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Flavans from *Iris tenuifolia* and their effects on β-amyloid aggregation and neural stem cells proliferation in vitro

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ABSTRACT

Two new flavans (**1**, **2**) and a new flavanone (**3**), together with three known compounds (**4**–**6**), were isolated from the roots of *Iris tenuifolia*. Their structures were elucidated by means of spectroscopic methods including 1D and 2D NMR techniques and mass spectrometry. Compounds **1**, **4**, and **6** were further confirmed by single-crystal X-ray diffraction analysis. Biological evaluation showed that compounds **1** and **4** were positive in inhibiting β-amyloid (Aβ) aggregation and promoting neural stem cells (NSCs) proliferation, respectively.

Iris is a genus of about 300 species of flowering plants, and is the largest and most complex genus of the family Iridaceae. It is widely distributed throughout the North Temperate Zone, about 60 species and 13 variations are found in China.[1] Many members of the genus have been used as traditional folk medicines for the treatment of various diseases, such as cancer, inflammation, bacterial and viral infections.[2–8] *Iris tenuifolia* Pall. is an important member of this genus, distributed in Inner Mongolia, Xinjiang Province, and other Gobi and sand-dune districts of China. Because of the extreme circumstance, *I. tenuifolia* has got some adaptable changes that different from other plants in the genus, and the chemical constituents of *I. tenuifolia* would have changed correspondingly. Additionally, previous phytochemical studies have shown the presence of flavanones, isoflavones, and benzils including several new compounds.[9,10] Thus, we took *I. tenuifolia* as biological materials. And in this report, we describe the isolation and structure determination of two novel flavans (**1**, **2**), a new flavanone (**3**), and three known compounds (**4**–**6**) (Fig. 1) from the roots of *I. tenuifolia*, while flavanones are the major components of *I. tenuifolia*,[5] and flavans are the first time to be found in this genus. However, we also investigated the inhibitory actions on Alzheimer's β-amyloid (Aβ) fibrils and positive effects on neural stem cells (NSCs) proliferation in vitro of compounds **1** and **4**, which have more in this species. The structures of compounds **1**, **4** and **6** were also confirmed by single-crystal X-ray diffraction analysis, and the crystal data were reported for the first time.

The fresh roots of *I. tenuifolia* (1.6 kg) collected in May 2008 from Xilingol League of Inner Mongolia autonomous region of China, were extracted three times with MeOH (5 L × 3) at room temperature, concentrated in vacuo to give a crude extract (241.8 g), which was partitioned between $CHCl_3$ and H_2O, yielding $CHCl_3$ (198.6 g) and H_2O layers (23.2 g). Part of the $CHCl_3$ layer (41.8 g) was fractionated on silica gel using a gradient of $CHCl_3$–MeOH (10:0–6:4) to yield fractions 1–6. Fraction 3 was precipitated as yellow crystals (**4**,[10,11] 79 mg). Fraction 4 was further fractionated on silica gel with $CHCl_3$–MeOH (10:0–0:10), followed by precipitating and recrystallizing ($CHCl_3$), to yield the white crystal mixture of **1** and **2**. Then the mixture was further purified on a Chiralcel OJ-H column (hexane–isopropanol = 1:1) with HPLC equipment, finally yielding **1**[12] (101 mg) and **2**[13] (14 mg). Fraction 5 was purified to produce **5**[10] (18 mg) and **6**[10,14] (37 mg) by silica gel ($CHCl_3$–MeOH, 9:1–7:3) and Sephadex LH-20 (MeOH) column chromatography in sequence. The H_2O layer (20 g) was fractionated on silica gel ($CHCl_3$–MeOH, 95:5–7:3) and open ODS column (H_2O–MeOH, 7:3–1:1), to yield **3**[15] (34 mg).

Compound **1** was obtained as white columnar crystals ($CHCl_3$). Its molecular formula was determined to be $C_{17}H_{16}O_5$ by the HR-ESI-MS peak at *m/z* 301.10377 $[M+H]^+$ (calcd for 301.10760). The ^1H NMR and ^{13}C NMR spectra (Table 1) indicated a flavan skeleton[16,17] deduced from the five aromatic protons at δ 5.97 (d, $J = 2.3$ Hz, H-8), 6.11 (d, $J = 2.3$ Hz, H-6), 6.68 (d, $J = 7.4$ Hz, H-5′),

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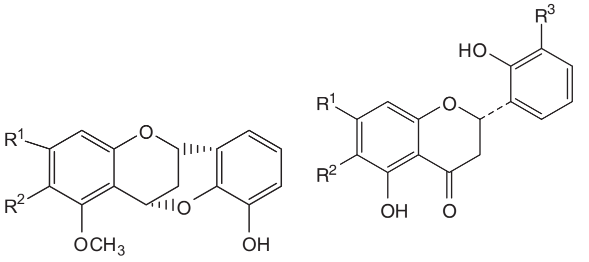

3 R^1 = R^2 = OCH$_3$, R^3 = OH
4 R^1 , R^2 = OCH$_2$O, R^3 = OH
5 R^1 , R^2 = OCH$_2$O, R^3 = H
6 R^1 = OCH$_3$, R^2 = H , R^3 = OH

1 R^1 = OCH$_3$, R^2 = H
2 R^1 , R^2 = OCH$_2$O

Figure 1. The structures of compounds **1**–**6** isolated from *I. tenuifolia*.

6.70 (d, *J* = 1.6 Hz, H-4′), and 6.80 (dd, *J* = 1.5, 7.2 Hz, H-6′) in the downfield, three acyclic protons at δ 2.08 (dt, *J* = 2.5, 14.0 Hz, H-3a), 2.16 (dt, *J* = 2.5, 14.0 Hz, H-3b), and 5.34 (br s, H-2), and the absence of signals for C=O in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum also showed the resonances of two methoxy singlets at δ 3.67 (s) and 3.80 (s). HMBC correlations between these two singlets and C-5 and C-7, respectively, indicated the location of the methoxy groups. DEPT and HSQC spectra indicated the presence of methylene at δ 2.08, 2.16 (H-3a, 3b) and δ 26.5 (C-3). Further analysis of the HMBC spectrum confirmed the structure (Fig. 2). The HMBC correlations between H-4 (δ 5.62, 1H, br s) and C-2′ (δ 146.1) indicated that the methine at 4-position had a bond linking C-2′ indirectly. All these data deduced compound **1** as a 4-*O*-2′-cycloflavan. The structure of compound **1** was confirmed by single-crystal X-ray diffraction (Fig. 3), and the deposition number is CCDC 796429.[12] Thus, compound **1** was determined to be 3′-hydroxy-5,7-dimethoxy-4-*O*-2′-cycloflavan.

Compound **2** was obtained as colorless columnar crystals (CHCl$_3$). Its molecular formula was determined to be $C_{17}H_{14}O_6$ by positive HR-ESI-MS at *m/z* 337.06908 [M+Na]$^+$ (calcd for 337.06881). Comparing the ^{1}H and ^{13}C NMR data of compound **2** with those of compounds **1** (Table 1), **2** was deduced to have the same structural skeleton as compound **1**. The signals at δ 5.85 (d, *J* = 0.8 Hz) and δ 5.96 (d, *J* = 0.8 Hz) in the ^{1}H NMR were assigned to a methylenedioxy group. A methoxy group with a singlet at δ 3.99 (s) was assigned to C-5 by HMBC correlations (Fig. 2). Compound **2** was thus determined to be 3′-hydroxy-5-methoxy-6,7- methylenedioxy-4-*O*-2′-cycloflavan.

Compound **3** was isolated as yellow amorphous powder, and has the molecular formula $C_{17}H_{16}O_7$, demonstrated by HR-ESI-MS at *m/z* 331.07858 [M−H]$^-$ (calcd for 331.08178). In the ^{1}H NMR spectrum for compound **3** (Table 1), characteristic resonances at δ 2.59 (dd, *J* = 2.0, 16.5 Hz, H-3b), 2.94 (dd, *J* = 16.2, 16.4 Hz, H-3a) and another at δ 5.63 (dd, *J* = 2.0, 13.0 Hz, H-2) indicate that compound **3** has a flavanone skeleton.[10] The HMBC correlations between H-2 and C-1′, C-2′, and C-6′ indicate that the B-ring is linked to C-2. The ^{1}H NMR spectrum also showed the resonances of two methoxy singlets at δ 3.69 (s) and 3.78 (s), and the HMBC correlations between these two singlets and C-6, C-7, respectively, indicate the location of the methoxy groups. All the assignments of hydroxyl groups were confirmed by ^{13}C NMR and 2D NMR (HSQC and HMBC; Table 1, Fig. 2). These results indicate that the hydroxyl group attached to the A-ring is located at the 5-position, and those attached to the B-ring are located at the 2′- and 3′-positions. Therefore, compound **3** was determined to be 5,2′,3′-trihydroxy-6,7-dimethoxyflavanone.

All known compounds were identified by comparison to the reported spectroscopic data. And the structures of compounds **4** and **6** were confirmed by single-crystal X-ray diffraction (Fig. 3), and this is the first time to report the crystal data, the deposition numbers are CCDC 796430 and CCDC 796431, respectively.[11,14]

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, the abundance of β-amyloid's (Aβ) polymerization has been identified as one of the major characteristics, resulting in the absence of neural stem cells (NSCs).[18,19] Molecules that prevent the aggregation of Aβ or promote the proliferation of NSCs could be therapeutic value in AD.[20–24] Besides, recent studies have shown that polyphenols have potent anti-amyloidogenic activites in vitro, and the compounds isolated from *I. tenuifolia* have similar chemical groups as above, so we also investigated the inhibitory actions on Aβ aggregation and positive effects on NSCs proliferation of the compounds.

Table 1
^{1}H and ^{13}C NMR data for compounds **1**, **2**, and **3** in DMSO-d_6

Position	**1**[a]		**2**[b]		**3**[a]	
	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
2	5.34 (1H, br s)	67.2	5.29 (1H, br s)	66.5	5.63 (1H, dd, 2.0, 13.0)	74.3
3a	2.08 (1H, dt, 2.5, 14.0)	26.5	2.07 (1H, dt, 2.8, 14.0)	25.9	2.94 (1H, dd, 16.2, 16.4)	44.2
3b	2.16 (1H, dt, 2.5, 14.0)		2.17 (1H, dt, 2.8, 14.4)		2.59 (1H, dd, 2.0, 16.5)	
4	5.62 (1H, br s)	61.5	5.59 (1H, br s)	61.4		189.3
5		159.3		140.6		154.6
6	6.11 (1H, d, 2.3)	91.8		129.8		136.6
7		161.9		149.9		158.1
8	5.97 (1H, d, 2.3)	93.4	6.13 (1H, s)	92.1	6.27 (1H, s)	100.0
9		155.0		148.5		159.9
10		103.5		106.2		108.4
1′		122.5		121.9		126.4
2′		146.1		145.8		142.8
3′		142.2		141.6		145.5
4′	6.70 (1H, d, 1.6)	116.5	6.66 (1H, d, 7.8)	116.1	6.80 (1H, d, 7.6)	115.5
5′	6.68 (1H, d, 7.4)	120.1	6.70 (1H, dd, 2.6, 8.1)	119.8	6.70 (1H, t, 7.7)	119.6
6′	6.80 (1H, dd, 1.5, 7.2)	121.4	6.78 (1H, dd, 2.3, 6.9)	120.8	6.90 (1H, d, 7.7)	117.4
–O–CH$_2$–O–			5.85 (1H, d, 0.8) 5.96 (1H,d, 0.8)	100.8		
5-OCH$_3$	3.67 (3H, s)	56.1	3.99 (3H, s)	59.8		
6-OCH$_3$					3.69 (3H, s)	61.1
7-OCH$_3$	3.80 (3H, s)	55.6			3.78 (3H, s)	61.6

[a] Spectra were measured in DMSO-d_6 (^{1}H, 500 MHz and ^{13}C, 125 MHz).
[b] Spectra were measured in DMSO-d_6 (^{1}H, 400 MHz and ^{13}C, 100 MHz).
[c] Chemical shifts (δ) are expressed in ppm.
[d] *J* values (in Hz) are in parentheses.