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Original Article

Flavonoids from *Capsella bursa-pastoris* and their hepatoprotective activities *in vitro*

Qinge Ma[a,*], Yongming Guo[a], Rongrui Wei[b,*], Zhipei Sang[a], Wenmin Liu[a], Li Gao[c], Taotao Liu[a]

[a] College of Chemistry and Pharmaceutical Engineering, Nanyang Normal University, Nanyang, Henan, China
[b] College of Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu, China
[c] Zhang Zhongjing College of Chinese Medicine, Nanyang Institute of Technology, Nanyang, Henan, China

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ABSTRACT

Two new flavonoids (**1** and **2**), named 4′,7-dihydroxy-5-hydroxymethyl-8-prenylflavonoid and 4′,7-dihydroxy-5-hydroxymethyl-6,8-diprenylflavonoid, together with seven known flavonoids (**3**–**9**) were isolated from the aerial parts of *Capsella bursa-pastoris* (L.) Medik., Brassicaceae, for the first time. The chemical structures of the purified compounds (**1**–**9**) were identified by their spectroscopic data and references. Moreover, compounds (**1**–**9**) were evaluated for their hepatoprotective activities against D-galactosamine induced toxicity in WB-F344 cells by using a MTT colorimetric method. As a result, compounds **2**, **3**, **6**, and **9** (10 μM) exhibited moderate hepatoprotective activities.

Introduction

Capsella bursa-pastoris (L.) Medik. is an annual or biennial herb belonging to the Brassicaceae family, and used as a popular vegetable in Chinese folk. Meanwhile, *C. bursa-pastoris* has always been served for a medicinal plant to threat conjunctivitis, vomit, metrorrhagia, and hydropsy (Wang et al., 2014). Diverse groups of biological activities are reported to be present in the different plant parts of *C. bursa-pastoris* which possessed anti-tumor (Kelko et al., 1976), anti-inflammatory (Yue et al., 2007), antioxidant (Zhang et al., 2008), anti-microbial (Yang et al., 2010), and anti-hypertensive (Huang et al., 2005) activities. Previous phytochemical investigations of *C. bursa-pastoris* exhibited the presence of amino acids (Xu et al., 2004a,b), flavonoids (Song et al., 2009), alkaloids (Pan, 2006), and essential oils (Gao and Zhou, 2009).

After consulting a large number of references, we found that there have few reports on hepatoprotective activities of *C. bursa-pastoris*, which prompted us to study its hepatoprotective effect. We carried out a bioassay-guided investigation of *C. bursa-pastoris* in order to evaluate its hepatoprotective activity. As a result, nine compounds (**1**–**9**), including two new compounds, named 4′,7-dihydroxy-5-hydroxymethyl-8-prenylflavonoid (**1**) and 4′,7-dihydroxy-5-hydroxymethyl-6,8-diprenylflavonoid (**2**), were isolated and identified from the active fractions (EtOAc fraction) of *C. bursa-pastoris*. The known compounds (**3**–**9**) were obtained from *C. bursa-pastoris* for the first time. All the compounds (**1**–**9**) were evaluated for their hepatoprotective activities, which were tested against D-galactosamine induced toxicity in WB-F344 cells by using the MTT colorimetric method (Ma et al., 2014).

Materials and methods

Plant material

Capsella bursa-pastoris (L.) Medik., Brassicaceae, was harvested from Nanyang, Henan province, China, in March 2014. This plant was identified by Dr. Su Zhang of Wuyang Weisen Biological Medicine Co., Ltd,. A voucher specimen (No. JC-201403) has been deposited in Nanyang Normal University.

Extraction and isolation

The dried aerial parts of *C. bursa-pastoris* (11 kg) were extracted with 70% EtOH (17 l ×3) three times, each time for 30 min. The extract was concentrated by rotary evaporator under reduced pressure resulting in a black extractum (1.1 kg). The combined extracts were successively partitioned with petroleum ether,

* Corresponding authors.
E-mails: maqinge2006@163.com (Q. Ma), weirongrui2011@163.co (R. Wei).

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EtOAc, and *n*-butanol to yield three parts: petroleum ether extract (108.5 g), EtOAc extract (237 g), and *n*-butanol extract (320.7 g). It was found that the EtOAc extract exhibited potential hepatoprotective activity according to bioassay-guided investigation. Therefore, the EtOAc part was subjected to column chromatography (silica gel, 100–200 mesh) and eluted with a solvent of petroleum ether/EtOAc (10:1, 6:1, 3:1, 1:1, 1:2) to obtain five fractions: A (28.4 g), B (33.6 g), C (48.1 g), D (40.4 g), and E (47.5 g), respectively.

The fraction B was fractionated on column chromatography (silica gel, 200–300 mesh) and eluted with petroleum ether/EtOAc (9:1 → 6:1 → 4:1, v/v) to obtain three sub-fractions: B-a, B-b, B-c, separately. The separation of B-b (9.05 g) was chromatographed on silica gel (100–200 mesh, 200–300 mesh) and Sephadex LH-20, repeatedly, yielded **3** (11.56 mg), **6** (9.68 mg), and **9** (10.21 mg). Similarly, the fraction C was applied to a silica gel CC and eluted with petroleum ether/EtOAc (5:1 → 3:1 → 1:1, v/v) to give three sub-fractions: C-a, C-b, C-c, separately. These sub-fractions were chromatographed over Sephadex LH-20 and silica gel CC (100–200, 200–300 mesh) eluting with suitable mobile phases, yielded **1** (8.05 mg), **2** (9.32 mg), **5** (11.36 mg), and **7** (13.20 mg). The fraction D was separately purified by MPLC (30–100% MeOH–H_2O), silica gel (200–300 mesh), and Sephadex LH-20 (MeOH in H_2O, 95%), and yielded **4** (9.55 mg) and **8** (12.25 mg).

4′,7-dihydroxy-5-hydroxymethyl-8-prenylflavonoid (1): pale yellow powder; mp 131.5–132.8 °C; UV (MeOH) λ_{max}: 206, 255, 288, and 337 nm; IR ν_{max} 3429.8, 2952.4, 1657.8, 1608.5, 1357.4, and 1004.2 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 1; HR-ESI-MS: *m/z* 375.6853 $[M+Na]^+$ (calcd. for $C_{21}H_{20}O_5Na$, 375.6851).

4′,7-dihydroxy-5-hydroxymethyl-6,8-diprenylflavonoid (2): pale yellow powder; mp 135.1–136.7 °C; UV (MeOH) λ_{max}: 206, 258, 286, and 335 nm; IR ν_{max} 3430.3, 2950.7, 1657.2, 1605.8, 1359.1, and 1003.6 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 1; HR-ESI-MS: *m/z* 443.1562 $[M+Na]^+$ (calcd for $C_{26}H_{28}O_5Na$ 443.1568).

General experimental procedures

The UV and IR spectra were measured by Australia GBC UV-916 spectrophotometer and Nicolet 5700 FT-IR spectrometer with KBr pellets, separately. The melting points were measured on WRX-4 microscopic melting point apparatus (Shanghai Suoguang Electric Technlogy Co., Ltd, China) which was uncorrected. The 1D & 2D NMR spectral data were run on Bruker-400 with TMS as internal standard (Ma et al., 2015). The HR-ESI-MS data were measured by Agilent 1100 series LC/MSD ion trap mass spectrometer. MPLC was carried out on a BUCHI Sepacore spectrometer with DAD detector (BUCHI Labortechnik AG, Switzerland) (Ma et al., 2013). Column chromatography was performed on silica gel (100–200, 200–300) mesh (Qingdao Yuminyuan Silica-Gel Reagent Factory, Qingdao, China), and Sephadex LH-20 (Amersham Pharmacia Biotech Co., Ltd., Tokyo, Japan).

Hepatoprotective assay

The hepatoprotective activities of compounds (**1**–**9**) were evaluated for using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay in WB-F344 rat hepatic epithelial stem-like cells according to the procedure described previously (Feng et al., 2013; Xu et al., 2004a,b). The WB-F344 cell lines were fostered with 3% fetal calf serum, penicillin (100 units/ml), and 100 units/ml streptomycin in 5% CO_2 at 37 °C in Dulbecco's modified eagle medium (DMEM). They were put the 96-well microplate and precultured for 24 h. The fresh medium (200 μl) containing bicyclol and test samples were added and the cells were cultured for 1 h (Hsiao et al., 2013). The cultured cells were measured for cytotoxic effects which exposed to 40 mM D-galactosamine for 24 h. The medium was replaced for the serum-free medium (0.5 mg/ml MTT) for 3.5 h incubation. After removing of the medium and adding DMSO (150 μl/well) into the microplate, the formazan crystals were redissolved. At last, the optical density (OD) was measured at 492 nm by a microplate

Table 1
^1H NMR (400 MHz, DMSO-d_6), ^{13}C NMR (100 MHz, DMSO-d_6) and key HMBC correlations of compounds **1**–**2**.

No.	1			2		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	–	–	–	–	–	–
2	–	164.5	–	–	164.3	–
3	6.75(s)	104.2	C-4	6.74(s)	104.3	C-4
4	–	180.2	–	–	180.4	–
4a	–	112.2	–	–	112.2	–
5	–	142.6	–	–	142.4	–
5-CH_2	4.58(s)	46.2	C-4a,C-6	4.60(s)	46.1	C-4a,C-6
6	6.78(s)	108.1	C-8	–	116.3	–
7	–	162.0	–	–	160.2	–
8	–	111.8	–	–	118.3	–
8a	–	158.6	–	–	158.1	–
1′	–	123.1	–	–	123.3	–
2′	7.85(d,8.5)	128.6	C-2,C-4′	7.84(d,8.5)	128.7	C-2,C-4′
3′	6.86(d,8.5)	115.7	–	6.86(d,8.5)	115.8	–
4′	–	157.5	–	-	157.7	–
5′	6.86(d,8.5)	115.7	–	6.86(d,8.5)	115.8	–
6′	7.85(d,8.5)	128.6	C-1,C-4′	7.84(d,8.5)	128.7	C-1,C-4′
1″	3.48(d,6.6)	22.8	C-8a, C-3‴	3.50(d,6.6)	22.7	C-7, C-3″
2″	5.20(t,6.6,3.0)	122.7	C-8	5.21(t,6.6,3.0)	122.8	–
3″	–	132.0	–	–	132.2	–
4″-CH_3	1.65(s)	25.1	–	1.66(s)	25.2	–
5″-CH_3	1.71(s)	18.6	–	1.73(s)	18.8	–
1‴	–	–	–	3.48(d,6.9)	22.9	C-8a, C-3‴
2‴	–	–	–	5.19(t,6.9,3.1)	123.0	C-8
3‴	–	–	–	–	133.0	–
4‴-CH_3	–	–	–	1.64(s)	25.1	–
5‴-CH_3	–	–	–	1.70(s)	18.7	–

reader. Therefore, the inhibition was calculated by the following formula (Li et al., 2006):

$$\text{Inhibition}\,(\%) = \left[\frac{(OD_{(sample)} - OD_{(control)})}{(OD_{(normal)} - OD_{(control)})}\right] \times 100$$

Results and discussion

Chemistry

Compound **1** was obtained as a pale yellow powder, and its molecular formula was deduced to be $C_{21}H_{20}O_5$ by HR-ESI-MS data at *m/z* 375.6853 $[M+Na]^+$ (calcd. for $C_{21}H_{20}O_5Na$, 375.6851), implying twelve degrees of unsaturation. Its UV spectrum showed the absorptions at λ_{max} 206, 255, 288, and 337 nm, which indicated the absorption peaks characteristic of the 8-prenylflavone skeleton (Hossain and Rahman, 2015). The IR absorptions indicated the existence of hydroxyl (3429.8 cm^{-1}), carbonyl (1657.8 cm^{-1}), aromatic ring (1608.5 cm^{-1}), methyl (1357.4 cm^{-1}) functional groups.

The ^1H NMR spectrum of compound **1** showed an AA′BB′ system at δ_H 7.85 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 6.86 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), and two singlet signals at δ_H 6.75 (1H, s, H-3), 6.78 (1H, s, H-6) in the aromatic region, combined with 15 aromatic carbons in the ^{13}C NMR spectrum implied the existence of the flavone structure (Table 1). A typical singlet δ_H 4.58 (2H, s, 5-CH_2) was observed in the high field of ^1H NMR spectrum, which indicated a hydroxymethyl group (Chen et al., 2015) was in compound **1**. Moreover, a group complex signals at δ_H 3.48 (2H, d, *J* = 6.6 Hz, H-1″), 5.20 (1H, t, *J* = 6.6, 3.0 Hz, H-2″), 1.65 (3H, s, 4″-CH_3), 1.71 (3H, s, 5″-CH_3) were shown in the ^1H NMR spectrum, which suggested the presence of a prenyl group in compound **1**. The structure of **1** was established on the HMBC correlations of H-3/C-4; 5-CH_2/C-4a, C-6; H-6/C-8; H-2′/C-2, C-4′; H-6′/C-2, C-4′; H-1″/C-8a, C-3″; H-2″/C-8 (Fig. 1) and the ^1H–^1H COSY correlations of H-2′/H-3′; H-5′/H-6′; H-1″/H-2″ (Fig. 1). Therefore, compound **1** was elucidated as 4′,7-dihydroxy-5-hydroxymethyl-8-prenylflavonoid.

Compound **2** was isolated as a pale yellow powder. Its molecular formula was determined to be $C_{26}H_{28}O_5$ by HR-ESI-MS: *m/z* 443.1562 $[M+Na]^+$ (calcd. for $C_{26}H_{28}O_5Na$ 443.1568). According to the spectral data of UV (MeOH) λ_{max}: 206, 258, 286, 335 nm and IR ν_{max} 3430.3, 2950.7, 1657.2, 1605.8, 1359.1, 1003.6 cm^{-1}, we concluded that compound **2** was an analog of compound **1** (Hossain and Rahman, 2015).

Compared with the ^1H NMR and ^{13}C NMR spectral data of compound **1**, it was concluded that the spectral data of compound **2** were closely comparable to those of compound **1** (Table 1). The only difference was the presence of two prenyl groups: δ_H 3.50 (2H, d, *J* = 6.6 Hz, H-1″), 5.21 (1H, t, *J* = 6.6, 3.0 Hz, H-2″), 1.66 (3H, s, 4″-CH_3), 1.73 (3H, s, 5″-CH_3); δ_H 3.48 (2H, d, *J* = 6.9 Hz, H-1‴), 5.19 (1H, t, *J* = 6.9, 3.1 Hz, H-2‴), 1.64 (3H, s, 4‴-CH_3), 1.70 (3H, s, 5‴-CH_3)

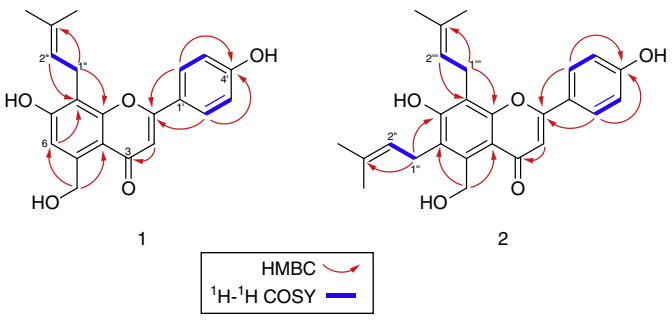

Fig. 1. Key HMBC (H → C) and ^1H–^1H COSY correlations of compounds **1**–**2**.

Table 2
Hepatoprotective effects of selective compounds against D-galactosamine-induced toxicity in WB-F344 cells.[a]

Compound	Cell survival rate (% of normal)	Inhibition (% of control)
Normal	100.0 ± 7.3	–
Control	32.2 ± 2.1	–
Bicyclol[b]	53.4 ± 6.5[c]	31.3
2	63.7 ± 8.2[c]	46.5
3	49.9 ± 4.6[d]	26.1
6	51.9 ± 4.1[d]	29.1
9	61.2 ± 5.7[c]	42.8

[a] Results were expressed as means ± SD (*n* = 3; for normal and control, *n* = 6).
[b] Positive control substance.
[c] $p < 0.05$, significantly different from control by Student's *t*-test.
[d] $p < 0.01$, significantly different from control by Student's *t*-test.

in compound **2**. Some important correlations of H-3/C-4; 5-CH_2/C-4a, C-6; H-2′/C-2, C-4′; H-6′/C-2, C-4′; H-1″/C-7, C-3″; H-1‴/C-8a, C-3‴; H-2‴/C-8 in the HMBC spectrum and correlations of H-2′/H-3′; H-5′/H-6′; H-1″/H-2″; H-1‴/H-2‴ in the ^1H–^1H COSY spectrum were seen in Fig. 1. Therefore, compound **2** was determined as 4′,7-dihydroxy-5-hydroxymethyl-6,8-diprenylflavonoid.

In addition, other seven known compounds (**3**–**9**) were identified by comparison of their spectroscopic data with those reported in the literature. Their structures were determined as chrysoeriol-7-*O*-β-D-glucopyranoside (**3**) (Zhang et al., 2005), acacetin-7-*O*-β-D-glucopyranoside (**4**) (Zhang et al., 2005), quercetin (**5**) (Shabrawy et al., 2014), sinensetin (**6**) (Hossain and Rahman, 2015), licoflavonol (**7**) (Kwon et al., 2010), icaritin (**8**) (Gao et al., 2013), and 6,8-diprenylgalangin (**9**) (Jain and Zutshi, 1973). Furthermore, the known compounds (**3**–**9**) were isolated from this plant for the first time.

Hepatoprotective activities

Compounds (**1**–**9**) were evaluated for their hepatoprotective activities against D-galactosamine induced WB-F344 cell damage with the bicyclol (hepatoprotective activity drug) as the positive drug (Ma et al., 2014). The screening results of hepatoprotective activities were exhibited in Table 2, the inhibition (%) of compounds **2**, **3**, **6**, and **9** based on the computing formula with values of 46.5, 26.1, 29.1, and 42.8, respectively. However, compounds **1**, **4**, **5**, **7**, and **8** exhibited no hepatoprotective activities which determined by the screening results of bioactivities. We concluded that the active compounds contained 2-prenyl, 4-prenyl, and 5′-OCH_3 played positive roles in the mediating their hepatoprotective activities. The study of structure–activity relationship of the active compounds from *C. bursa-pastoris* which needed further research. Furthermore, all the values of the active compounds were expressed as means ± SD of three experiments. The significance of unpaired observations between normal or control and tested samples was determined by Student's *t*-test (Li et al., 2006). Differences were considered significant at $p < 0.05$ and $p < 0.01$ (Lin et al., 2011). Therefore, compounds **2**, **3**, **6**, and **9** (10 μM) exhibited moderate hepatoprotective activities (Table 2).

Conclusion

In this work, nine flavonoids (**1**–**9**) were isolated and identified by their spectral data and references from *C. bursa-pastoris*. Among then, compound (**1** and **2**) were new 5-hydroxymethyl flavonoids, and the known compounds (**3**–**9**) were obtained from this plant for the first time. Moreover, all the compounds (**1**–**9**) were evaluated for their hepatoprotective activities against D-galactosamine induced WB-F344 cell damage with the bicyclol as the positive

control substance. As a result, compounds **2**, **3**, **6**, and **9** (10 μM) exhibited moderate hepatoprotective activities.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Author's contribution

QGM, YMG, and ZPS contributed in carrying out the extraction and isolation of pure compounds, and participated in drafting the manuscript. WML, LG, and TTL contributed to chromatographic analysis, designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. RRW carried out the hepatoprotective activity measurement. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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