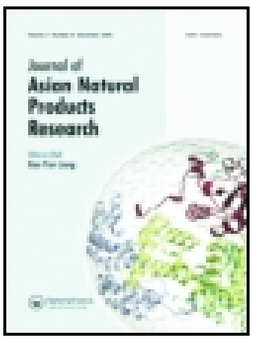

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Two new biflavonones from *Coreopsis tinctoria*

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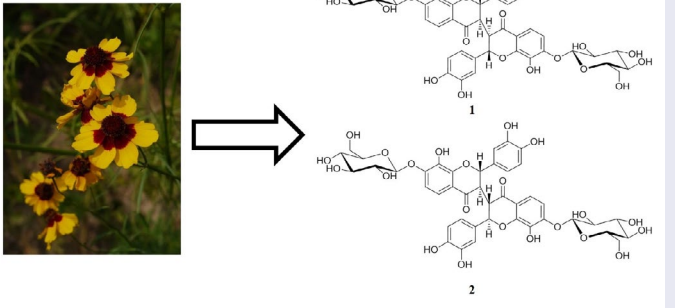
ABSTRACT

Two new biflavonone compounds, sikokianin D (**1**) and sikokianin E (**2**), were isolated from the capitulum of *Coreopsis tinctoria*. The structures of these compounds were elucidated by spectroscopic techniques including NMR, HRESIMS and circular dichroism (CD).



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Compositae; *Coreopsis tinctoria*; biflavonones; chemical constituents

1. Introduction

Coreopsis tinctoria is an annual plant that belongs to Chrysanthemum family. It grows in the Karakorum Mountains at an altitude of above 3000 m in southern Xinjiang of China [1]. Its capitulum has been consumed as tea, and generally used as folk medicine in treating diabetes, hyperlipaemia, and hypertension because of its effects in reducing blood pressure and vasorelaxant [2–5], as well as antioxidant [6] and anti-coagulating activities [7]. Several flavonoids, phenolic acids [8], bisabolane-type sesquiterpenoids [9], and C_{14}-polyacetylene glycosides [10] were reported from this plant previously. Considering its notable pharmacological properties, we made a phytochemical research on the capitulum and obtained two novel biflavonones, sikokianin D (**1**) and sikokianin E (**2**) (Figure 1). Herein, we report the isolation and structure elucidation of **1** and **2**.

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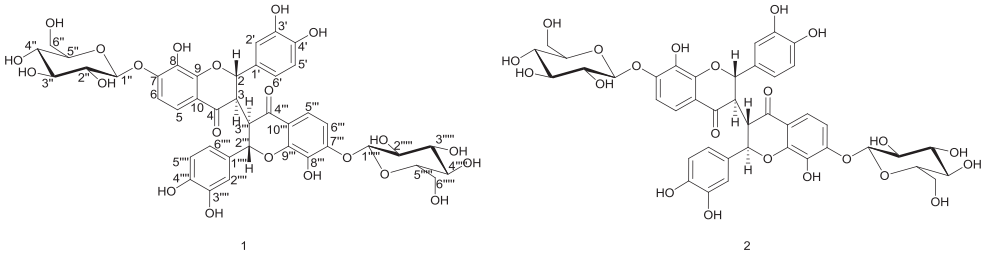

Figure 1. Structures of compounds **1** and **2**.

Table 1. ^{1}H NMR (400 Hz) and ^{13}C NMR (100 Hz) spectral data of compounds **1** and **2** in DMSO-d_6 (δ in ppm).

	1		**2**	
Position	δH	δC	δH	δC
2,2‴	5.72 d (12.0)	84.3	5.72 d (12.0)	84.3
3,3‴	2.66 d (12.0)	51.0	2.66 d (12.0)	50.9
4,4‴	–	191.7	–	191.7
5,5‴	7.22 d (8.8)	117.1	7.21 d (8.8)	117.0
6,6‴	6.88 d (8.8)	109.5	6.88 d (8.8)	109.7
7,7‴	–	151.1	–	151.1
8,8‴	–	135.2	–	135.2
9,9‴	–	150.4	–	150.5
10,10‴	–	116.4	–	116.5
1′,1⁗	–	127.5	–	127.5
2′,2⁗	6.59 s	115.8	6.58 d (2)	115.8
3′,3⁗	–	145.4	–	145.4
4′,4⁗	–	146.3	–	146.3
5′,5⁗	6.75 d (8.0)	115.8	6.75 d (8.8)	115.7
6′,6⁗	6.34 d (8.0)	118.6	6.34 d (8.8, 2.0)	118.6
1″,1⁗⁗	4.82 d (6.8)	101.7	4.83 d (6.8)	101.6
2″,2⁗⁗	3.28–3.30, m	73.3	3.28–3.30, m	73.3
3″,3⁗⁗	3.28–3.30, m	75.9	3.28–3.30, m	76.0
4″,4⁗⁗	3.20–3.22, m	69.9	3.20–3.22, m	69.9
5″,5⁗⁗	3.38–3.40, m	77.4	3.38–3.40, m	77.4
6″,6⁗⁗	3.49, dd (11,5.5) 3.73,brd (11)	60.8	3.49, dd (11,5.5) 3.72,brd (11)	60.9

2. Results and discussion

Compounds **1** and **2** were obtained as brown amorphous powder. The molecular formula, $C_{42}H_{42}O_{22}$, was deduced from HR-ESI-MS (*m/z* 897.2064 $[M-H]^-$; calcd. 897.2089). The chemical reaction with HCl–Mg reagent indicated that **1** is a flavonoid. The IR spectrum showed the presence of OH groups (3409 cm^{-1}), conjugated C=O groups (1670 cm^{-1}), and aromatic rings (1619 and 1522 cm^{-1}). The UV spectrum showed a maximum absorption at 290 nm, which is characteristic of a flavonone. By analysis of ^{13}C-NMR data, the presence of two C=O at δ_C 191.7 was established. The C (2 and 2‴) at δ_C 84.3, and C (3 and 3‴) at δ_C 51.0 signals in the ^{13}C-NMR spectrum suggested that **1** consisted of two flavonone units [11].

The ^{1}H-NMR spectrum (Table 1) showed two methine groups (δ_H 5.72, 2H, d, J = 12.0 Hz; δ_H 2.66, 2H, d, J = 12.0 Hz) in the rings C and C‴ of biflavanone. The ten aromatic H-atom

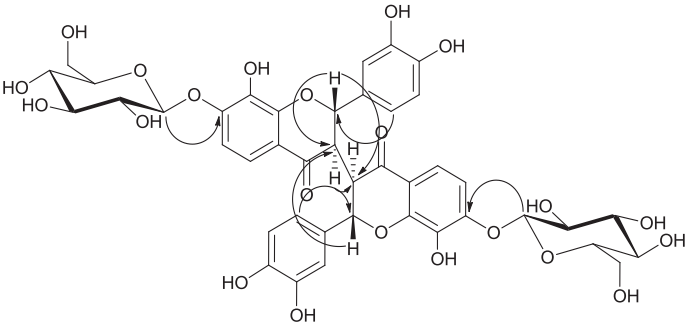

Figure 2. Key HBMC correlations for compound **1**.

signals (δ_H 6.34–7.22) indicated the presence of two sets of typical 7, 8-dioxygenated A and A‴ rings at δ_H 7.22 (2H, d, J = 8.8 Hz) and δ_H 6.88 (2H, d, J = 8.8 Hz), and two sets of ABX spin system attributed to 1,3,4-trisubstituted aromatic B and B‴ rings at δ_H 6.59 (2H, brs), 6.75 (2H, d, J = 8.0 Hz) and δ_H 6.34 (2H, d, J = 8.0 Hz). Linkage of the B ring to the C ring was established at C (2 and 2‴) by HMBC experiment (Figure 2), in which H–C (2′ and 2‴′) at δ_H 6.59 (2H, s) and H–C (6′and 6‴′) at δ_H 6.34 (2H, d, J = 8.0 Hz) correlated with C (2 and 2‴) at δ_C 84.3. The presence of signals of H–C (3 and 3‴) at δ_H 2.66 (2H, d, J = 12.0 Hz) showed that **1** has a structure dimerized at the C-3 position of the two flavonone units [12,13]. Additionally, a series of typical signals of sugar residues were recognized, including the anomeric H-atom at δ_H 4.82 (2H, d, J = 6.8 Hz), which revealed a β-configuration present in the sugar residue on the basis of the coupling constant [14]. The sugar residues of **1** and **2** were determined as D-glucose by acid hydrolysis and further GC analysis of its corresponding acetylated derivative [15]. Appearance of two peaks in HPLC spectrum suggested that **1** and **2** are possible to have different absolute configurations (Figure 3). The comparison of the NMR data between **1** and **2** revealed they had the same planar structure, indicating they should be stereoisomers. The J-values of H–C (2 and 2‴) at δ_H 5.72 (2H, d, J = 12.0 Hz) and H–C (3 and 3‴) at δ_H 2.66 (2H, d, J = 12.0 Hz) suggested **1** and **2** both have the *trans-trans* configurations [11]. In order to establish the absolute configurations at C-2 and C-3 in **1** and **2**, the circular dichroism curves of **1** and **2** were measured. The CD data of **1** exhibited two strong Cotton effects at 329 nm ($\Delta\varepsilon$ = + 13.889) corresponding to $n\rightarrow\pi^*$ transition and at 290 nm ($\Delta\varepsilon$ = −25.359) corresponding to $\pi\rightarrow\pi^*$ transition for the carbonyl groups. The positive sign of the former predicted the configuration 2*S*, 3*R* for 2, 3-diequatorial substituents (J = 12.0 Hz) on basis of the octant rule, modified for aryl ketones [16,17]. The structure of **1** was elucidated to be (2*S**, 2‴*S**, 3R*, 3‴R*)-[3, 3‴-bi-4H-1-benzopyran]-4, 4‴- dione, 7, 7‴-bis (β-D-glucopyranosyloxy)- 2, 2‴, 3, 3‴-tetrahydro-8, 8‴-dihydroxy-2, 2‴-bis (3′,4′-hydroxyphenyl), and named sikokianin D. Differently, the CD of **2** showed mesomeric effects, at 329 nm ($\Delta\varepsilon$ = + 0.184) and at 290 nm ($\Delta\varepsilon$ = + 0.010), indicating that the aglycone of **2** was *meso*-form [18]. Thus, The structure of **2** was identified as (2*S**, 2‴*R**, 3*R**, 3‴*S**)-[3, 3‴-bi-4H-1-benzopyran]-4, 4‴-dione, 7, 7‴-bis (β-D-glucopyranosyloxy)-2, 2‴, 3, 3‴-tetrahydro-8, 8‴-dihydroxy-2, 2‴-bis (3′, 4′-hydroxyphenyl), and named sikokianin E.

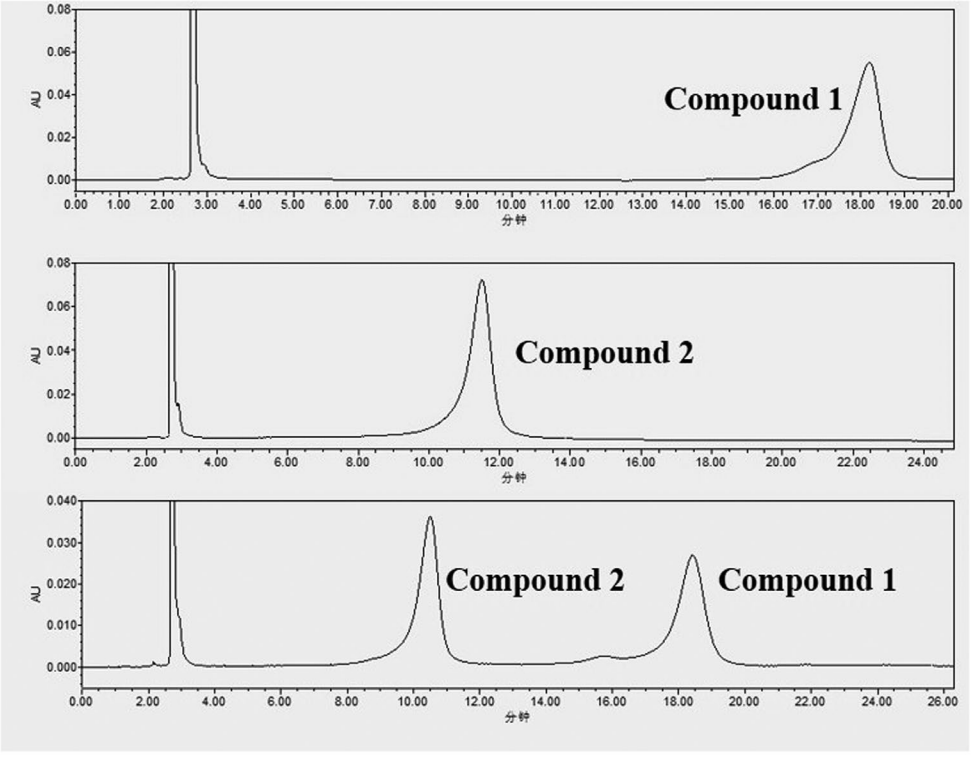

Figure 3. The HPLC analysis of **1** and **2**.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined with a Perkin-Elmer-341 polarmeter (Perkin-Elmer, San Jose, CA, U.S.A). UV-vis spectra were recorded using a Techcomp 8500 spectrometer (Techcomp, Shanghai, China). IR spectra were recorded in KBr pellets on a Nicolet-NEXUS-670-FTIR spectrophotometer (Nicolet, Madison, WI, U.S.A). NMR spectra were obtained with a Varian INOVA-400/500 instrument (Varian, Palo alto, CA, U.S.A). The ^{1}H and ^{13}C NMR chemical shifts were relative to solvent signals at $\delta_{H/C}$ 2.49/39.5 (DMSO-d_6), using tetramethylsilane as internal standard. MS was measured on a Waters Q-Tof micro Ya019 mass spectrometer (Waters, Lee-derville, WA, U.S.A). CD spectra were measured by MOS 450 detector (Bio-Logic Co., Claix, France). Preparative HPLC was obtained on a Shimadzu HPLC system consisting of two LC-8A pumps and an SPD-M10A detector. For preparative purposes, a Shimadzu PRC-ODS (15 μm, i.d. 20 mm × 250 mm, Shimadzu, Kyoto, Japan) was used. Analytical HPLC was carried out on Agilent Technologies 1260 Infinity Series LC system (U.S.A) equipped with a binary pump, DAD detector and chromatographic column (4.6 × 250 mm; 5 μm; Xridge C-18 column; Waters; Milford, U.S.A). Analytical GC was carried out on Agilent 6890N system (H2 flame ionization detector; Agilent Minneapolis, MN, U.S.A) and a capillary column (30 m × 0.32 mm × 0.25 μm; Abel AB-5; Appleton, WI, U.S.A). Column chromatography (CC) was carried out on silica gel (100–200 or 200–300 mesh; Shanghai Sanpont Co., Ltd, Shanghai, China), D101 macroporous resin (Cangzhou

Bon Adsorber Technology Co., Ltd, Cangzhou, China), C18 reversed-phase silica gel (50 mesh, YMC Co., Ltd, Kyoto, Japan). Silica gel HSGF254 (Yantai Jiangyou Guijiao Kaifa Co., Ltd, Yantai, China) was used for thin layer chromatography (TLC). Fractions were monitored by TLC on silica-gel plates sprayed with 10% H_2SO_4 in EtOH, followed by heating.

3.2. Plant material

The capitula of *Coreopsis tinctoria* were collected in August 2012 in Xinjiang Uygur Autonomous Region of China. The plant was identified by Prof. Deyun Kong, Shanghsai Institute of Pharmaceutical Industry, China, and the voucher specimen (NO. 20121201) was deposited at the Herbarium of the Shanghsai Institute of Pharmaceutical Industry, China. The search for chemical constituents from *C. tinctoria* has been an ongoing project in our laboratory.

3.3. Extraction and isloation

The powder of dried capitula (2 kg) was extracted with water under reflux for 3 h (1–20 L). The water extract was subjected to macroporous adsorption resin (D101) chromatography column and eluted with H_2O, 30, 50, 70, and 95% EtOH to obtain five fractions (Fr.1~5). Fr.2 (30% EtOH, 132 g) was subjected to polyamide column chromatography (CC) and eluted with H_2O/EtOH (100:0–5:95, v/v) to obtain five fractions (Fr.2-1~Fr.2-5). Fr.2-2 (30% EtOH, 56 g) was subjected to silica gel CC with elution of a gradient of petroleum ether-ethyl acetate, followed by EtOAc–MeOH gradient, to afford ten fractions (Fr.2-2-1~Fr.2-2-10). Fr.2-2-6 (1.3 g) was subjected to C18 reversed-phase silica gel with elution of a gradient of H_2O/MeOH (95:5–30:70) to afford nine fractions (Fr.2-2-6-1~Fr.2-2-6-9). Fr.2-2-6-2 (90 mg) was separated by semipreparative reversed-phase (RP) HPLC (acetonitrile/H_2O, 18:82, v/v; 3 ml/min) to afford **1** (7 mg, t_R: 12 min) and **2** (8 mg, t_R: 18 min).

3.3.1. Compound 1

A brown amorphous powder; $[\alpha]_D^{20}$ + 20.3 (*c* 0.08, H_2O). UV (H_2O) λ_{max} (log ε) nm: 290 (4.35); IR (KBr) ν_{max} cm^{-1}: 3409, 3344, 1670, 1619, 1522, 1461, 1367, 1322, 1265, 1211, 1190, 1083, 1063, 989, 892, 781, 634, 576 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 897.2064 $[M–H]^-$ (calcd for $C_{42}H_{41}O_{22}$, 897.2089). CD (H_2O) $\Delta\varepsilon_{329\ nm}$ +13.889, $\Delta\varepsilon_{290\ nm}$ −25.359, $\Delta\varepsilon_{224\ nm}$ +46.178.

3.3.2. Compound 2

A brown amorphous powder; $[\alpha]_D^{20}$ + 68.5 (*c* 0.08, H_2O). UV (H_2O) λ_{max} (log ε) nm: 290 (4.29); IR (KBr) ν_{max} cm^{-1}: 3408, 3344, 1670, 1619, 1522, 1461, 1367, 1322, 1265, 1223, 1190, 1083, 1063, 989, 892, 781, 634, 576; for ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 897.2072 $[M–H]^-$ (calcd for $C_{42}H_{41}O_{22}^-$, 897.2089). CD (H_2O) $\Delta\varepsilon_{329\ nm}$ +0.184, $\Delta\varepsilon_{290\ nm}$ +0.010, $\Delta\varepsilon_{224\ nm}$ +0.008.

3.4. Acid hydrolysis of 1 and 2

Compounds **1** and **2** (5 mg each) were refluxed in 2 mol/L HCl (2 ml) for 3 h. The mixture was neutralized with $NaHCO_3$ and then partitioned with ethyl acetate. To the dried aqueous layer, pyridine and acetic anhydride (1 ml each) were added, and the mixture was kept

overnight. The acetylated derivatives were subjected to GC analysis to identify the sugars. Conditions for GC: AB-5 (30 m × 0.32 mm × 0.25 μm) column; column temp.: from 100 to 250 °C, programmed increase 10 °C/min and keeping 250 °C for 5 min; injector and detector temp., 250 °C; injection volume, 2.0 ml; split ratio, 1:20; carrier gas, N_2 at 1 ml/min [15]. D-glucose (t_R: min) was detected from **1** and **2** (identical to authentic materials).

3.5. HPLC analysis of 1 and 2

Around 2 mg of compound **1**, **2** and two compounds mixture were dissolved in 1.0 ml of mobile phase and filtered through a Millipore (0.45 μm) filter prior to injection. Chromatographic analysis was conducted at 30 °C and a flow rate of 1 ml/min. The mobile phase consisted of acetonitrile (A) and 0.1% phosphoric acid in water (B); the eluting gradient was used as follows: 0–30 min, 14.0% (A).

Disclosure statement

No potential conflict of interest was reported by the authors.

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