), 3.67 (3H, s), and ^{13}C NMR (125 MHz, pyridine-d_5) spectral data are given in Table 1. HRMS *m/z*: 1674.7302 $[M]^+$(calcd for $C_{79}H_{118}O_{38}$, 1674.7301). ESIMS *m/z*: 1697.5 $[M + Na]^+$, 1673 $[M - H]^-$.

3.4 Acid hydrolysis

The saponin (10 mg) was heated with 2 M HCl–MeOH (10 ml) under reflux for 3 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The water layer was neutralized with Na_2CO_3, concentrated, and subjected to TLC analysis with authentic samples D-glucose, D-fucose, D-xylose, L-arabinose, L-rhamnose, and D-apiose, and developed with CH_2Cl_2–MeOH–H_2O (15:6:1) and H_2O–MeOH–AcOH–EtOAc (15:15:20:65). Detection was carried out with aniline phthalate spray.

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