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Saffloflavonesides A and B, Two Rearranged Derivatives of Flavonoid *C*-glycosides with a Furan−Tetrahydrofuran Ring from *Carthamus tinctorius*

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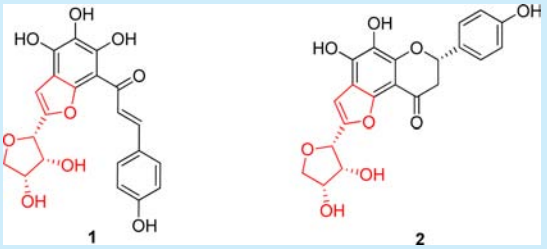
Supporting Information

ABSTRACT: Two new rearranged derivatives of flavonoid *C*-glycosides, saffloflavonesides A (**1**) and B (**2**), were isolated from the florets of *Carthamus tinctorius*. Their structures were determined using UV, IR, HRESIMS, and 1D and 2D NMR data and by comparing experimental and calculated electronic circular dichroism (ECD) spectra. Compounds **1** and **2** were unprecedented chalcone and flavanone derivatives possessing a furan conjoining tetrahydrofuran ring. A potential biosynthetic pathway was proposed. At 10 μM, **1** and **2** both showed strong inhibitory activity against PC12 cell damage induced by rotenone.



Flavonoids are a familiar and important class of plant secondary metabolites that are widely distributed in nature and serve many functions.[1] Flavonoids have long been recognized to possess a diverse range of biological activities in vitro and in vivo, e.g., anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities.[2] The florets of *Carthamus tinctorius* (Compositae), a well-known traditional Chinese medicine, are used to treat stroke, gynecological disease, coronary heart disease, angina pectoris, and hypertension.[3] To date, approximately 40 flavonoid derivatives, including flavonoid *C*-glycosides and *O*-glycosides, have been isolated from *C. tinctorius*.[4] Of these derivatives, hydroxysafflor yellow A (HSYA), a quinochalcone *C*-glycoside, is the main active component of safflower.[5] In our search for additional constituents from the florets of *C. tinctorius*, two new flavonoid *C*-glycoside derivatives, saffloflavonesides A (**1**) and B (**2**), were isolated (Figure 1). Their structures were determined by UV, IR, HRESIMS, and 1D and 2D NMR data and by comparing experimental and calculated electronic circular dichroism (ECD) spectra. Compounds **1** and **2** were unusual chalcone and flavanone derivatives sharing a furan with a tetrahydrofuran ring substituent. This unusual substituent may be derived from the rearrangement of a *C*-glycoside, and is an important building block for the synthesis of structurally diverse *C*-glycoside derivatives. At 10 μM, **1** and **2** showed strong inhibitory activity against PC12 cell damage induced by rotenone.

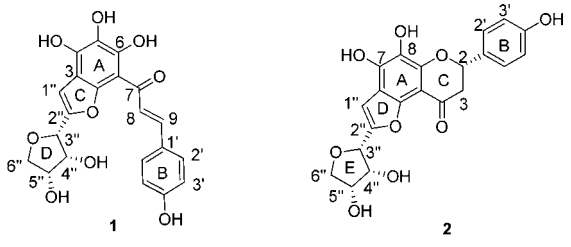


Figure 1. Structures of saffloflavonesides A (**1**) and B (**2**).

Compound **1** was obtained as a pale yellow powder. Its molecular formula $C_{21}H_{18}O_9$ was deduced from the $[M + Na]^+$ ion at m/z 437.0849 (calcd 437.0843) from HRESIMS analysis, corresponding to 13 degrees of unsaturation. The IR spectrum showed the presence of hydroxy groups (3273 cm^{-1}), carbonyl groups (1627 cm^{-1}), and aromatic rings (1604 and 1513 cm^{-1}).

The ^1H NMR spectrum of **1** (Table 1) showed a pair of obvious trans-olefinic proton signals at δ_H 8.04 (1H, d, J = 15.5 Hz) and 7.85 (1H, d, J = 15.5 Hz), aromatic proton signals in an AA′BB′ system at δ_H 7.62 (2H, d, J = 8.5 Hz) and 6.88 (2H, d, J = 8.5 Hz), and a phenolic hydroxy proton resonance at δ_H 10.19 (1H, br s), which suggested the existence of a *trans-p*-hydroxycinnamoyl group in **1**. Furthermore, an olefinic proton signal at δ_H 6.92 (1H, s), three oxymethenyl proton signals at δ_H 4.73 (1H, d, J = 6.5 Hz), 4.30 (1H, m), and 4.24 (1H, m), and two oxymethylene protons at δ_H 4.12 (1H, dd, J = 9.0, 4.5 Hz) and 3.75 (1H, dd, J = 9.0, 2.5 Hz) were observed. Additionally, a characteristic downfield proton signal at δ_H 14.42 (1H, s) suggested the presence of an internal hydrogen bond attributable to 6-OH. The ^{13}C NMR spectrum of **1** exhibited a total of 21 carbon signals (Table 1), and the carbon resonances for a chalcone skeleton were observed: a carbonyl

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Table 1. ^{1}H and ^{13}C NMR Assignments for 1 and 2 in DMSO-d_6

	1		2	
no.	δ_H	δ_C	δ_H	δ_C
1		101.1		
2		146.9	5.49, dd (2.5, 12.0)	79.3
3		110.7		
3ax			3.12, dd (12.0, 16.5)	43.5
3eq			2.69, dd (2.5, 16.5)	
4		148.5		188.0
5		128.5		146.6
6		154.7		111.8
7		189.5		145.4
8	8.04, d (15.5)	121.6		128.1
9	7.85, d (15.5)	144.4		150.7
10				101.5
1′		126.1		129.2
2′,6′	7.62, d (8.5)	131.0	7.38, d (8.5)	128.2
3′,5′	6.88, d (8.5)	116.6	6.79, d (8.5)	114.9
4′		160.8		157.4
1″	6.92, s	103.6	6.87, s	102.2
2″		153.4		154.1
3″	4.73, d (6.5)	77.0	4.64, d (6.0)	76.5
4″	4.30, m	75.6	4.21, overlap	74.4
5″	4.24, m	71.2	4.21, overlap	70.3
6″α				
	4.12, dd (9.0, 4.5)	73.3	4.08, dd (9.0, 4.0)	72.5
6″β	3.75, dd (9.0, 2.5)		3.66, dd (9.0, 2.5)	
4-OH	10.19, brs			
6-OH	14.42, s			
7-OH			10.43, brs	
8-OH			8.33, brs	
4′-OH	10.19, brs		9.53, brs	
4″-OH	5.29, brs		5.15, brs	
5″-OH	5.09, brs		5.00, brs	

^{1}H NMR (500 MHz) (δ in ppm, J in Hz), ^{13}C NMR (125 MHz).

carbon signal at δ_C 189.5, a pair of olefinic carbon signals at δ_C 121.6 and 144.4, and 12 aromatic carbon signals.[6] Intriguingly, the six remaining carbon signals included a pair of olefinic carbon signals at δ_C 146.9 and 103.6 and four carbon signals bearing oxygen at δ_C 77.0, 75.6, 73.3, and 71.2. In the HMBC spectrum (Figure 2), the cross peaks between the oxymethylene proton signal H-6″ at δ_H 4.12 and 3.75 and C-3″, C-4″, and C-5″, as well as the correlations of H-4″ with H-3″ and H-5″ and that of H-5″ with H-6″, in the ^{1}H−^{1}H COSY experiment revealed the presence of a tetrahydrofuran ring (ring D) in **1**, and the cross peaks of H-1″ at δ_H 6.92 and C-2, C-3, and C-4 and H-3″ at δ_H 4.73 and C-1″ as well as the

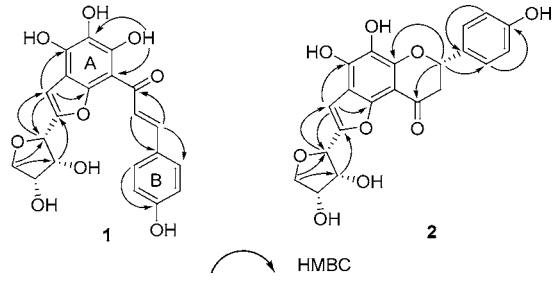

Figure 2. Key HMBC correlations of **1** and **2**.

remaining single degree of unsaturation implied that **1** contained another furan ring (ring C). However, no proton signal and four downfield carbon signals in addition to those of C-1 and C-3 in ring A were observed in the NMR spectrum of **1**, suggesting that ring-A of **1** was tetra-oxygenated. Thus, compound **1** was confirmed to be a chalcone sharing an uncommon tetrahydrofuran−furan ring.

Because ROESY correlations in five-membered rings are normally not sufficiently reliable when determining the orientation of the protons at adjacent carbons, the absolute configurations of C-3″, C-4″, and C-5″ of the tetrahydrofuran ring D in **1** were established by the plausible biogenetic pathway and by comparison of the experimental CD spectrum with calculated ECD data. The unique biogenetic origin of the tetrahydrofuran−furan ring in **1** can plausibly be traced back to a flavonoid *C*-glycoside isolated from *C. tinctorius*. Thus, the chirality at C-4″ and C-5″ in **1** was determined be the same, 4″*R* and 5″*R*, as the corresponding carbons of D-glucose because the configurations at C-4″ and C-5″ remained unchanged during rearrangement of the *C*-glycoside. Furthermore, the absolute configuration of C-3″ in **1** was established by comparison of the experimental CD spectrum and the calculated ECD data. Therefore, only two diastereoisomers (**1a**, 3″*S*,4″*R*,5″*R*; **1b**, 3″*R*,4″*R*,5″*R*) remained (Figure S1, Supporting Information). A systematic conformational analysis was performed for **1a** and **1b** using a MMFF94 molecular mechanics force field calculation. The optimized conformations of **1a** and **1b** were obtained (Tables S1 and S2, Supporting Information) using the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G(d) level. The overall calculated ECD spectra of **1a** and **1b** were generated by Boltzmann weighting of their lowest energy conformers. The overall pattern of the calculated ECD spectrum of **1a** attributable to the 3″*S*-diastereomer was consistent with the experimental data for **1** throughout the entire range of wavelengths under investigation (Figure 3). The

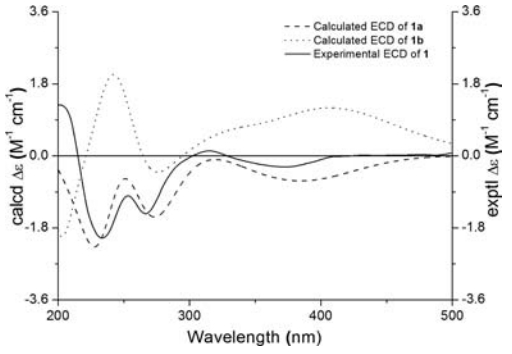

Figure 3. Experimental ECD spectrum of **1** and calculated ECD of **1a** and **1b** in MeOH.

correlation of H-3″ with H-4″ and H-5″ in the ROESY experiment (Figure 4) supported this conclusion. Therefore, the absolute configuration of **1** was established as 3″*S*,4″*R*,5″*R*. The structure of **1** was determined to be that depicted, and compound **1** was named saffloflavoneside A.

Compound **2** was obtained as a white amorphous powder. Its IR spectrum showed absorptions for hydroxy, carbonyl, and aromatic ring groups at 3313, 1655, 1597, and 1518 cm^{-1}, respectively. The UV spectrum exhibited absorptions at 248 and 310 nm. The same molecular formula as **1**, $C_{21}H_{18}O_9$, was

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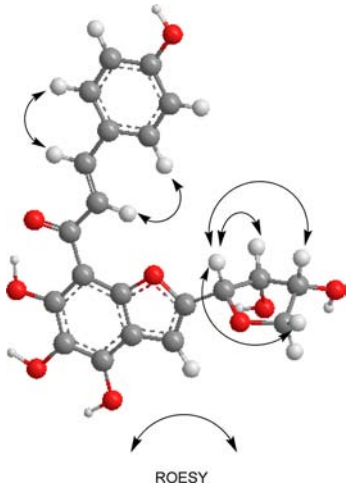


Figure 4. Key ROESY correlations of **1**.

deduced on the basis of the HRESIMS at m/z 415.1032 $[M + H]^+$ and 437.0850 $[M + Na]^+$, indicating that **2** is an isomer of **1**.

The NMR data for **2** (Table 1) were similar to the data for **1** except for the obvious differences resulting from C_3 being part of the flavone $C_6-C_3-C_6$ skeleton. In the ^{1}H NMR spectrum of **2** (Table 1), the characteristic aliphatic proton signals at δ_H 2.69 (1H, dd, J = 16.5 and 2.5 Hz), 3.12 (1H, dd, J = 16.5 and 12.0 Hz), and 5.49 (1H, dd, J = 2.5 and 12.0 Hz) for H-3eq, H-3ax, and H-2, respectively, as well as two corresponding carbon signals at δ_C 43.5 and 79.3 (Table 1) in the HSQC spectrum suggested that the aglycon of **2** was flavanone.[7] The absence of a proton signal and four downfield carbon signals in addition to C-1 and C-3 in ring A, similar to **1**, were observed in the NMR spectrum of **2**, which suggested that **2** possesses a tetraoxygenated ring A. The absence of obvious downfield hydroxy signals in the ^{1}H NMR spectrum of **2** and the presence of more upfield carbonyl carbon signals at δ_C 188.0 in the ^{13}C NMR spectrum of **2** confirmed that a furan ring (ring D) formed at C-5 rather than C-7 of flavanone.[8]

The absolute configurations of C-3″, C-4″, and C-5″ in tetrahydrofuran ring A in **2** were determined to be the same, 3″*S*, 4″*R*, and 5″*R*, as in **1** according to its plausible biogenetic pathway. Considering that the cotton effects at C-2 and C-3″ may have interfered with the respective CD spectra, a comparison of the experimental CD spectrum and the calculated ECD data was applied to confirm the absolute configuration of C-2 in **2**. A systematic conformational analysis and optimization was performed for **2a** (2*S*,3″*S*,4″*R*,5″*R*) and **2b** (2*R*,3″*S*,4″*R*,5″*R*) (Figure S5 and Tables S3 and S5, Supporting Information) using the same method used for **1**. Comparison of the theoretically calculated and experimental ECD curves (Figure 5) permitted the assignment of the absolute configuration of **2** as 2*S*,3″*S*,4″*R*,5″*R*. Thus, **2** was determined to have the depicted structure and was named saffloflavoneside B.

Flavonoid *C*-glycosides are considered to be among the characteristic constituents of *C. tinctorius,* and 11 quinochalcone *C*-glycosides have been isolated.[4a−c] Structurally, **1** and **2** are rearranged derivatives of flavonoid *C*-glycoside. Their most intriguing feature is the existence of an unusual furan−tetrahydrofuran ring. We isolated several rearranged derivatives of flavonoid *C*-glycoside with six−five member and five−five member dioxaspirocycle moieties from *C. tinctorius*[4a] and

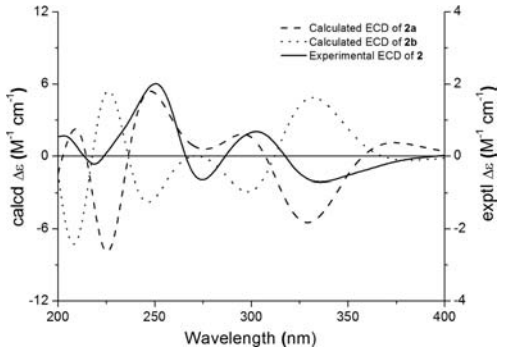
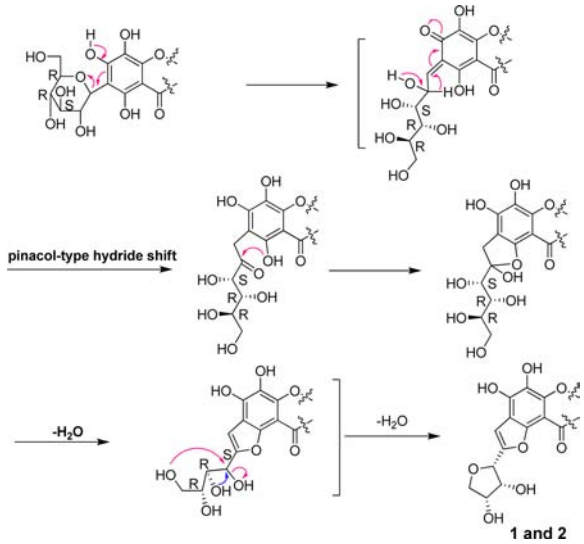


Figure 5. Experimental ECD spectrum of **2** and calculated ECD of **2a** and **2b** in MeOH.

Crataegus pinnatifida Bge. var. *major* N.E.Br.[9] Obviously, **1** and **2** represent a novel type of rearranged derivative of flavonoid *C*-glycosides. A plausible biogenetic pathway is proposed and shown in Scheme 1. The furan ring is potentially

Scheme 1. Hypothetical Biogenetic Pathway of 1 and 2



derived from the *C*-glycoside flavone through the ring opening, a pinacol-type hydride shift,[10] intramolecular nucleophilic addition, and dehydration. The formation of the tetrahydrofuran moiety with the 3″*S* configuration is followed by intramolecular nucleophilic substitution involving neighboring group (4″-OH) participation. This unusual scaffold is also important as a building block for the synthesis of structurally diverse *C*-glycoside derivatives.

In the in vitro neuroprotective assays,[11] **1** and **2** exhibited significant inhibition against PC12 cell damage induced by rotenone. Compared with the cell viability of 54.1 ± 2.8% in the model, **1** and **2** showed increased viability of 60.1 ± 2.6% and 69.5 ± 3.7% at 10 μM, respectively, using coenzyme Q10 as a positive control (cell viability of 59.0 ± 2.2%).

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, physicochemical properties, 1D and 2D NMR, MS, and IR spectra, and the ECD calculation data of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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