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Triterpenoid Saponins and Oligosaccharides from the Roots of *Polygala tenuifolia* Willd.

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[ABSTRACT] AIM: To study the chemical constituents and bioactivities of the roots of *Polygala tenuifolia* Willd.. **METHODS**: Bioactivity-guide was applied to study the chemical constituents of *P. tenuifolia*, and the chemical structures were determined by application of spectroal data (NMR, MS). **RESULTS**: One new, four known triterpenoid saponins and six oligosaccharides were obtained and their structures were identified as 3-*O*-*β*-D-glucopyranosyl-presenegenin 28-*O*-*α*-L-arabinopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-[3-*O*-3, 4, 5-trimethoxycinnamoyl] -*β*-D-fucopyranosyl ester (onjisaponin Wg, **1**), onjisaponins Fg (**2**), Ng (**3**), R (**4**), and O (**5**), tenuifolioses A (**6**), B (**7**), C(**8**), I (**9**), K (**10**), and G (**11**). **CONCLUSION:** Compound **1** is a new saponin. Compounds **6** and **7** showed neuroprotective activity against glumate and serum deficiency at the concentration of 1×10^{-5} mol·L^{-1}. Compounds **7** and **8** potentiated basal synaptic transmission in the dentate gyrus of anesthetized rats. The NMR data of **2-5** were reported for the first time.

[KEY WORDS] *Polygala tenuifolia* Willd.; Triterpenoid saponin; Onjisaponin Wg: Oligosaccharide; Neuroprotection

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1 Introduction

Polygala tenuifolia Willd. (Polygalaceae) has been used as a traditional Chinese medicine for thousands of years as expectorant, tonic, tranquillizer, and for treatment of dementia, amnesia, neurasthenia [1]. We have reported the isolation of six new triterpenoid saponins with neuroprotec- tive effect [2]. Furthermore, polygalasaponin XXXII could enhance hippocampus-dependent learning and memory, possibly through the improvement of synaptic transmission, activation of the MAP kinase cascade and enhancement of BDNF level [3]. In our ongoing studies on the bioactive chemical constituents of the roots of *P. tenuifolia*, one new, four known saponins and six known oligosaccharides were isolated. This paper discribes the isolation and structural elucidation of the new triterpenoid saponin, as well as the neuroprotective effect and the potentiating effect on basal synaptic transmission in the dentate gyrus of anesthetized rats of oligosaccharides.

2 Experimental

2.1 *General experimental procedures*

Optical rotations were determined on a Perkin-Elmer 241 digital polarimeter. IR spectra were recorded on a Nicolet 5700 spectrometer as FT-IR-Microscope. NMR spectra were acquired on a Varian Inova-500 spectrometer using TMS as internal standard. ESI-MS and HREI-MS were performed on an Agilent 1100 series LC/MSD Trap SL mass spectrometer and an Autospec Ultima-Tof mass spectrometer, respectively. Reversed-phase HPLC was performed on YMC-Pack ODS-A column (YMC Co., Ltd.). Silica gel (100−200 and 200−300 mesh, Qingdao) was used for column chromatography and silica gel GF_{254} (Qingdao) for TLC analysis.

2.2 *Plant material*

The roots of *Polygala tenuifolia* Willd. were collected in Shanxi Province, China, in June, 2004. The plant was identified by Professor LI Xian-En. A voucher specimen (No. 2004005) was deposited in the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing.

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These authors have no any conflict of interest to declare.

2.3 *Extraction and isolation*

Dried roots of *P. tenuifolia* Willd. (3.0 kg) were powdered and extracted three times with 95% EtOH for 2 h each time, affording 0.68 kg residue after solvent removement under reduced pressure. The residue was subjected to silica gel column chromatography (100−200 mesh) eluted successively with $CHCl_3$, EtOAc, acetone and MeOH. The MeOH extract (PM, 380 g) was applied to macroporous resin (D101, 2 kg) column chromatography and eluted with H_2O, 30% EtOH, 60% EtOH and 95% EtOH (F-a−F-d), respectively. The 60% EtOH fraction (F-c, 77.2 g) was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH-H_2O (70 : 30 : 5) to afford 10 fractions. Fractions 2 (6.0 g) and 8 (4.5 g) were separated by preparative MPLC (40%−80% MeOH) and then purified repeatedly by semi-preparative HPLC to afford **1**−**11** (10, 20, 25, 25, 18, 347, 170, 165, 25, 15 and 20 mg, respectively).

2.4 *Onjisaponin Wg (**1**):*

White powder, $[\alpha]_D^{20}$ − 4.0 (*c* 0.10, MeOH). IR (KBr)*υ*: 3 397, 2 938, 1 746, 1 679, 1 633, 1 504, 832, 720 cm^{-1}. ^1H and ^{13}C NMR data, see Table 1. ESI-MS *m/z* 1 587 [M −

Table 1 NMR data of compound 1 in pydridine-d_5 (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR; [a] overlap with other signals)

Position	^1H NMR	^{13}C NMR	Position	^1H NMR	^{13}C NMR
1		44.2	C-3 sugar		
2	4.70 m	69.2	Glc-1	5.07 d (7.5)	105.5
3	4.61 d (3.0)	85.9	2	4.13 [a]	75.2
4		52.8	3	4.17 [a]	78.3
5		52.5	4	4.18 [a]	71.5
6		21.2	5	3.91 [a]	78.3
7		33.0	6	4.44 dd (11.5, 2.0)	62.6
				4.31 dd (11.5, 5.0)	
8		41.1	C-28 sugar		
9		49.2	Fuc-1	6.24 d (8.0)	94.3
10		37.0	2	4.94 t (9.0)	73.8
11		23.6	3	5.62 dd (9.5, 2 .5)	78.1
12	5.78 m	128.0	4	4.06 [a]	69.7
13		138.6	5	4.38 [a]	72.1
14		47.8	6	1.51 d (6.5)	16.8
15		24.4	Rha-1(Fuc-2)	5.86 br s	101.9
16		24.0	2	4.75 br s	71.7
17		46.8	3	4.48 [a]	81.8
18	3.23 br d (13.0)	42.1	4	4.41 [a]	78.3
19		45.2	5	4.37 [a]	68.6
20		30.8	6	1.65 d (6.0)	18.9
21		33.7	Xyl-1(Rham-4)	5.24 d (8.0)	105.3
22		32.2	2	3.92 t (8.5)	75.2
23		180.9	3	4.11 [a]	86.3
24	1.99 s	14.2	4	4.07 [a]	69.2
25	1.58 s	17.5	5	3.34 t (11.0)	66.5
26	1.12 s	18.9	Ara-1 (xyl-3)	5.14 d (7.5)	104.7
27	3.79 br d (11.5)	64.5	2	4.45 m	78.3
	4.00 br d (11.5)		3	4.05 m	74.7
28		176.5	4	4.15 m	69.2
29	0.78 s	33.0	5	3.56 d (11.5)	67.0
30	0.94 s	24.0	Api-1(Rham-3)	5.98 d (4.5)	111.8
Cin.			2	4.05 [a]	79.7
1		130.3	3	4.20 [a]	77.8
2	6.86 s	106.3	4	4.13 [a]	74.7
3		154.0	5	4.16 d (12.0)	64.6
4		141.0		4.40 d (12.0)	
5		154.0			
6	6.86 s	106.3			
7	7.94 d (16.0)	117.5			
8	6.72 d (16.0)	146.0			
9		166.5			
OMe	3.74 s (3H)	56.2			
	3.74 s (3H)	56.2			
	3.87 s (3H)	60.6			

H]$^-$, 1 612 [M + Na]$^-$; HRESI-MS *m/z* 1 611.679 5 [M + Na]$^+$ (calcd. for $C_{75}H_{112}O_{36}Na$, 1 611.682 6).

2.5 *Neuroprotective effects assay* [2]

2.5.1 *PC12 cells culture*

PC12 cells were purchased from the Cells Center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and maintained in DMEM medium supplemented with 5% FBS and 5% HS. The flask was incubated at 37 °C in a humidified, 5% CO_2 atmosphere.

2.5.2 *MTT assay for survival rate of PC12 cells injured by serum deficiency with* ***6***−***9***

After 80% culture surface was covered by PC12 cells, the cells were suspended and diluted to 5 × 10^{-4}/mL with DMEM medium with 5% FBS and 5% HS. The cells were inoculated into 96-well plate (100 μL per well) And the plates were incubated at 37 °C in a humidified, 5% CO_2 atmosphere for 24 h. The original culture medium was replaced with the medium containing 1% FBS and 1% HS with or without compounds. The cells were cultured for another 24 h. The cells of the control group were treated with serum without compounds. Subsequently, 10 μL MTT (5 mg·mL^{-1}) was added into each well and cultured in the incubator for 4 h. A solubilization solution (100 mL, 10% SDS in 0.01 mol·L^{-1} HCl) was added into each well. The plate was incubated overnight at 37 °C. The optical density of the cells was measured at 570 nm using a microtiter plate reader. The survival rate of the blank group is regarded as 100%. The survival rate of PC12 cells was calculated by the following formula: Survival rate = ($A_{\text{model with sample}}$ / $A_{\text{blank serum}}$) × 100%. When the survival rate of the test compound exceeds that of the model compound, it was regarded to have neuroprotective effect.

2.5.3 *MTT assay for survival rate of the PC12 cells injured by glutamate*

In the current work, glutamate at a final concentration of 10 mmol·L^{-1} was used as an inducer for neurotoxicity of PC12 cells, with the test compounds added in each treated groups. After 24 h, MTT assay was performed to measure the survival rate of PC12 cells. The survival rate of PC12 cells was calculated by the following formula:

Survival rate = ($A_{serum\ with\ sample}$ / $A_{blank\ serum}$) × 100%

2.6 *Electrophysiological assays* [3]

The evoked potentials were recorded as described previously. Briefly, male Wistar rats were anesthetized with urethane carbamate (1.5 g·kg^{-1}, ip) before being fixed on a SR-6N stereotaxic apparatus (Narishige, Japan). Three holes were sequentially drilled at 0.8, 3.8, and 7.5 mm posterior to Bregma and 1.8, 2.5, and 4.2 mm lateral to the mid-line to allow the placement of an outer guide cannula, a monopolar recording electrode and a bipolar stimulating electrode, respectively. The cannula was placed into the lateral cerebral ventricle at a depth of 2.5–3.0 mm; the recording electrode was placed in the granular cell layer of dentate gyrus at a depth of 3.0–3.5 mm; and the stimulating electrode was lowered into the perforant path (PP) to a depth of 3.0−3.5 mm. The synaptic responses were monitored on a VC-11 memory oscilloscope (Nihon Kohden, Japan). The amplitude of population spike (PS) was used to assess the excitation level of the granular cell population in the dentate gyrus. An evoked response was generated in the granular cell layer by stimulating the PP at low frequency (0.033 Hz) with single constant current pulses (150 μs in duration) triggered by a SEN-7203 electrical stimulator (Nihon Kohden, Japan) through a SS-202J isolator (Nihon Kohden, Japan). After input / output curve determination, the baseline responses were evoked by a stimulus with an intensity that producing 20% of the maximal PS amplitude.

3 Results and Discussion

3.1 *Identification of compound* ***1***

Onjisaponin Wg (**1**) was assigned a molecular formula of $C_{75}H_{112}O_{36}$, from the pseudo-molecular ion at *m/z* 1 611.679 5 [M + Na]$^+$ in the positive HRESI-MS. Its IR spectrum showed absorption bands of hydroxyl (3 397 cm^{-1}), carboxyl (1 679 cm^{-1}) and phenyl (1 633, 1 512, 834, 722 cm^{-1}) groups. Five tertiary methyl groups (δ_H 0.78, 0.94, 1.12, 1.58 and 1.99), one pair of hydroxymethyl [δ_H 3.79 (br d, *J* = 11.5 Hz) and 4.00 (d, *J* = 11.5 Hz)] and one trisubstituted olefinic proton [δ_H 5.78 (m)] were observed in its ^{1}H NMR spectrum together with the information from the ^{13}C NMR data (five sp^3 carbons at δ_C 14.2, 17.5, 18.9, 24.0, 33.0 and two sp^2 olefinic carbons at δ_C 128.0 and 138.6) assigned the aglycone of **1** as presenegenin [2, 4-6]. The proton signals at δ_H 3.74 (6H, s), 3.87 (3H, s), 6.86 (2H, s), 6.72 (d, *J* = 16.0 Hz) and 7.94 (d, *J* = 16.0 Hz) suggested the presence of a 3, 4, 5-trimethoxycinnamic acid moiety in **1**. The ^{13}C NMR spectrum showed six signals for anomeric carbons at δ_C 94.3, 101.9, 104.7, 105.3, 105.5 and 111.8, respectively, and the corresponding anomeric protons at δ_H 6.24 (d, *J* = 8.0 Hz), 5.86 (br s), 5.14 (d, *J* = 7.5 Hz), 5.24 (d, *J* = 8.0 Hz), 5.07 (d, *J* = 8.0 Hz), and 5.98 (d, *J* = 4.5 Hz) were also shown in ^{1}H NMR spectrum. Comparison of the ^{1}H and ^{13}C NMR spectra data with those of onjisaponin W [2] indicated that the difference between the two saponins is the disappearance of the HMG signal in **1**.

The sequencing of the oligosaccharides chain was finally achieved by analysis of HMBC data. For pentasaccharide chain linked to the C-28 of the aglycone, the HMBC spectrum showed the correlations between H-1 (δ_H 5.14, d, *J* = 7.5 Hz) of terminal arabinose and C-3 (δ_C 86.3) of xylose, H-1 (δ_H 5.24, d, *J* = 8.0 Hz) of xylose and C-4 of rhamnose (δ_C 78.3), H-1 (δ_H 5.98, d, *J* = 4.5 Hz) of apiose and C-3 (δ_C 81.8) of rhamnose, H-1 (δ_H 5.86, br s) of rhamnose and C-2 of fucose (δ_C 73.8), and H-1 (δ_H 6.24, d, *J* = 8.0 Hz) of fucose and C-28 (δ_C 176.5). An HMBC correlation was observed between H-1 (δ_H 5.07, d, *J* = 7.5 Hz) of glucose and C-3 (δ_C

85.8) of the aglycone, connecting the glucose unit to C-3 of the aglycone. The 3, 4, 5-trimethoxycinnamic acid moiety was attached to C-3 of fucose by HMBC correlation between H-3 [δ_H 5.62 (dd, J = 9.5, 2.5 Hz)] of fucose and the carboxylic carbon (δ_C 166.5) of 3, 4, 5-trimethoxycinnamic acid, as well as significantly downfield chemical shift of H-3. Therefore, the structure of **1** was elucidated as 3-*O*-*β*-D-glucopyranosyl-presenegenin 28-*O*-*α*-L-arabinopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-[3-*O*-3, 4, 5-trimethoxycinnamoyl]-*β*-D-fucopyranosyl ester.

The structures of onjisaponin Fg (**2**), Ng (**3**), R (**4**), and O (**5**) were previously determinded by LC-ESI-MS^n [6], but the NMR data of those compounds were not reported. Here we report the NMR data of those compounds for the first time (Tables 2 and 3). Compounds **6-11** were identified as tenuifolises A−G (**6**−**11**) by comparison of their NMR and MS data with those reported in the literature [7-8].

Table 2 ^{1}H NMR data of compounds 2−5 (500 MHz in pydridine-d_5; [a] overlap with other signals)

Position	2	3	4	5
Aglycone				
2	4.72 m	4.68 m	4.71 m	4.68 m
3	4.60 d (3.5)	4.62 d (3.5)	4.62 d (3.0)	4.60 d (3.0)
12	5.88 m	5.75 m	5.75 m	5.73 m
18	3.25 br d (11)	3.20 br d (12.5)	3.22 d (12.0)	3.22 br d (10.0)
24	1.95 s	1.96 s (3H)	1.99 s	1.95 s (3H)
25	1.56 s	1.53 s (3H)	1.52 s	1.50 s (3H)
26	1.12 s	1.08 s (3H)	1.08 s	1.12 s (3H)
27	3.83 br d (10.5)	3.78 br d (10.5)	3.80 br d (10.5)	3.79 br d (10.5)
	4.03 br d (10.5)	4.05 br d (10.5)	4.02 br d (10.5)	4.06 br d (10.5)
29	0.76 s	0.76 s	0.77 s	0.78 s
30	1.02 s	0.92 s	0.93 s	0.94 s
C-3 sugar				
Glu-1	5.03 d (7.5)	5.05 d (7.5)	5.06 d (7.5)	5.06 d (7.5)
2	4.12 [a]	4.12 [a]	4.12 [a]	4.14 [a]
3	4.15 [a]	4.13 [a]	4.165 [a]	4.13 [a]
4	4.18 [a]	4.14 [a]	4.20 [a]	4.22 [a]
5	3.91 [a]	3.92 dd (8.5, 5.0)	3.91 [a]	3.90 [a]
6	4.29 dd (11.5, 5.5)	4.28 dd (11.5, 5.5)	4.26 dd (11.5, 5.0)	4.30 dd (11.5, 5.0)
	4.45 dd (12.5)	4.48 dd (12.5)	4.46 dd (11.5, 2.5.)	4.44 dd (11.5)
C-28 sugar				
Fuc- 1	6.28 d (8.0)	6.18 d (8.0)	6.28 d (8.0)	6.14 d (7.5)
2	4.62 t (9.5)	4.64 t (9.0)	5.00 t (9.0)	4.70 t (9.0)
3	4.41 [a]	4.18 [a]	4.50 [a]	4.46 [a]
4	5.88 br s	5.75 br s	5.75 br s	5.73 br s
5	4.46 [a]	4.10 [a]	4.10 [a]	4.11 [a]
6	1.33 d (6.0)	1.34 d (6.0)	1.37 d (6.5)	1.35 d (6.0)
Rha-1(Fuc-2)	5.85 s	6.21 s	6.17	5.96 s
2	4.76 br s	5.00 br s	4.70 br s	4.84 br s
3	4.42 [a]	4.54 [a]	4.46 [a]	4.45 [a]
4	4.51 [a]	4.59 [a]	4.37 [a]	4.54 [a]
5	4.33 [a]	4.50 [a]	4.33 [a]	4.45 d (3.0)
6	1.72 d (6.0)	1.72 d (6.0)	1.74 d (5.5)	1.72 d (6.0)

(Continuous Table 2)

position	**2**	**3**	**4**	**5**
Rha-1(Fuc-3)	5.53 s			5.84 s
2	4.78 m			4.64 br s
3	4.64 m			4.45 [a]
4	4.20 [a]			4.25 [a]
5	4.38 [a]			4.38 [a]
6	1.75 d (6.0)			1.78 d (6.0)
Xyl-1(Rham-4)	5.29 d (7.0)	5.31 d (8.0)	5.26 d (7.5)	4.97 d (7.5)
2	3.98 t (8.5)	4.01 t (8.5)	4.03 t (8.5)	3.94 t (8.5)
3	4.10 [a]	4.17 [a]	3.89 [a]	4.14 [a]
4	4.22 t (8.5)	4.05 t (8.5)	4.20 t [a]	4.05 [a]
5	3.50 t (11.0)	3.49 t (11.0)	3.38 t (11.0)	3.45 t (11.0)
	4.20 [a]	4.20 [a]	4.21 [a]	4.23 [a]
Gal-1 (Xyl-4)			4.93 d (8.0)	4.97 d (7.5)
2			4.48 m	4.47 m
3			4.15 m	4.16 m
4			4.49 m	4.51 m
5			4.16 m	4.16 m
6			4.37 br d (12.5)	4.24 br d (12.5)
			4.42 br d (12.5)	4.44 br d (12.5)
Ara-1 (xyl-3)		5.15 d (7.0)		
2		4.48 m		
3		4.05 m		
4		4.20 m		
5		3.64 (d, 12.0)		
		4.25(d, 12.0)		
Api-1 (Rham-3)	6.29 d (3.5)	5.99 d (3.5)	6.05 d (3.5)	
2	4.23 [a]	4.64 [a]	4.23 [a]	
4	4.28 [a]	4.38 [a]	4.08 [a]	
	4.26 [a]	4.49 [a]	4.12 [a]	
5	4.40 d (11.0)	4.51 d (11.0)	4.13 d (12.0)	
	4.54 d (11.0)	4.47 d (11.0)	4.23 d (12.0)	
Cinn-2 (Fuc-2/4)	7.39 d (8.5)	6.84 s	6.84 s	6.83 s
3	6.97 d (8.5)			
5	6.97 d (8.5)			
6	7.39 d (8.5)	6.84 s	6.84 s	6.83 s
7	7.94 d (16.0)	7.95 d (16.0)	7.95 d (16.0)	7.98 d (16.0)
8	6.54 d (16.0)	6.61 d (16.0)	6.62 d (16.0)	6.72 d (16.0)
OMe	3.66 s (3H)	3.80 s (6H)	3.76 s (6H)	3.78 s (6H)
		3.88 s (3H)	3.86 s (3H)	3.92 s (3H)
HMG-2	3.04 d (2H, 17.5)	3.04 d (2H,17.5)		
4	3.12 d (2H, 17.5)	3.12 d (2H,17.5)		
CH_3	1.70 s (3H)	1.70 s (3H)		

Table 3 ^{13}C NMR data of compounds 2−5 (125 MHz in pydridine-d_5)

Position	2	3	4	5	Position	2	3	4	5
1	44.2	44.7	44.2	44.1	5	78.3	78.7	78.3	78.3
2	70.0	69.6	70.1	70.2	6	62.7	62.9	62.6	62.6
3	85.9	86.2	85.9	85.9	C-28 sugar				
4	52.8	53.2	52.9	52.8	Fuc-1	94.9	94.7	94.5	94.6
5	52.5	52.9	52.6	52.4	2	73.2	76.6	76.5	75.5
6	21.2	21.7	21.3	21.4	3	79.9	74.6	78.3	77.2
7	33.1	33.4	33.0	33.0	4	76.6	75.2	76.6	75.2
8	41.2	41.5	41.1	41.1	5	70.5	71.8	70.8	70.9
9	49.2	49.6	49.2	49.2	6	16.9	17.0	16.6	17.0
10	37.0	37.3	37.0	37.0	Rha-1(Fuc-2)	102.2	102.7	102.2	101.8
11	23.5	23.9	23.6	23.5	2	71.7	71.8	71.7	71.7
12	127.7	128.2	127.7	127.7	3	82.5	82.4	82.3	81.3
13	138.9	139.0	139.0	138.8	4	78.1	78.7	78.6	78.3
14	47.9	48.2	47.9	47.9	5	68.9	68.8	68.3	68.4
15	24.5	24.8	24.4	24.4	6	18.7	19.7	18.9	18.6
16	24.1	24.4	24.0	23.9	Rha-1(Fuc-3)	104.8			105.1
17	46.9	47.2	46.9	47.0		72.2			72.3
18	42.1	42.3	41.9	42.0		78.3			78.3
19	45.5	45.6	45.4	45.4		73.6			73.4
20	30.8	31.1	30.8	30.7		70.7			70.1
21	33.8	34.0	33.7	33.8		18.7			18.5
22	32.2	32.7	32.4	32.2	Xyl-1(Rham-4)	105.1	105.2	104.8	105.3
23	180.7	181.3	181.3	180.8	2	75.6	75.0	75.0	76.5
24	14.2	14.6	14.3	14.2	3	78.4	86.8	76.5	78.3
25	17.6	17.8	17.4	17.4	4	70.2	69.6	78.3	78.3
26	18.7	19.2	18.9	18.8	5	67.2	66.9	64.7	64.9
27	64.4	64.9	64.7	64.3	Ara-1(xyl-3)		105.9		
28	176.5	177.0	176.7	176.6	2		73.0		
29	33.1	33.4	33.0	33.0	3		74.6		
30	24.1	24.4	24.0	23.9	4		69.6		
Cinn.					5		67.5		
1	127.4	130.7	130.4	130.2	Gal-1(Xyl-4))			104.4	
2	130.5	106.7	106.3	106.4	2			78.3	
3	114.7	141.4	154.1	154.0	3			74.1	
4	145.6	154.4	141.0	140.9	4			69.9	
5	114.7	141.4	154.1	154.0	5			77.3	
6	130.5	106.7	106.3	106.4	6			62.2	
7	115.7	118.0	117.7	117.2	Api-1(Rham-3)	111.4	111.9	111.7	
8	145.6	146.3	145.9	146.3	2	78.3	78.3	78.3	
9	167.2	167.9	167.6	167.1	3	77.8	78.7	77.3	
OMe	55.3	56.6	56.3	56.2	4	74.3	74.3	74.4	
		56.6	56.3	56.2	5	66.4	67.2	64.5	
		61.0	60.7	60.7	HMG-1	171.5	171.9		
C-3 sugar					2	46.2	46.7		
Glc-1	105.3	105.6	106.3	106.7	3	70.0	70.4		
2	75.2	74.6	74.4	74.4	4	46.3	46.8		
3	78.3	78.7	78.6	78.3	5	174.8	175.0		
4	71.5	71.8	71.5	71.7	CH_3	28.2	28.6		

3.2 *Neuroprotective effects of compounds* **6**–**9**

The antagonistic action of **6**–**9** on neurotoxicity induced by glutamate and serum deficiency on PC12 cells was investigated *in vitro* by MTT method (Tables 4−6) [2]. Compounds **6** and **7** showed neuroprotective effect against glumate and serum deficiency at a concentration of 1×10^{-5} mol·L^{-1}, while compounds **8** and **9** showed no protective effect. When the concentration decreased to 1×10^{-6} mol·L^{-1}, compounds **6** and **7** showed no protective effect against serum deficiency.

Table 4 Effect of 6-9 on the survival rate of PC12 cells injured by serum deficiency (1×10^{-5} mol·L^{-1})

Group	OD	Proliferation rate (%)
control	0.551 4 ± 0.028 8	100
model	0.504 9 ± 0.008 6$^{\#}$	91.6
7	0.534 7 ± 0.009 9**	97.0
8	0.528 6 ± 0.026 7	95.9
9	0.452 3 ± 0.008 5	82.0
control	0.614 5 ± 0.059 9	100
model	0.473 8 ± 0.025 6$^{\#}$	77.1
6	0.520 4 ± 0.024 5*	84.7

$^{\#}P < 0.05$ *vs* control; *$P < 0.05$ *vs* model; **$P < 0.01$ *vs* model

Table 5 Effect of 6 and 7 on the survival rate of PC12 cells injured by glumate (1×10^{-5} mol·L^{-1})

Group	OD	Proliferation rate (%)
control	0.680 4 ± 0.027 0	100
model	0.599 3 ± 0.021 0$^{\#\#}$	88.1
6	0.631 0 ± 0.010 1*	92.7
7	0.701 9 ± 0.006 1***	103.2

$^{\#}P < 0.01$ *vs* control; * $P < 0.05$ *vs* model; ** $P < 0.01$ *vs* model; ***$P < 0.001$ *vs* model.

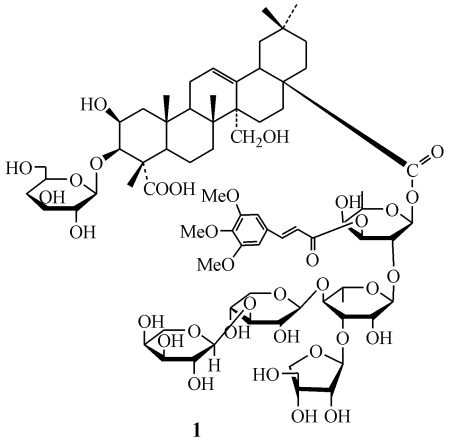
Table 6 Effect of 6 and 7 on the survival rate of PC12 cells injured by serum deficiency (1×10^{-6} mol·L^{-1})

group	OD	Proliferation rate (%)
control	0.440 3 ± 0.018 6	100
model	0.388 3 ± 0.000 3 $^{\#}$	88.2
6	0.382 2 ± 0.018 0	86.8
7	0.407 6 ± 0.019 6	92.6

$^{\#}P < 0.05$ *vs* control

3.3 *Effects of tenuifolisoses B (***7***) and C (***8***) on basal synaptic transmission in the dentate gyrus of anesthetized rats*

After *icv* administration of 2 μmol·L^{-1} tenuifolioses A−C, the PS amplitude of groups TB and TC increased to (178.47 ± 57.09)% and (177.67 ± 38.92)% of baseline, respectively, within 60 min. The PS amplitude of TB and TC increased more than 30% over baseline (which lasted more than 30 min), revealed the LTP (long-term potentiation) in the hippocampal dentate gyrus (Figure 3). Thus, tenuifolioses B (**7**) and C (**8**) can potentiate basal synaptic transmission in the dentate gyrus of anesthetized rats, indicating the potential effect of **7** and **8** on hippocampal-dependent learning and memory.



1

Fig. 1 Structure of compound 1

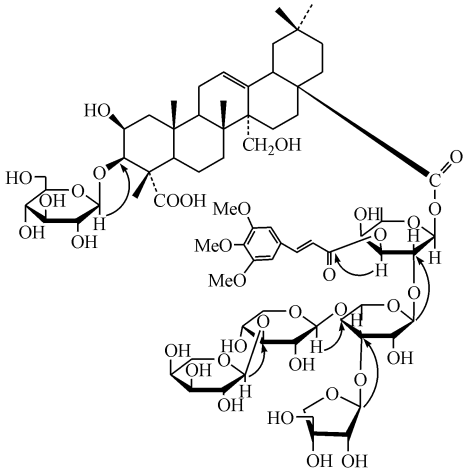


Fig. 2 Main HMBC (arrows) correlations of 1

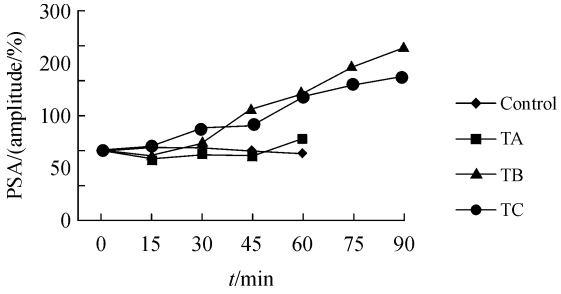


Fig. 3 Time course of effects for tenuifolioses A (TA), B (TB), and C (TC) on the basal synaptic responses following application of low-frequency stimulation (1/30 Hz, 1.0 mA) in the denate gyrus of anesthetized rats after *icv* administration of a final brain concentration of 2 μmol·L^{-1}. $\bar{x}$ ± s, n = 3-6

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远志中的三萜皂苷和寡糖多酯类成分

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【摘　要】 **目的**: 研究远志的抗老年痴呆活性成分。**方法**: 采用活性追踪的方法, 利用硅胶柱层析、反相中压色谱、制备高效液相等多种色谱技术分离单体化合物; 并利用多种光谱技术确定化合物的结构。**结果**: 从远志的根中分离得到了 5 个三萜皂苷类化合物 Onjisaponin Wg (**1**), Fg (**2**), Ng (**3**), R (**4**) 和 O (**5**)及 6 个寡糖多酯类化合物 tenuifolioses A (**6**), B (**7**), C(**8**), I (**9**), K (**10**), 和 G (**11**)。**结论**: 化合物 **1** 为新化合物, 化合物 **2-5** 首次报道其核磁数据; 化合物 **6** 和 **7** 显示了 PC12 细胞的保护作用; 化合物 **7** 和 **8** 可增强大鼠齿状回突触的传递作用, 表明具有改善大鼠学习记忆的活性。

【关键词】 远志; 三萜皂苷; 寡糖多酯; 神经细胞(PC12 细胞)保护; 突触传递

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