Flavonoids from *Carthamus tinctorius*

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Two new flavonoids, (2*S*)-4′,5-dihydroxyl-6,7-di-*O*-β-*D*-glucopyranosyl flavanone (1) and 6-hydroxykaempferol 6,7-di-*O*-β-*D*-glucopyranoside (2), were isolated from *Carthamus tinctorius*. Their structures were elucidated by spectroscopic means including 2D NMR, ESIMS and CD.

Keywords *Carthamus tinctorius*, compositae, isolation, structure elucidation, flavonoids

Introduction

Carthamus tinctorius L. (Compositae) is a widely used traditional Chinese medicine having the function of promoting blood circulation by removing blood stasis.[1] Among its constituents, polyacetylenes,[2] serotonin derivatives,[3] steroids,[4] lignans,[5,6] alkane diols,[7,8] flavonoids,[9,10] semi-quinone chalcone[11] and cycloheptenone oxide derivative[12] have been reported. During the course of our inverstigation, two new flavonoids, (2*S*)-4′,5-dihydroxyl-6,7-di-*O*-β-*D*-glucopyranosyl flavanone (**1**) and 6-hydroxykaempferol 6,7-di-*O*-β-*D*-glucopyranoside (**2**), were isolated from its *n*-BuOH extract accompanied by the two known compounds, 6-hydroxyapigenin 6,7-diglucoside (**3**)[13] and 6-hydroxykeampferol 3,6,7-triglucoside (**4**).[9] Compound **3** was isolated from the plant firstly. This paper describes the isolation and structure elucidation of compounds **1** and **2**.

Results and discussion

Compound **1** was a white amorphous powder. The molecular formula was established as $C_{27}H_{32}O_{16}$ by HRESIMS measurement. Its EIMS displayed the fragment ions at *m/z* 288 $[M-2Glu]^+$ and 168 $[M-2Glu-p$-hydroxyphenylethylene$]^+$. The IR spectrum suggested the presence of conjugated carboxyl (ν: 1649 cm^{-1}) and hydroxyl groups which were due to sugar moieties (ν: 3363, 1074 br cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) showed the presence of the flavanone core structure with a *p*-hydroxyl-phenyl group at δ 7.32 (H-2′ and H-6′), 6.79 (H-3′ and H-5′) and 9.60 (OH-4′). The downfield chemical shift of OH-5 (δ 11.96) suggested that it should be formed an internal hydrogen bond with the carbonyl carbon C-4 (δ 197.6). Furthermore, the characteristic signals of flavanone were revealed at δ 5.47 (dd, $J=2.5$, 13.0 Hz) for Hβ-2, at δ 2.69 (dd, $J=2.5$, 17.2 Hz) for Hβ-3 and at δ 3.30 (dd, $J=13.0$, 17.2 Hz) for Hα-3. Particularly, in the UV spectrum, the absorption band II at 283 nm and band I at 341 nm (sh) were observed, which were strong evidence for flavanones. Two anomeric protons at δ 4.56 (d, $J=7.2$ Hz, H_{Glc}-1′) and δ 4.89 (d, $J=7.3$ Hz, H_{Glc}-1″) demonstrated the presence of sugar moieties. The above facts suggested that **1** should be a glucoside of flavanone. Comparison with authentic sample by TLC, acid hydrolysis of **1** yielded glucose. In the ^1H NMR spectrum, the *J* values of the anomeric signals ($J=7.2$ Hz, H_{Glc}-1′; $J=7.3$ Hz, H_{Glc}-1″) indicated that the two glucosyl moieties exhibited β-configuration. In the HMBC spectrum, the presence of the cross-peaks between C-6 and HGlc-1′ as well as C-7 and H_{Glc}-1″ confirmed that two glucosyl moieties were attached to the C-6 and C-7, respectively. In addition, the significant NOE was observed between Hβ-2 and Hβ-3 indicating that the *p*-hydroxylphenyl group and Hα-3 were *syn*-form. The absolute configuration at C-2 was determined as *S* by observing the

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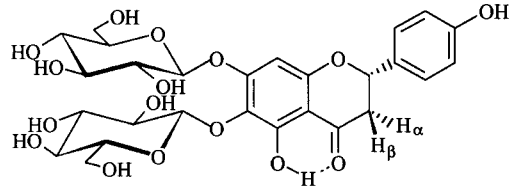
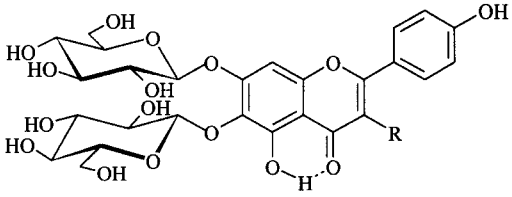
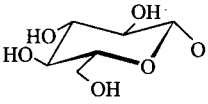
CD spectrum, which displayed a positive Cotton effect at 342 nm and a negative Cotton effect at 300 nm.[14-16] On the basis of the above evidence, the structure of **1** was elucidated to be (2*S*)-4′, 5-dihydroxyl-6, 7-di-*O*-β-*D*-glucopyranosyl flavanone.



1



2 R = OH ; **3** R = H ; **4** R =



Compound **2** was isolated as light yellow prisms. The molecular formula was determined as $C_{27}H_{30}O_{17}$ by HRESIMS, and its EIMS showed the fragment ion at *m/z* 302 $[M-2Glu]^+$. The IR spectrum indicated the presence of hydroxyl groups which were due to sugar moieties (ν: 3404, 1064 br cm^{-1}). The characteristic absorption band I of 3-hydroxyl flavonol at 365 nm was observed in the UV spectrum. Therefore **2** was a glucoside of flavonol. The IR, ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectral data of **2** were similar to those of **4**, especially the ^{13}C NMR data, which suggested that **2** would be the same aglycone as **4**. Acid hydrolysis of **2** obtained glucose identified by TLC comparison with authentic sample. In the ^1H NMR spectra, the *J* values of the anomeric protons at δ 4.87 (*J* = 7.4 Hz, H_{Glc}-1′) and at δ 5.03 (*J* = 7.4 Hz, H_{Glc}-1″) indicated that the glucosyl moieties exhibited β-configuration. In the HMBC spectrum, the presence of cross peaks between H_{Glc}-1′ and C-6 as well as H_{Glc}-1″ and C-7 further proved that the two glucosyl groups were attached to the C-6 and C-7, respectively. Considering the above results, the structure of **2** was determined as 6-hydroxykaempferol 6,7-di-*O*-β-*D*-glucopyranoside.

Experimental

The melting points (uncorrection) were determined on a Buechi 510 melting point apparatus. The $[\alpha]_D^{25}$ values were obtained on a DIP-181 digital polarimeter. The UV spectra were taken on a Varian Cary 300 Bio spectrophotometer. The IR spectra were recorded on a Nicolet 750 instrument. The NMR spectra were measured on a Bruker AM-400 spectrometer, with TMS as internal standard in DMSO-d_6. The ESIMS were taken on a LCQ DECA mass spectrometer. The HRESIMS was obtained on an APEX mass spectrometer. The CD were measured on a JASCO J-715 spectropolarimeter.

Table 1 ^1H NMR spectral data for compounds **1**—**4** *

Position	1	2	3	4
Hβ-2	5.47 (dd, *J* = 2.5, 13.0 Hz)			
Hα-3	3.30 (dd, *J* = 13.0, 17.2 Hz)*		6.87 (s)	
Hβ-3	2.69 (dd, *J* = 2.5, 17.2 Hz)			
8	6.35 (s)	7.01 (s)	7.06 (s)	6.99 (s)
H-2′	7.32 (d, *J* = 8.5 Hz)	8.06 (d, *J* = 8.9 Hz)	7.96 (dd, *J* = 6.9, 2.0 Hz)	8.05 (d, *J* = 9.0 Hz)
H-3′	6.79 (d, *J* = 8.5 Hz)	6.94 (d, *J* = 8.9 Hz)	6.94 (dd, *J* = 6.9, 2.0 Hz)	6.89 (d, *J* = 9.0 Hz)
H-5′	6.79 (d, *J* = 8.5 Hz)	6.94 (d, *J* = 8.9 Hz)	6.94 (dd, *J* = 6.9, 2.0 Hz)	6.89 (d, *J* = 9.0 Hz)
H-6′	7.32 (d, *J* = 8.5 Hz)	8.06 (d, *J* = 8.9 Hz)	7.96 (dd, *J* = 6.9, 2.0 Hz)	8.05 (d, *J* = 9.0 Hz)
OH-3		9.52 (s)		
OH-5	11.96 (s)	12.51 (s)	13.05 (s)	12.65 (s)
OH-4′	9.60 (s)	10.14 (s)	10.04 (s)	10.26 (s)
H_{Glc}-1				5.47 (d, *J* = 7.3 Hz)
H_{Glc}-1′	4.56 (d, *J* = 7.2 Hz)	4.87 (d, *J* = 7.4 Hz)	4.88 (d, *J* = 7.4 Hz)	4.87 (d, *J* = 7.3 Hz)
H_{Glc}-1″	4.89 (d, *J* = 7.3 Hz)	5.03 (d, *J* = 7.4 Hz)	5.04 (d, *J* = 7.4 Hz)	5.04 (d, *J* = 7.7 Hz)

* H signal at δ 3.30 was overlapped by the solvent DMSO-d_6.

Table 2 ^{13}C NMR spectral data for compounds **1**—**4**

Position	1	2	3	4
C-2	78.8 (d)	147.5 (s)	164.3 (s)	157.1 (s)
C-3	42.3 (t)	135.7 (s)	102.8 (d)	133.2 (s)
C-4	197.6 (s)	176.1 (s)	182.3 (s)	177.8 (s)
C-5	154.7 (s)	151.5 (s)	152.6 (s)	152.3 (s)
C-6	126.9 (s)	128.4 (s)	129.2 (s)	128.9 (s)
C-7	158.1 (s)	155.9 (s)	156.1 (s)	156.0 (s)
C-8	94.8 (d)	94.2 (d)	94.7 (d)	94.4 (d)
C-9	158.5 (s)	151.3 (s)	152.3 (s)	151.6 (s)
C-10	103.4 (s)	105.6 (s)	105.8 (s)	106.2(s)
C-1′	128.6 (s)	121.4 (s)	121.0 (s)	120.9 (s)
C-2′	128.5 (d)	129.5 (d)	128.5 (d)	131.0 (d)
C-3′	115.2 (d)	115.4 (d)	115.9 (d)	115.2 (d)
C-4′	157.8 (s)	159.3 (s)	161.3 (s)	160.1 (s)
C-5′	115.2 (d)	115.4 (d)	115.9 (d)	115.2 (d)
C-6′	128.5 (d)	129.5 (d)	128.6 (d)	131.0 (d)
3-Glucosyl moiety				
G-1				100.8 (d)
G-2				74.3 (d)
G-3				76.4 (d)
G-4				69.9 (d)
G-5				77.5 (d)
G-6				60.9 (t)
6-Glucosyl moiety				
G-1′	103.8 (d)	103.4 (d)	103.3 (d)	103.4 (d)
G-2′	73.3 (d)	73.3 (d)	73.4 (d)	73.4 (d)
G-3′	76.3 (d)	75.8 (d)	76.3 (d)	76.4 (d)
G-4′	69.6 (d)	69.6 (d)	69.7 (d)	69.8 (d)
G-5′	77.2 (d)	77.1 (d)	77.3 (d)	77.2 (d)
G-6′	60.6 (t)	60.6 (t)	60.8 (t)	60.8 (t)
7-Glucosyl moiety				
G-1″	100.7 (d)	100.9 (d)	100.9 (d)	100.6 (d)
G-2″	74.1 (d)	74.0 (d)	74.1 (d)	74.2 (d)
G-3″	76.3 (d)	76.2 (d)	75.9 (d)	75.9 (d)
G-4″	69.8 (d)	69.7 (d)	69.6 (d)	69.7 (d)
G-5″	77.2 (d)	77.2 (d)	77.1 (d)	77.2 (d)
G-6″	60.9 (t)	60.7 (t)	60.7 (t)	60.7 (t)

Plant material

The flower petals of *Carthamus tinctorius* were collected in Sichuan province, China, and authenticated by Prof. Lan Xu. A voucher specimen (No. 77) has been deposited at Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation

The air-dried flower petals (6 kg) were extracted with water, and extracted with EtOAc, followed by *n*-BuOH. The *n*-BuOH fraction (150 g) was chromatographied on a silica gel column using gradient elution with $CHCl_3$-MeOH-H_2O ($V:V:V$, 15:1:0, 8:1:0, 4:1:

0.1, 2:1:0.1, MeOH). Five fractions were collected. The MeOH fraction was subjected to a polyamide column with H_2O, H_2O-MeOH ($V:V$, 1:1) and MeOH successively. The fraction (H_2O-MeOH) was chromatographied on a silica gel column repeatedly, and **4** (25 mg) was obtained. The fraction $CHCl_3$-MeOH-H_2O ($V:V:V$, 2:1:0.1) was chromatographied on a polyamide eluted with H_2O-MeOH ($V:V$, 3:1), and then repeatedly on a silica gel column using $CHCl_3$-MeOH-H_2O ($V:V:V$, 4:1:0.1) as solvent. The purification by Sephdex LH20 chromatography with methanol was subjected, then compounds **1** (80 mg), **2** (30 mg), **3** (40 mg) and **4** (25 mg) were obtained, respectively.

1 White amorphous powder, m.p. 207 ℃ (dec.), $[\alpha]_D^{25}$ − 51.2 (*c* 0.108, MeOH); UV-vis (MeOH) λ_{max}: 283, 341 nm; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; IR (KBr) ν: 3363, 2895, 1649, 1448, 1283, 1074 (br), 835 cm^{-1}; EIMS (70 eV) *m/z* (%): 168 ([M − 2Glu − *p*-hydroxyphenylethylene]$^+$, 100); 288 ([M − 2Glu]$^+$, 48); ESIMS (negative ion) *m/z* (%): 611 ([M − H]$^-$, 100), 449 ([M − Glu − H]$^-$, 14); ESIMS (positive ion) *m/z* (%): 613 ([M + H]$^+$, 30), 289 ([M − 2Glu + H]$^+$, 25), 451 ([M − Glu + H]$^+$, 48), 630 ([M + NH_4^+], 100), 1242 ([2M + NH_4^+], 70); CD: $[\theta]_{342}$ + 3450, $[\theta]_{300}$ − 7000, $[\theta]_{280}$ + 800, $[\theta]_{260}$ − 2500, $[\theta]_{250}$ + 2000 (c 1.60×10^{-3}); HRESIMS (positive): calcd for $C_{27}H_{33}O_{16}$ 613.1762, found 613.1763.

2 Light yellow prisms, m.p. 187 ℃ (dec.), $[\alpha]_D^{25}$ − 66.2 (*c* 0.0803, MeOH); UV-vis (MeOH) λ_{max}: 365, 269, 257 nm; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; IR (KBr) ν: 3404, 2933, 1649, 1610, 1552, 1512, 1481, 1356, 1290, 1182, 1064 br, 839 cm^{-1}; EIMS (70 eV) *m/z* (%): 302 ([M − 2Glu]$^+$, 70); ESIMS (negative ion) *m/z* (%): 625 ([M − H]$^-$, 100), 463 ([M − Glu − H]$^-$, 18), 301 ([M − 2Glu − H]$^-$, 5); ESIMS (positive ion) *m/z* (%): 627 ([M + H]$^+$, 100), 649 ([M + Na]$^+$, 25), 465 ([M − Glu + H]$^+$, 30); HRESIMS (positive): calcd for $C_{27}H_{31}O_{17}$ 627.1555, found 627.1554.

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