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A NOVEL COMPOUND FROM *Flos carthami* AND ITS BIOACTIVITY

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*A novel compound, 4-{1′-hydroxy-1′-mercapto-1′-[1″-2″(N→O)-isoquinolyl]}yl-1-benzoic acid (**1**), together with six known compounds, 6-hydroxykaempferol-3-*O*-β-*D*-glucopyranoside (**2**), rutin (**3**), quercetin-3-O-β-*D*-glucopyranoside (**4**), kaempferol-3-O-β-*D*-glucopyranoside (**5**), cartormin (**6**), hydroxysafflor yellow A (**7**), were isolated by chromatography from the* n-*BuOH fraction of 50% ethanol extraction of* Flos carthami. *Their structures were elucidated on the basis of spectral analysis and comparison with published data. Among them, compound **1** was shown to possess a weak protective effect against cerebral ischemic damage in rats.*

Key words: *Flos carthami*, 4-{1′-hydroxy-1′-mercapto-1′-[1″-2″(N→O)-isoquinolyl]}yl-1-benzoic acid, cerebral ischemic damage.

Flos carthami, dried flower petals of *Carthamus tinctorius* L., is an important crude drug in traditional Chinese medicine for promoting blood circulation and removing obstruction in the channels. It has long been used in the prevention and treatment of cardiovascular diseases and thrombosis in China [1]. Clinical experience from many hospitals in China has proved that *Flos carthami* also has good efficacy in the prevention and treatment of cerebrovascular diseases including cerebral thrombus, cerebral embolism, cerebral ischemia, and lacuna embolism [2]. So far as we know, there are studies reporting the chemical constituents of it, including flavonoids, such as apigenin, scutellarin, kaempferol and its ramification [3, 4], quercetin and its glycosides [5], alkane diols [6], lignans [7], and pigments such as carthamin, safflor yellow A [8], safflor yellow B, hydroxysafflor yellow A [9, 10], tinctormine [11], and cartormin [12]. In the present paper, we report a novel compound and six known compounds from *Flos carthami*. The structure of them were elucidated as 4-{1′-hydroxy-1′-mercapto-1′-[1″-2″(N→O)-isoquinolyl]}yl-1-benzoic acid (**1**), 6-hydroxykaempferol-3-*O*-*β*-D-glucopyranoside (**2**), rutin (**3**), quercetin-3-*O*-*β*-D-glucopyranoside (**4**), kaempferol-3-*O*-*β*-D-glucopyranoside (**5**), cartormin (**6**), and hydroxysafflor yellow A (**7**), respectively. Furthermore, the protective effect of compound **1** on a transient focal cerebral ischemia-reperfusion model in rats was assayed primarily. The results demonstrated that compound **1** had a weak protective effect against cerebral ischemic damage.

Compound **1** was isolated as a red powder and had a molecular composition of $C_{17}H_{13}NO_4S$, assigned by HR-ESI-MS at 327.0562 $[M]^+$; caculated: 327.0565. The ^1H NMR (DMSO-d_6, δ, ppm) spectrum showed the presence of ten aromatic protons. The ^{13}C NMR-DEPT (DMSO-d_6, δ, ppm) spectrum revealed the presence of seven quaternary carbons and ten methane carbons. In the ^1H–^1H COSY spectrum the presence of cross peaks between δ 8.57 and 7.63 (see Table 1), between 7.63 and 7.48, and between 7.48 and 7.79 indicated that the four protons were in the same aromatic ring. The presence of cross peaks between 2H (d) signals 7.82 (2H, d) and 7.76 (2H, d) indicated that the four protons were in the same aromatic ring. The connection of the proton and carbon signals in the NMR spectra of compound **1** were assigned by HMQC.

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TABLE 1. ^{1}H NMR and ^{13}C NMR Data of Compound **1** (DMSO-d_6, ppm, J/Hz)

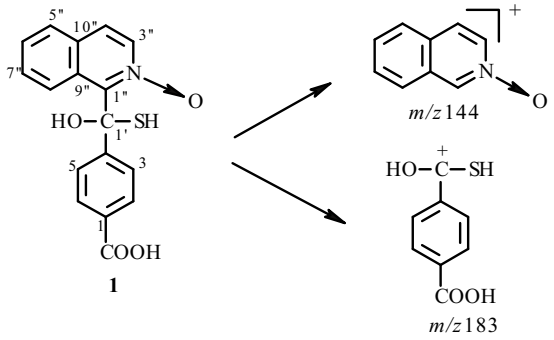
Position	δ_C	δ_H	Position	δ_C	δ_H
1″	129.4		10″	132.7	
2″			1′	79.1	
3″	140.2	7.97 (1H, d, J = 10)	1	147.5	
4″	124.0	6.92 (1H, d, J = 9)	2	127.1	7.76 (2H, d, J = 9)
5″	121.4	8.57 (1H, d, J = 8)	3	118.0	7.82 (2H, d, J = 9)
6″	129.1	7.63 (1H, t)	4	144.7	
7″	125.9	7.48 (1H, t)	5	118.0	7.82 (2H, d, J = 9)
8″	128.8	7.79 (1H, d, J = 7)	6	127.1	7.76 (2H, d, J = 9)
9″	127.8		COOH	169.5	

TABLE 2. Effect of Compound **1** against Cerebral Ischemic Damage on Rats

MeOH, Group	Neurological	Moisture content of brain tissue, %		Infarct volume, %
		left hemisphere	right hemisphere	
A	0	76.73±0.44	76.42±0.57	0
B	1.7±0.6	77.83±0.45	82.47±0.76[a]	31.01±9.02
C	1.1±0.5*	77.44±0.56	81.38±0.67*	22.76±8.65
D	0.8±0.7*	78.75±0.64	81.06±0.82*	15.98±6.14**

A: sham-operation; B: vehicle; C: compound **1**, 10 mg/kg; D: nimodipine, 2 mg/kg; *P<0.05; **P<0.01 vs the vehicle-treated group; [b]P<0.05; [a]P<0.01 vs left hemisphere.

In the HMBC spectrum the presence of cross peaks between ^{13}C (δ 147.5) and two protons ^{1}H 7.82 and ^{13}C (δ_C 144.7) and two protons ^{1}H 7.76 suggested that the six carbons [δ_C:147.5, 144.7, 127.1, 2CH, 118.0, 2CH] were in the same aromatic ring. The cross peaks between ^{13}C (δ_C 127.8) and ^{1}H 8.57, 7.48 and between ^{13}C (δ_C 132.7) and ^{1}H 7.79, 7.63 showed that the six carbons (δ_C 132.7, 127.8, 129.1, 128.8, 125.9, 121.4) were in the same aromatic ring. The presence of cross peaks between ^{13}C (δ_C 132.7) and ^{1}H 7.97 and 6.92 indicated that ^{13}C (δ_C 140.2, 124.0) was in the aromatic ring comprising C (δ_C 132.7 and 127.8), while -CH (δ_C 140.2) was connected with N. The presence of cross peaks between ^{13}C (δ_C 129.4) and ^{1}H (δ 8.57, 6.92) proved that C (δ_C 129.4) was connected with C (δ_C 127.8). So, the structure of compound **1** comprised *p*-phenyl and 1-isoquinolyl. The ^{13}C (δ_C 79.1), which connected with -OH and -SH, was connected with the two fragments. Signal ^{13}C (δ_C 169.5) relates to the carbonyl group. On the basis of these, the structure of compound **1** was elucidated as 4-{1′-hydroxy-1′-mercapto-1′-[1″-2″(N→O)-isoquinolyl]}yl-1-benzoic acid. The structure of compound **1** was proved by EI-MS analysis.



Furthermore, the protective effect of compound **1** on a transient focal cerebral ischemia-reperfusion model in rats was investigated in this paper. The results of compound **1** against cerebral ischemic damage on rats are shown in Table 2. The results showed that compound **1** can reduce the neurological score and the moisture content of brain tissue of the rats significantly. It can also reduce the infarct volume of the rats. This indicated that compound **1** had a weak protective effect against cerebral ischemic damage.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined on a Yanaco micromelting point apparatus uncorrected. UV spectra were measured with a Shimadzu 265-FW spectrophotometer in MeOH solution. EI-MS and HR-ESI-MS were taken on a Varian MAT-212 mass spectrometer and Q-TOF micro mass spectrometer, respectively. The ^{1}H NMR, ^{13}C NMR, and 2D NMR were recorded on a Bruker DMX-500 NMR spectrometer using TMS as an internal standard. For column chromatography, silica gel (200–300 mesh, Qingdao Haiyang Chemical Group Co. Ltd., Qingdao, R. P. China) and Sephadex LH-20 (Pharmacia) were used. Male Sprague-Dawley (SD) rats (250–280 g, Experimental Animal Center of the Second Military Medical University) were subjected to transient focal cerebral ischemia.

Plant Materials. The petals of *Carthamus tinctorius* L. were obtained from Jimusaer County, Xinjiang, P. R. China in August, 2004 and authenticated by associate Professor Mei-li Guo, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, China.

Extraction and Isolation. The air-dried flower petals of *Carthamus tinctorius* L. (15 kg) were extracted with 50% ethanol (300 L) at room temperature after maceration 24 h. The combined EtOH extracts were evaporated under reduced pressure to yield a viscous residue. The latter was suspended in H_2O and extracted with petroleum ether, CH_2Cl_2, and *n*-BuOH, to give 45, 65, and 150 g, respectively. The *n*-BuOH fraction was subjected to column chromatography on silica gel eluted with a $CHCl_3$–MeOH solvent system (ratio of 15:1 to 1:1). Combining the fractions with TLC (GF_{254}) monitoring, we obtained nine fractions. Then, fraction 3 (3.2 g) was subjected to CC on silica gel eluted with $CHCl_3$–MeOH (ratio of 8:1) to give compound **1** (60 mg). Fraction 4 (12.4 g) was subjected to CC on silica gel eluted with $CHCl_3$–MeOH (from 7:1 to 6:1) to give five fractions: fr.4-1, fr.4-2, fr.4-3, fr.4-4, and fr.4-5. Fraction 4-2 was chromatographed on silica gel eluted with $CHCl_3$–MeOH (7:1) to give compound **5** (80 mg). Fraction 4-4 was purified by Sephadex LH-20 and eluted with 40% aqueous MeOH to give compound **4** (100 mg) and compound **2** (30 mg). Fraction 6 (8.6 g) was subjected to CC on silica gel and eluted with $CHCl_3$–MeOH (ratio of 4:1) to give three fractions: fr.6-1, fr.6-2, and fr.6-3. Fraction 6-2 was subjected to chromatography on Sephadex LH-20 and eluted with 40% aqueous MeOH to obtain compound **3** (30 mg) and compound **6** (50 mg). Fraction 9 (22.3 g) was subjected to chromatography on Sephadex LH-20 and eluted with water to give two fractions: fr.9-1 and fr.9-2. Fraction 9-1 was purified on Sephadex LH-20 and eluted with water repeatedly to give compound **7** (150 mg).

Compound 1, red powder, mp 125–127°C. FAB-MS, *m/z*: 327 $[M]^+$. EI-MS, m/z: 183 (40), 169 (39), 159 (100), 144 (17), 130 (49). UV (MeOH, λ_{max}, nm): 486. The ^{1}H NMR and ^{13}C NMR data are given in Table 1.

6-Hydroxykaempferol-3-*O*-*β*-D-glucopyranoside (2), $C_{21}H_{20}O_{12}$, yellow needle crystal (MeOH), mp 257–259°C. ESI-MS, *m/z*: 463 $[M-H]^+$, 302 $[M-Glc]^+$. ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 12.42 (OH), 8.04 (2H, d, J = 9, H-2′,6′), 6.89 (2H, d, J = 9, H-3′,5′), 6.55 (1H, s, H-8), 5.46 (1H, d, J = 8, H-1″), 3.0–5.0 (m). ^{13}C NMR (DMSO-d_6, δ, ppm): 177.53 (C-4), 159.73 (C-4′), 156.17 (C-2), 153.47 (C-7), 148.97 (C-9), 146.48 (C-5), 132.92 (C-3), 130.85 (C-6′,C-2′), 128.95 (C-6), 121.21 (C-1′), 115.05 (C-3′,C-5′), 104.38 (C-10), 101.02 (C-1″), 93.52 (C-8), 77.42 (C-5″), 76.43 (C-3″), 74.19 (C-2″), 69.90 (C-4″), 60.83 (C-6″).

Rutin (3), $C_{27}H_{30}O_{16}$, yellow powder, mp 213–215°C.^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 12.58, 10.86, 9.72 (OH), 7.54 (1H, d, H-6′), 7.51 (1H, s, H-2′), 6.83 (1H, d, H-5′), 6.38 (1H, d, H-8), 6.18 (1H, d, H-6), 5.33 (1H, d, J = 7.5, H-1″, Glc-1), 4.36 (1H, s, H-1″, Rha-1), 3.0–4.0 (m), 0.97 (3H, d, Rha-6). ^{13}C NMR (DMSO-d_6, δ, ppm): 177.4 (C-4), 164.1 (C-7), 161.2 (C-5), 156.6 (C-9), 156.4 (C-2), 148.3 (C-3′), 144.7 (C-4′), 133.3 (C-3), 121.6 (C-1′), 121.2 (C-6′), 116.3 (C-2′), 115.2 (C-5′), 103.9 (C-10), 101.2 (C-1-Glc), 100.7 (C-1-Rha), 98.6 (C-6), 93.6 (C-8), 76.4 (C-3-Glc), 75.9 (C-5-Glc), 74.0 (C-2-Glc), 71.8 (C-4-Rha), 70.5 (C-3-Rha), 70.3 (C-2-Rha), 69.9 (C-4-Glc), 68.2 (C-5-Rha), 67.0 (C-6-Glc), 17.7 (C-6-Rha).

Quercetin-3-*O*-*β*-D-glucopyranoside (4), $C_{21}H_{20}O_{12}$, yellow powder, mp 224–225°C. EI-MS, *m/z*: 302 $[M-Glc]^+$ (100), 286 (58), 137 (16), 57 (38). ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 12.63 (OH), 7.60 (H, s, H-6′), 7.58 (2H, d, J = 8, H-2′), 6.87 (2H, d, J = 9, H-5′), 6.44 (1H, s, H-8), 6.22 (1H, s, H-6), 5.47 (H, d, J = 7), 3.1–3.6 (m). ^{13}C NMR (DMSO-d_6, δ, ppm): 177.46 (C-4), 164.07 (C-7), 161.14 (C-5), 156.37 (C-2), 156.23 (C-9), 148.40 (C-4′), 144.77 (C-3′), 133.42 (C-3), 121.64 (C-6′), 121.20 (C-1′), 116.24 (C-5′), 115.18 (C-2′), 104.05 (C-10), 100.95 (C-1″), 98.65 (C-6), 93.56 (C-8), 77.53 (C-5″), 76.50 (C-3″), 74.08 (C-2″), 69.97 (C-4″), 60.96 (C-6″).

Kaempferol-3-*O*-*β*-D-glucopyranoside (5), $C_{21}H_{20}O_{11}$, yellow needle crystal (MeOH), mp 192–193°C. EI-MS, *m/z*: 286 $[M-Glc]^+$ (100), 121 (18). ^{1}H NMR (CD_3OD, δ, ppm, J/Hz): 12.63, 10.88, 10.19 (OH), 8.06 (2H, d, J = 9, H-2′,6′), 6.91 (2H, d, J = 9, H-3′,5′), 6.46 (1H, d, J = 3, H-8), 6.24 (1H, d, J = 2, H-6), 5.41 (1H, m, H-1″, Glc-1), 3.0–5.0 (m). ^{13}C NMR (CD_3OD, δ, ppm): 177.52 (C-4), 164.10 (C-7), 161.29 (C-5), 161.01 (C-4′), 159.96 (C-9), 156.39 (C-2), 133.33 (C-3), 130.94 (C-2′, C-6′), 121.00 (C-1′), 115.15 (C-3′, C-5′), 104.10 (C-10), 100.99 (C-1″), 98.71 (C-6), 93.73 (C-8), 77.49 (C-3″), 76.54 (C-5″), 74.23 (C-2″), 69.93 (C-4″), 60.87 (C-6″).

Cartormin (6), $C_{27}H_{29}NO_{13}$, yellow needle crystal (MeOH), mp > 230°C. ESI-MS, *m/z*: 1172 $[2M+Na]^+$, 598 $[M+Na]^+$, ^1H NMR (DMSO-d_6, δ, ppm, J/Hz): 17.87 (1H, s, OH-5), 11.64 (1H, s, -NH-), 10.03 (1H, s, OH-13), 7.63 (1H, d, J = 16, H-9), 7.55 (2H, d, J = 8, H-11, H-15), 7.35 (1H, d, J = 15, H-8), 6.83 (2H, d, J = 8, H-12, H-14), 6.35 (1H, s, H-16), 4.52 (1H, d, J = 8, H-18), 4.12 (1H, m, H-21), 4.08 (1H, m, H-20), 4.04 (1H, m, H-19), 3.61 (1H, m, H-21), 2.80–3.50 (7H, m). ^{13}C NMR (DMSO-d_6, δ, ppm): 196.01 (C-1), 185.61 (C-5), 180.30 (C-7), 159.83 (C-13), 142.09 (C-3), 141.10 (C-9), 134.91 (C-17), 130.49 (C-11, C-15), 126.16 (C-10), 118.73 (C-8), 115.84 (C-12, C-14), 114.70 (C-4), 109.11 (C-6), 103.19 (C-16), 84.02 (C-22), 79.44 (C-26), 78.38 (C-24), 78.13 (C-2), 76.58 (C-18), 75.84 (C-19), 72.74 (C-21), 70.41 (C-20), 69.19 (C-25), 69.04 (C-23), 60.63 (C-27).

Hydroxysafflor yellow A (7), $C_{27}H_{32}O_{16}$, yellow powder, mp 184–186°C.

Establishment of the Transient Focal Cerebral Ischemia-Reperfusion Model. Male Sprague-Dawley (SD) rats weighting 250–270 g were anesthetized with 17.5% chloral hydrate (350 mg/kg) intraperitoneally. The right common carotid artery, external carotid artery (ECA), and internal carotid artery (ICA) were isolated via a ventral midline incision. A 50-mm length of monofilament nylon suture (diameter 0.22–0.24 mm), with its tip rounded by heating near a flame, was introduced into the ECA lumen and advanced into the ICA (about 18–22 mm in depth) to block the origin of the MCA. Sham-operated animals were not exposed to I/R. After 2 h ischemia, the nylon suture was withdrawn to establish reperfusion [13]. Sham-operated rats were operated using the same surgical process but with no suture inserted. Rats were treated with saline, nimodipine 1.2 mg/kg, and compound **1** 10 mg/kg (i.v.) through the tail vein after 1 h ischemia.

Neurological Deficits and Quantification of Infarct Volume. Neurological examinations were performed 24 hours after reperfusion. The neurological findings were scored on a 5-point modified scale: (0) no neurological deficit; (1) failure to extend the left forepaw fully; (2) turning to left; (3) circling to left; (4) unable to walk spontaneously; (5) stroke-related death. Then, the rats were anesthetized with an overdose of chloral hydrate and decapitated. The brains were removed and sectioned coronally at 2 mm intervals. Sections were immersed in 2% 2,3,5-triphenyltetrazolium hydrochloride (TTC) in saline for 20 min at 37°C and then fixed for 30 min in 4% paraformaldehyde. Five sections per brain were analyzed for infarct size using a computerized image analysis system (smartscape 2002, China). The infarct area in each section was calculated by subtracting the residual uninfarcted, TTC-stained area of the ischemic (right) hemisphere from the total area of the nonischemic (left) hemisphere. The data were presented as mean±SE and analyzed with Student′s t test.

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