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Two New Quinochalcones from the Florets of *Carthamus tinctorius*

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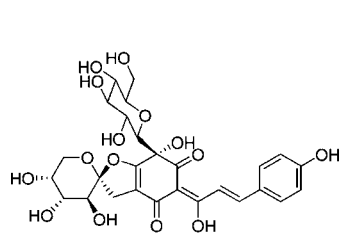
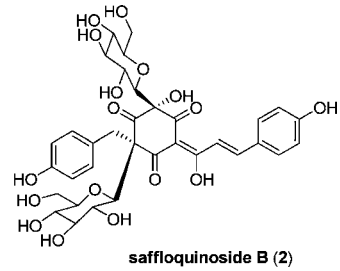
ABSTRACT



saffloquinoside A (1)



saffloquinoside B (2)

Two new quinochalcone compounds, named saffloquinoside A (1) and saffloquinoside B (2), were isolated from the florets of *Carthamus tinctorius*. Their unusual structures including their absolute stereochemistry were elucidated based on UV, IR, HRESIMS, 1D and 2D NMR data, and CD spectrum. Saffloquinoside A has an uncommon six−five member dioxaspirocycle and saffloquinoside B has a cyclohexatrione skeleton with a benzyl group and two *C*-glycosyl units. Saffloquinoside A exhibited middling anti-inflammatory activity.

The florets of *Carthamus tinctorius* (Compositae) have been used as a remedy for stroke, gynecological disease, coronary heart disease, angina pectoris, and hypertension in Chinese folk medicine.[1,2] Up to date, many constituents such as flavonoids,[3,4] alkaloids,[5] and lignans,[5e,6] were isolated from this plant. Among them, quinochalcone glycosides of flavonoids are considered as the characteristic constituents of *Carthamus tinctorius* and 10 quinochalcone glycosides have been obtained.[3] In the course of studying chemical components from the flowers of this plant, two new quinochalcone glycosides, named saffloquinoside A (**1**) and saffloquinoside B (**2**), were isolated successfully. Saffloquinoside A has an uncommon six−five member dioxaspirocycle and saffloquinoside B has a cyclohexatrione skeleton with a benzyl group

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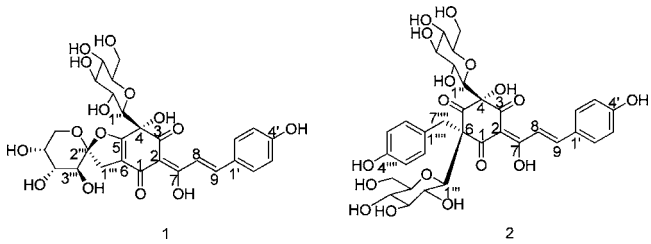

Figure 1. Structures of saffloquinoside A (**1**) and B (**2**).

and two *C*-glycosyl units. To our knowledge, structural types in **1** and **2** were found for the first time. In addition, saffloquinoside A (**1**) exhibited middling anti-inflammatory activity.

The florets of *Carthamus tinctorius* L. (7.0 kg) were exhaustively extracted with H_2O under refluxed conditions. The H_2O extracts were then concentrated under reduced pressure to give a residue (1500 g). The residue was dissolved in H_2O again, then chromatographing over macroporous adsorbent resin (HP-20) column. After eluting with H_2O, then the adsorbed constituents were eluted with 10% ethanol, 30% ethanol, and 50% ethanol, respectively. The 30% ethanol part (50.0 g) was chromatographed over Sephadex LH-20 eluting with $H_2O-MeOH$ (from 100:0 to 0:100) to give 30 fractions. Fr.15 and Fr.18 were further purified by reversed-phase preparative HPLC with CH_3OH-H_2O (55:45, 3 mL/min) as mobile phase to yield **1** (20.0 mg) and **2** (25.0 mg) (Figure 1), respectively.

Compound **1** was obtained as a yellow powder.[7] The IR spectrum of **1** showed the presence of hydroxyl groups (3381 cm^{-1}), carbonyl groups (1625 cm^{-1}), and aromatic rings (1598 and 1521 cm^{-1}). A molecular formula of $C_{27}H_{29}O_{15}$ was deduced on the basis of HRESIMS at *m*/*z* 593.1517 $[M - H]^-$.

The ^1H NMR spectrum of **1** (Table 1) showed *trans* olefinic hydrogen signals at δ 7.68 (1H, d, $J = 16.0$ Hz) and 7.48 (1H, d, $J = 16.0$ Hz), AA′BB′ system proton signals at δ 7.54 (2H, d, $J = 8.0$ Hz) and 6.83 (2H, d, $J = 8.0$ Hz), and a phenolic hydroxyl proton signal at δ 10.11 (1H, br s), which suggested the existence of a *trans*-*p*-hydroxycinnamoyl group in **1**. Additionally, a very characteristic downfield proton signal at δ 17.42 (1H, br s) was assignable to an enolic hydroxyl owning to forming an internal hydrogen bond. Furthermore, methylene proton signals at δ 3.17 (1H, d, $J = 15.5$ Hz) and 2.59 (1H, d, $J = 15.5$ Hz), a carbinol hydroxyl proton signal at δ 6.05 (1H, br s), and seven hydroxyl proton signals and some protons of saccharide moieties at δ 2.7−5.5 ppm were observed. This evidence suggested that **1** was a quinochalcone glycoside derivative in combination with the absorption maxima at 243 and 404 nm in the UV−vis spectrum.[3d−f] The ^{13}C NMR spectrum of **1** showed 27 carbon signals (Table 1). Comparing the NMR data of **1** with those of the corresponding signals of hydroxysafflor yellow A,[3d] the significant difference was carbon chemical shifts of quinocycle, two olefinic signals in the *trans*-*p*-hydroxycinnamoyl group and a set of carbon signals of the saccharide moiety. Careful analysis of the HMBC spectrum (Table 1), which showed that the enolic hydroxyl at δ 17.42 correlated with C-2, C-7, and C-8, revealed that an enolic *p*-hydroxycinnamoyl group existed in **1** and it linked to C-2 of the quinocycle unit via a double bond. Coupled with the presence of two carbonyl carbons (C-1 and C-3), the quinocycle unit in **1** should be the cyclohexaendione moiety, instead of cyclohexadienone such as hydroxysafflor yellow A. Furthermore, a set of carbon signals of the sugar moiety resonating at δ 34.9 (C-1‴), 109.5 (C-2‴), 70.2 (C-3‴), 69.5 (C-4‴), 68.6 (C-5‴), and 66.1 (C-6‴) in the ^{13}C NMR spectrum and the carbon signal at δ 34.9 (C-1‴) being directly attached to the protons at δ 3.17 (1H, d, $J = 15.5$ Hz) and 2.59 (1H, d, $J = 15.5$ Hz) in the HSQC spectrum suggested the sugar moiety existed as a fructopyranose form in **1**. The connectivity of the aglycon and the fructopyranose moiety was established on the basis of the following evidence. A downfield quaternary carbon at δ 109.5 (C-2‴) of the fructopyranose should be linked to the C-5 of the aglycon by the oxygen atom due to the upfield shift signal of C-5 compared with the corresponding signal of hydroxysafflor yellow A, while the methylene of the fructopyranose moiety was directly attached at C-6 of the quinocycle unit based on the correlations of H-1‴a (δ 3.17) and H-1‴b (δ 2.59) with C-5 (δ 173.1), and H-1‴b (δ 2.59) with C-6 (δ 116.6) in the HMBC experiment. This signifies that a six−five member dioxaspirocycle moiety was formed. The relative configuration of fructopyranose moiety in **1** was determined from the ROESY spectrum. In the ROESY spectrum, the correlations of H-1‴a and H-1‴b with H-3‴ indicated that the fructopyranose moiety was β-D-form in **1**[8] coupling with the fact that fructose from natural products was D-form. In addition, the correlations between H-1″/C-5 and OH-4/C-4, C-1″ in the HMBC experiment demonstrated that a glucopyranosyl moiety and a OH group were on C-4 of the quinocycle unit. The C-4 stereochemistry of **1** was elucidated from its CD spectroscopic data. In the CD spectrum, **1** exhibited an identical negative cotton effect at around 270 nm with carthamin reported in the literature.[9] Thus the absolute stereochemistry at the C-4 position in **1** was determined to be *S*. Finally, compound **1** was identified as depicted and named saffloquinoside A.

(7) Saffloquinoside A (**1**): $[\alpha]^{25}_D$ −24.6 (*c* 0.04, MeOH); UV (MeOH) λ_{max} 404 (ϵ 17 681), 314 (ϵ 3 472), 243 (ϵ 7 259), 202 (ϵ 4 586) nm; IR (KBr) ν_{max} 3381, 2935, 1625, 1598, 1521, 1439, 1252, 1171, 1088, 964, 918, 832, 727 cm^{-1}; ESIMS *m*/*z* 595 $[M + H]^+$, 635 $[M + H_2O + Na]^+$; HRESIMS *m*/*z* 593.1517 $[M - H]^-$ (calcd 593.1501); CD (MeOH) $\lambda_{extremum}$ ($\Delta\epsilon$) 262 (−4.23).

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data and HMBC Correlations for Compounds **1** and **2** (125, 500 MHz, DMSO-d_6)

	1			**2**			
no.	δ_C	δ_H (mult)	HMBC[a]	δ_C	δ_H (mult)	δ_H[b] (mult)	HMBC[a]
1	187.5			196.8			
2	107.7			112.8			
3	193.8			188.6			
4	77.5	6.05 br s (OH)	3, 4, 5, 1″	89.7	5.06 br s (OH)		3, 4, 5, 1″
5	173.1			201.7			
6	116.6			63.6			
7	179.2	17.42 br s (OH)	2, 7, 8	182.4	17.83 br s (OH)		1, 2, 7, 8
8	117.9	7.48 d (16.0)	7, 9, 1′	118.3	7.13 d (16.0)	7.11 d (16.0)	7, 1′
9	142.4	7.68 d (16.0)	7, 1′, 2′, 6′	143.6	7.72 d (16.0)	7.71 d (16.0)	7, 8, 2′, 6′
1′	126.0			125.9			
2′,6′	130.6	7.54 d (8.0)	4′	131.4	7.60 d (8.5)	7.59 d (8.5)	9, 4′
3′,5′	116.0	6.83 d (8.0)	1′, 4′	115.9	6.81 d (8.5)	6.81 d (8.5)	1′, 4′
4′	160.2	10.11 brs (OH)	3′, 5′	160.5	10.15 brs (OH)		3′, 4′, 5′
1″	83.0	3.51 overlap	5	77.7	4.61 overlap	4.60 d (8.5)	5, 3″, 5″
2″	69.8	3.51 m	1″	69.1	3.32 m		3″
3″	78.1	3.12 m	4″, 5″	78.3	3.17 m		
4″	69.9	2.89 m	3″, 5″	71.0	2.86 dd (9.0, 6.0)		3″, 5″
5″	81.1	3.02 m	6″	82.0	3.09 m		4″
6″	61.8	3.63 m		61.9	3.81 m		5″
		3.31 m	5″		3.44 m		
1‴	34.9	3.17 d (15.5)	5, 2‴, 3‴	79.1	3.84 d (10.0)	3.84 d (10.5)	1, 5, 6
		2.59 d (15.5)	5, 6, 2‴				
2‴	109.5			71.5	3.24 m		3‴
3‴	70.2	3.65 m	5‴	78.0	3.07 m		1‴
4‴	69.5	3.71 m	3‴	68.7	3.00 m		
5‴	68.6	3.79 m	6‴	78.1	3.24 m		3‴
6‴	66.1	3.91 m 3.60 m	5‴	60.7	3.55 m 3.38 m		5‴
1⁗				125.0			
2⁗, 6⁗				130.8	6.63 d (8.5)	6.63 d (7.5)	3⁗, 4⁗, 5⁗
3⁗, 5⁗				114.7	6.46 d (8.5)	6.46 d (7.5)	1⁗, 2⁗, 4⁗, 6⁗
4⁗				156.1	9.13 br s (OH)		3⁗, 4⁗, 5⁗
7⁗				43.5	3.17 d (13.0) 3.01 d (13.0)		1, 5, 6, 1⁗, 2⁗, 6⁗

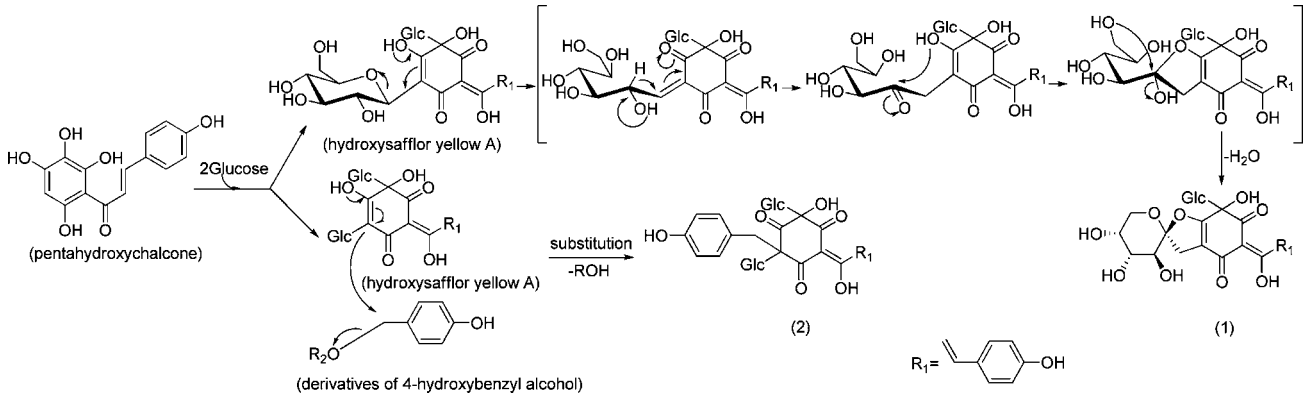
[a] H to C correlations [b] In DMSO-d_6 + D_2O.

Compound **2** was also obtained as a yellow powder.[10] The IR of **2** showed the presence of hydroxyl groups (3383 cm^{-1}), carbonyl groups (1726, 1668 cm^{-1}), and aromatic rings (1583 and 1516 cm^{-1}). The molecular formula of $C_{34}H_{37}O_{17}$ was determined by HRESIMS at *m/z* 717.2001 $[M - H]^-$ (calcd 717.2025).

The ^{1}H NMR spectrum of **2** (Table 1) also showed a set of enolic *p*-hydroxycinnamoyl group hydrogen signals at δ 7.72 (1H, d, $J = 16.0$ Hz, H-9) and 7.13 (1H, d, $J = 16.0$ Hz, H-8), δ 7.60 (2H, d, $J = 8.5$ Hz, H-2′,6′) and 6.81 (2H, d, $J = 8.5$ Hz, H-3′,5′), δ 10.15 (1H, br s, OH-4′), and δ 17.83 (1H, br s, OH-7). Additionally, two anomeric protons at δ 4.61 (1H, overlap) and δ 3.84 (1H, $J = 10.0$ Hz) and 8 hydroxyl hydrogen signals of saccharide moieties at δ 4.0−5.5 ppm were observed in the ^{1}H NMR of **2**, which suggested that **2** has the same two *C*-glycosyl moieties as hydroxysafflor yellow A. The differences between **2** and hydroxysafflor yellow A were the presence of a set of AA′BB′ system proton signals at δ 6.63 (2H, d, $J = 8.5$ Hz) and 6.46 (2H, d, $J = 8.5$ Hz), a phenolic hydroxyl proton signal at δ 9.13 (1H, br s), and methylene proton signals at δ 3.17 (1H, d, $J = 13.0$ Hz) and 3.01 (1H, d, $J = 13.0$ Hz) in **2**. Coupled with 6 carbon signals of a phenyl moiety at δ 125.0 (C-1⁗), 130.8 (C-2⁗, C-6⁗), 114.7 (C-3⁗, C-5⁗), 156.1 (C- 4⁗), and a methylene signal at δ 43.5 (C-7⁗) in the ^{13}C NMR spectrum and the 106 difference in mass spectrum, a *p*-hydroxybenzyl moiety could be concluded in **2**. The ^{13}C NMR spectrum of **2** also displayed 6 carbon signals of the quinocycle moiety at δ 196.8 (C-1), 112.8 (C-2), 188.6 (C-3), 89.7 (C-4), 201.7 (C-5), and 63.6 (C-6). Considering the existence of three carbonyl carbon signals, the quinocycle moiety could be presumed as a cyclohexatrione form in **2**. Above all the results, the structure of **2** consisted of an enolic *p*-hydroxycinnamoyl group, a cyclohexatrione unit, two glucopyranose moieties, and a *p*-hydroxybenzyl moiety, while the *p*-hydroxybenzyl moiety should be attached at C-6 of the cyclohexatrione unit by the

(10) Safflquinoside B (**2**): $[\alpha]^{25}_D$ −215 (*c* 0.07, MeOH); UV λ_{max} (MeOH) 389 (ϵ 4 415), 282 (ϵ 1 293), 222 (ϵ 2 671), 205 (ϵ 3 519) nm; IR (KBr) ν_{max} 3383, 2932, 1726, 1668, 1616, 1583, 1516, 1441, 1405, 1248, 1171, 1088, 932, 906, 834 cm^{-1}; ESIMS *m/z* 741 $[M + Na]^+$, 1459 $[2M + Na]^+$; HRESIMS *m/z* 717.2001 $[M - H]^-$ (calcd 717.2025).

Scheme 1. Proposed Biogenesis of Saffloquinoside A (**1**) and B (**2**)



correlations between H-7‴′ with C-1, C-5 and C-6 in the HMBC spectrum (Table 1).

Unfortunately, the stereochemistry at C-4 and C-6 of **2** was not directly concluded from its CD spectroscopic data because the cotton effects at C-4 and C-6 interfered with each other in the CD spectrum. So, the NOE experiment (DMSO-d_6 + D_2O as solvent, the ^{1}H NMR data in Table 1) was applied to confirm its relative configurations at C-4 and C-6. When irradiating the anomeric proton signal at δ 3.84 (1H, d, J = 10.5 Hz, H-1‴) of one glucopyranosyl unit at C-6, an enhancement of the anomeric proton signal at δ 4.60 (1H, d, J = 8.5 Hz, H-1″) of another glucopyranosyl unit at C-4 was observed, which suggested that two glucopyranosyl units were on the same side of cyclohexatrione unit. Furthermore, the chirality at the C-4 asymmetric center should be *S* based on the fact that presumably all the related quinochalcone glycosides obtained from the safflower were *S*.[9a,11] Thus, the absolute stereochemistry at the C-6 position in **2** was concluded to be *R*. Therefore, compound **2** was elucidated as depicted and named saffloquinoside B.

The most intriguing feature of **1** is the unusual 5,6-spirocycle with a five-member ring formed by fusion of the fructopyranose and the cyclohexaendione. This type of spirocycle has not been previously found in the quinochalcone glycosides. In addition, the feature of **2** is that the quinocycle unit bears a very special cyclohexatrione. Hypothetical biosynthesis of **1** and **2** is proposed as shown in Scheme 1. The spirocycle ring in **1** is possibly derived from the glucose of hydroxysafflor yellow A through a series of rearrangements including pinacol-type hydride shift,[12] while **2** could be formed by intermolecular substitution between hydroxysafflor yellow A and derivatives of 4-hydroxybenzyl alcohol.

In the vitro bioactive assays, compounds **1** and **2** were evaluated against inhibitory effect on release of β-glucuronidase from rat polymorphonuclear neutrophils (PMNs) induced by the platelet-activating factor (PAF). Compound **1** exhibited anti-inflammatory activity and the inhibitory rate was 54.3% (10^{-5} mol/L), but **2** did not show anti-inflammatory activity and the inhibitory rate was only 8.3% (10^{-5} mol/L), using ginkgolide B (BN52021, IC_{50}: 5.45 × 10^{-6} mol/L) as a positive control.

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Supporting Information Available: MS, HRMS, IR, UV, 1D and 2D NMR, and CD spectra of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Safflomin C: 4*S* [$\lambda_{extremum}$ ($\Delta\epsilon$) 266.5 (−7.72)]. Safflor yellow A: 4*S* [$\lambda_{extremum}$ ($\Delta\epsilon$) 255.5 (−3.23)]. Cartormin: 4*S* [$\lambda_{extremum}$ ($\Delta\epsilon$) 263.5 (−7.39)]. Hydroxysafflor yellow A: 4*S* [$\lambda_{extremum}$ ($\Delta\epsilon$) 268 (−11.93)]. Anhydrosafflor yellow B: 4*S* [$\lambda_{extremum}$ ($\Delta\epsilon$) 270 (−8.98)], 4″*S* [$\lambda_{extremum}$ ($\Delta\epsilon$) 287 (−7.76)]. (These quinochalcone glycosides were isolated from the safflower by us.)

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