Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Hepatoprotective phenylethanoid glycosides from Cirsium setosum

Qinge Ma, Yongming Guo, Baomin Luo, Wenmin Liu, Rongrui Wei, Chunxia Yang, Chenghua Ding, Xuefeng Xu & Minghui He

To cite this article: Qinge Ma, Yongming Guo, Baomin Luo, Wenmin Liu, Rongrui Wei, Chunxia Yang, Chenghua Ding, Xuefeng Xu & Minghui He (2015): Hepatoprotective phenylethanoid glycosides from Cirsium setosum, Natural Product Research, DOI: 10.1080/14786419.2015.1084304

To link to this article: http://dx.doi.org/10.1080/14786419.2015.1084304

NATURAL PRODUCT RESEARCH, 2015
http://dx.doi.org/10.1080/14786419.2015.1084304

Taylor & Francis
Taylor & Francis Group

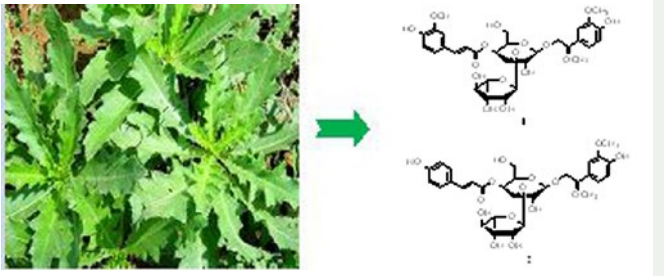
Hepatoprotective phenylethanoid glycosides from *Cirsium setosum*

Qinge Ma[a], Yongming Guo[a], Baomin Luo[a], Wenmin Liu[a], Rongrui Wei[b], Chunxia Yang[a], Chenghua Ding[a], Xuefeng Xu[a] and Minghui He[a]

[a]Department of Graduate Students, College of Chemistry and Pharmaceutical Engineering, Nanyang Normal University, Nanyang, China; [b]Department of Pharmacology, College of Pharmacy, China Pharmaceutical University, Nanjing, China

ABSTRACT

Two new phenylethanoid glycosides, namely β-D-glucopyranoside, 1″-*O*-(7*S*)-7-(3-methoxyl-4-hydroxyphenyl)-7-methoxyethyl-3″-α-L-rhamnopyranosyl-4″-[(8*E*)-7-(3-methoxyl-4-hydroxyphenyl)-8-propenoate] (**1**) and β-D-glucopyranoside, 1″-*O*-(7*S*)-7-(3-methoxyl-4-hydroxyphenyl)-7-methoxyethyl-3″-α-L-rhamnopyranosyl-4″-[(8*E*)-7-(4-hydroxyphenyl)-8-propenoate] (**2**), together with six phenylethanoid glycosides were isolated from *Cirsium setosum*. Their structures were elucidated by their spectroscopic data and references. Compounds **2**, **4**, **5**, **7** and **8** (10 μM) exhibited moderate hepatoprotective activities. Compounds (**3–8**) were obtained from this plant for the first time.



ARTICLE HISTORY
Received 20 May 2015
Accepted 28 July 2015

KEYWORDS
Cirsium setosum; phenylethanoid glycoside; hepatoprotective activity

1. Introduction

Cirsium setosum, belonging to the Asteraceae family, is well known as the name of 'Ci-Er-Cai'. It is widely distributed in China and has been used for thousands of years to treat haematuria, spitting of blood and uterine bleeding (Jiang et al. 2013). Previous phytochemical studies have revealed the presence of triterpenes (Li et al. 2012), lignans (Meng et al. 2009), flavonoids (Zhu & Zhang 2007), volatile oils (Małgorzata, Marek et al. 2015) and steroids (Sun et al. 2012). Moreover,

CONTACT Qinge Ma maqinge2006@163.com
The supplemental data for this article is available online at http://dx.doi.org/10.1080/14786419.2015.1084304.

C. setosum exhibit various pharmacological activities such as anti-inflammatory (Yang et al. 2006), antibacterial (Małgorzata, Anna et al. 2015) and sedative (Jiang et al. 2013) activities.

To the best of our knowledge, there have been no reports on hepatoprotective activities of *C. setosum*, which prompted us to investigate its further pharmacological potentials. During our systematic investigation on its chemical constituents, eight compounds (Figure S1) were isolated and identified from this plant. Among them, compounds **1** and **2** are two new phenylethanoid glycosides, and compounds (**3–8**) are known phenylethanoid glycosides isolated from *C. setosum* for the first time. Their structures were elucidated on the basis of the spectroscopic analysis and the reported references. Moreover, we evaluated the hepatoprotective activities of compounds (**1–8**), which were tested against D-galactosamine induced toxicity in HL-7702 cells using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method (Ma et al. 2013). Compounds **2**, **4**, **5**, **7** and **8** (10 μM) exhibited moderate hepatoprotective activities.

2. Results and discussion

2.1. Structure elucidation

Compound **1** was obtained as a yellow solid, with mp 154.5–155.8 °C, $[\alpha]^{22}_{D}$ −4.5° (c 0.80, MeOH), its UV (MeOH) spectrum showed absorbance at λ_{max}: 208, 288 and 330 nm. The molecular formula of compound **1** was determined as $C_{32}H_{42}O_{16}$ by HR-ESI-MS showed $[M+Na]^+$ at *m/z* 705.3581 (Calcd for $C_{32}H_{42}O_{16}Na$ 705.3587), indicating 12° of unsaturation. The IR spectrum showed the presence of hydroxyl (3405.5 cm^{-1}), ester carbonyl (1742.0, 1710.4 cm^{-1}) and methyl (1385.7 cm^{-1}) functionalities.

The ^{1}H NMR spectrum showed the presence of two ABX systems at δ_H 6.79 (1H, d, *J* = 1.5 Hz, H-2), 6.68 (1H, d, *J* = 8.0, Hz, H-5), 6.63 (1H, dd, *J* = 8.0, 1.5 Hz, H-6) and 7.16 (1H, d, *J* = 1.5 Hz, H-2′), 6.80 (1H, d, *J* = 8.0, Hz, H-5′), 7.02 (1H, dd, *J* = 8.0, 1.5 Hz, H-6′) (Table S1) in the aromatic field, which revealed two 1,3,4-trisubstituted benzene rings in compound **1**. Moreover, an AB system at δ_H 7.64 (1H, d, *J* = 15.8 Hz, H-7′), 6.39 (1H, d, *J* = 15.8 Hz, H-8′) showed in the ^{1}H NMR spectrum, indicated a trans-double bond in compound **1**. Therefore, we concluded that the caffeoyl fragment (Nan et al. 2013) was contained in compound **1**. In the middle field of ^{1}H NMR spectrum, there were two typical signals at δ_H 4.46 (1H, d, *J* = 7.5 Hz, H-7), 3.95 (2H, m, H-8), corresponding to δ_C 83.4 (C-7), 72.9 (C-8) of the ^{13}C NMR and HSQC spectra, which indicated the segment of $-CH_2-CHR-$ was in compound **1**. The data of ^{13}C NMR spectrum at δ_C 130.9 (C-1), 113.6 (C-2), 148.7 (C-3), 147.1 (C-4), 116.7 (C-5), 122.2 (C-6), 83.4 (C-7), 72.9 (C-8) (Table S1) revealed the fragment of 3-methoxyl-4-hydroxyphenyl phenylethyl alcohol in compound **1**. Additionally, three typical methoxyl signals were observed at δ_H 3.86(s, 6H), 3.23(s, 3H) and δ_C 56.8, 56.5 in the ^{1}H NMR and ^{13}C NMR spectra (Table S1).

The ^{13}C NMR spectrum showed at δ_C 103.8 (C-1″), 101.8 (C-1″), 62.6 (C-6″), 18.1 (C-6″) corresponding to the ^{1}H NMR spectrum displayed at δ_H 4.56 (1H, d, *J* = 7.8 Hz, H-1″), 5.01 (1H, d, *J* = 1.5 Hz, H-1″) and 1.24 (3H, d, *J* = 6.2 Hz, H-6″) which indicated the presence of the glucose and the rhamnose in compound **1** (Table S1). The other correlating data of compound **1** were confirmed by the experiment of HSQC, ^{1}H-^{1}H COSY and DEPT spectra. The planar structure of compound **1** was established on the basis of the correlations of H-6/C-4; H-7/C-2, C-6; H-6′/C-4′; H-7′/C-6′,C-9′; H-1″/C-8; H-4″/C-9′; H-6″/C-5″; H-1‴/C-3″ in the HMBC spectrum (Figure S2), and relative configuration was determined by the correlations of H-2/3-OCH_3, H-2′/3′-OCH_3, H-1″/7-OCH_3, H-5″/H-6″ in the NOESY spectrum (Figure S2). We measured the optical rotation of compound **1** with value $\alpha = -3.6°$, and its $[\alpha]^{22}_{D} = -4.5°$,

$[M]^{22}_{D=}$ −30.3°, $\Delta[M]^{22}_{D}$ = +89.5° (Yin & Liu 1997). The $\Delta[M]^{22}_{D}$ value of compound **1** was nearly equal to $\Delta[M]^{22}_{D}$ = +89.7° of *S*-suspensaside methyl ether (Guo et al. 2007). Therefore, the C-7 configuration of compound **1** was identified as 7*S*. Based on the above spectral evidences, the chemical structure of compound **1** was determined to be β-D-glucopyranoside, 1″-*O*-(7*S*)-7-(3-methoxyl-4-hydroxyphenyl)-7-methoxyethyl-3″-α-L-rhamnopyranosyl-4″-[(8*E*)-7-(3-methoxyl-4-hydroxyphenyl)-8-propenoate].

Compound **2** was isolated as a yellow solid, with mp 152.7–153.1 °C, $[\alpha]^{22}_{D}$ −4.7° (c 0.80, MeOH), its molecular formula was established as $C_{31}H_{40}O_{15}$ based on HR-ESI-MS at *m/z* 675.5341 $[M + Na]^+$ (Calcd for $C_{31}H_{40}O_{15}Na$, 675.5348), indicating 12° of unsaturation. Its UV (MeOH) spectrum showed absorbance at λ_{max}: 207, 288, 331 nm and IR spectrum indicated the presence of hydroxyl (3398.7 cm^{-1}), ester carbonyl (1743.4, 1711.8 cm^{-1}) and methyl (1388.0 cm^{-1}) functionalities. According to the data of HR-ESI-MS, UV and IR spectra, compound **2** was concluded to be an analogue (Nan et al. 2013) of compound **1**.

In the ^{1}H NMR spectrum, an AA′BB′ system at δ_H 7.40 (2H, d, *J* = 8.0 Hz, H-2′,6′), 6.78 (2H, d, *J* = 8.0 Hz, H-3′,5′) and an ABX system at δ_H 6.81 (1H, d, *J* = 1.5 Hz, H-2), 6.67 (1H, d, *J* = 8.0 Hz, H-5), 6.64 (1H, d, *J* = 8.0, 1.5 Hz, H-6) (Table S1) were observed in the aromatic proton field. All the fragments of compound **2** were connected by the experiments of DEPT, HSQC, HMBC and ^{1}H-^{1}H COSY spectra shown in Figure S2. In a similar way, the optical rotation of compound **2** was measured with value α = −3.8°, and its $[\alpha]^{22}_{D}$ = −4.7°, $[M]^{22}_{D=}$ −30.6°, $\Delta[M]^{22}_{D}$ = + 89.6° (Yin & Liu 1997). The $\Delta[M]^{22}_{D}$ value of compound **2** was nearly equal to $\Delta[M]^{22}_{D}$ = +89.7° of *S*-suspensaside methyl ether (Guo et al. 2007). Therefore, the C-7 configuration of compound **2** was identified as 7*S*. Consequently, compound **2** was determined as β-D-glucopyranoside, 1″-*O*-(7*S*)-7-(3-methoxyl-4-hydroxyphenyl)-7-methoxyethyl-3″-α-L-rhamnopyranosyl-4″-[(8*E*)-7-(4-hydroxyphenyl)-8-propenoate].

Additionally, other six known phenylethanoid glycosides were isolated and identified as cistanoside D (**3**) (Gross & Sticher 1987), acteoside (**4**) (Gross & Sticher 1987), β-D-glucopyranoside, 1″-*O*-7-(4-hydroxyphenyl)-7-ethyl-6″-[(8*E*)-7-(3,4-dihydroxyphenyl)-8-propenoate] (**5**) (Hiroko et al. 1987), calceolarioside B, (**6**) (Ersöz et al. 2002), dunalianoside C (**7**) (Zhao et al. 2008) and dunalianoside D (**8**) (Zhao et al. 2008) by comparison of their physical and spectroscopic data with those reported in the references.

2.2. Hepatoprotective activity

Compounds (**1–8**) were evaluated for their hepatoprotective activities against D-galactosamine-induced HL-7702 cell damage. As a result, compounds **2**, **4**, **5**, **7** and **8** (10 μM) showed moderate hepatoprotective activities (Table S2).

3. Experimental

3.1. General procedures

The melting points were recorded on a XT5B microscopic melting point apparatus (Beijing Tech electro-optical instrument factory, China) which were uncorrected. The optical rotations were measured on a Perkin-Elmer 241 polarimeter at 20 °C. The UV spectra were measured with an Australia GBC UV-916 spectrophotometer, and a Nicolet 5700 FT-IR spectrometer was used for scanning IR spectroscopy using KBr pellets. HR-ESI-MS data were measured using a *Q*-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer. 1D and 2D NMR spectra were recorded on

Bruker-400 with TMS as internal standard. Reversed-phase HPLC was performed using Agilent 1200 series with a DIKMA (4.6 × 250 mm) analytical column packed with C18 (5 μm). Column chromatography was performed on Sephadex LH-20 (Amersham Pharmacia, Sweden), silica gel H, 100–200 mesh and 200–300 mesh (Qingdao Marine Chemical Inc., Qingdao, China). TLC was performed on precoated silica gel GF254 plates, and the spots were visualised under UV light (254 or 356 nm) or by spraying with 10% H_2SO_4 in 95% EtOH followed by heating.

3.2. Plant material

The whole plant of *C. setosum* was collected from Xixia Country, Henan Province, China in October 2013. A voucher specimen (NO. NNU-201310) has been deposited in Nanyang Normal University. The plant material was dried, finely powdered and used for the successive extraction.

3.3. Extraction and isolation

The whole air-dried and powdered plant of *C. setosum* (10.0 kg) was extracted three times with 95% EtOH (15 L × 3) heating under reflux to give 1.2 kg of crude extract (Almeida et al. 2011). The combined extracts were successively partitioned with petroleum ether, ethyl acetate and *n*-butanol to yield three fractions: petroleum ether soluble fraction (104.5 g), ethyl acetate soluble fraction (188.4 g) and n-butanol soluble fraction (268.2 g). According to the results of bioassay-guided investigation of *C. setosum*, we found that the *n*-butanol soluble fraction showed potential hepatoprotective activity.

The *n*-butanol soluble fraction was performed on the macroporous adsorbent resin (Diaion-101) column, which was eluted with 20, 40, 60 and 95% ethanol to obtain four fractions: A (34.7 g), B (78.6 g), C (27.4 g) and D (13.5 g). The A part was purified on a Sephadex LH-20 column with a gradient system of MeOH/H_2O to give three sub-fractions: A–a, A–b, A–c. The separation of A–b (9.6 g) by silica gel (100–200 mesh or 200–300 mesh), repeatedly, yielded **4** (12.30 mg), **5** (11.25 mg) and **7** (13.20 mg). The A–c part was repeatedly chromatographed over Sephadex LH-20 and silica gel, successively, yielded **1** (8.52 mg), **2** (9.06 mg) and **3** (11.65 mg). Similarly, The B part was chromatographed over Sephadex LH-20 and silica gel (100–200 mesh or 200–300 mesh) eluting with different mobile phases, yielded **6** (11.20 mg) and **8** (10.66 mg). The structures of these compounds (**1–8**) are shown in Figure S1.

3.4. Acid hydrolysis of compounds 1–8

Compounds **1–8** (5 mg each) were treated in 5% HCl (0.5 mL) and heated at 90 °C for 2 h, respectively (Chang & Case 2005). After cooling, each reaction mixture was extracted with EtOAc, and the aqueous layer was neutralised with 0.1 M NaOH. As a result, glucose and rhamnose were obtained from compound (**1–4**), which were detected by thin layer chromatography (TLC) with authentic sugars. Similarly, we obtained glucose from compounds (**5–8**). The type of glucose and rhamnose were identified by TLC method with authentic sugars (Ma et al. 2014).

3.5. Hepatoprotective assay

The compounds (**1–8**) were evaluated for their hepatoprotective activities against D-galactosamine-induced toxicity in HL-7702 cells using a MTT colorimetric method. The HL-7702 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 3%

foetal calf serum, 100 units/mL penicillin and 100 units/mL streptomycin in 5% CO_2 and incubated at 37 °C, which were placed in a 96-well microplate and precultured for 24 h (Hsiao et al. 2013). The cultured cells were measured for cytotoxic effects which exposed to 40 mM D-galactosamine after 24 h. At last, the medium was replaced for the serum-free medium (0.5 mg/mL MTT) for 3.5-h incubation. The medium was removed and added DMSO (150 µL/well) into the microplate, and the formazan crystals were redissolved. The optical density (OD) was measured by a microplate reader at a wavelength of 492 nm, and the inhibition was calculated as inhibition (%) = $[(OD_{(sample)} - OD_{(control)})/(OD_{(normal)} - OD_{(control)})] \times 100$ (Liu et al. 2012).

3.6. Statistical analysis

We had evaluated the pharmacological activities of compounds (**1–8)** with the bicyclol (hepatoprotective activity drug) as the positive control for their hepatoprotective activities against D-galactosamine-induced toxicity in HL-7702 cells. The results of hepatoprotective activities were given in Table S2. The percentage of inhibition of compounds **2**, **4**, **5**, **7** and **8** (10 µM) were calculated using the following formula: inhibition (%) = $[(OD_{(sample)} - OD_{(control)})/(OD_{(normal)} - OD_{(control)})] \times 100$, respectively. Moreover, all the values 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 (Ma et al. 2014). The differences were considered significant at $p < 0.05$. Consequently, compounds **2**, **4**, **5**, **7** and **8** (10 µM) showed moderate hepatoprotective activities.

4. Conclusion

In this work, eight phenylethanoid glycosides (**1–8**) were isolated and identified from *C. setosum*. Compounds **1** and **2** were new phenylethanoid glycosides, compounds (**3–8**) were obtained from this plant for the first time. Among them, compounds **2**, **4**, **5**, **7** and **8** (10 µM) exhibited moderate hepatoprotective activities.

Acknowledgements

We express our appreciation to the Department of Instrumental Analysis of Nanyang Normal University for the UV, IR, NMR and MS. This work was supported by the Key Scientific Research Project of Colleges and Universities in Henan Province (NO.15A350009), the Special Project of Nanyang Normal University (NO.ZX2014044), the Graduate Students' Case Teaching Course Construction Project of Nanyang Normal University (NO.ALJX201502), and the Ideological and Political Education Project of Nanyang Normal University (NO.SZ2014015).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Key Scientific Research Project of Colleges and Universities in Henan Province [grant number 15A350009]; Special Project of Nanyang Normal University [grant number ZX2014044]; Graduate Students' Case Teaching Course Construction Project of Nanyang Normal University [grant number ALJX201502]; Ideological and Political Education Project of Nanyang Normal University [grant number SZ2014015].

Supplementary material

Experimental details relating to this article are available online, alongside Figures S1–S2 and Tables S1–S2.

References

Almeida JRGS, de Lima JT, de Oliveira HR, de Oliveira MR, Meira PRM, Lúcio ASSC, Barbosa Filho JMB, Quintans Júnior LJQ. 2011. Antinociceptive activity of discretamine isolated from *Duguetia moricandiana*. Nat Prod Res. 25:1908–1915.

Chang J, Case R. 2005. Phenolic glycosides and ionone glycoside from the stem of *Sargentodoxa cuneata*. Phytochemistry. 66:2752–2758.

Ersöz T, Tašdemï D, Çališ Ï. 2002. Phenylpropanoid glucosides from *Scutellaria galericulata*. Turkish J Chem. 26:465–471.

Gross GA, Sticher O. 1987. Phenylpropanoid glycosides isolated from *Scrophularia scopolii*. Phytochemistry. 26:2057–2061.

Guo H, Liu AH, Li L, Guo DA. 2007. Simultaneous determination of 12 major constituents in *Forsythia suspensa* by high performance liquid chromatography-DAD method. J Pharm Biomed Anal. 43:1000–1006.

Hiroko S, Yutaka S, Tokuo A. 1987. Cyanogenic and phenylpropanoid glucosides from *Prunus grayana*. Phytochemistry. 26:2363–2366.

Hsiao PC, Liaw CC, Hwang SY, Cheng HL, Zhang LJ, Shen CC, Hsu FL, Kuo YH. 2013. Antiproliferative and hypoglycemic cucurbitane-type glycosides from the fruits of *Momordica charantia*. J Agric Food Chem. 61:2979–2986.

Jiang H, Meng YH, Yang L, Wang QH, Yang BY, Liu C, Kuang HX. 2013. A new megastigmane glycoside from the aerial parts of *Cirsium setosum*. Chin J Nat Med. 11:534–537.

Li LL, Sun Z, Shang XY, Li JJ, Wang R, Zhu J. 2012. Triterpene compounds from *Cirsium setosum*. Chin J Chin Mater Med. 37:951–955.

Liu YF, Liang D, Luo H, Hao ZY, Wang Y, Zhang CL, Zhang QJ, Chen RY, Yu DQ. 2012. Hepatoprotective iridoid glycosides from the roots of *Rehmannia glutinosa*. J Nat Prod. 75:1625–1631.

Małgorzata K, Anna B, Anna M, Marcin C. 2015. Chemical compositions and antibacterial activity of extracts obtained from the inflorescences of *Cirsium canum* (L.) all. Nat Prod Res. 29:2059–2063. doi:10.1080/14786419.2015.1030341

Małgorzata K, Marek M, Joanna K. 2015. Chemical composition and variability of the volatile components from inflorescences of *Cirsium* species. Nat Prod Res. 29:1942–1944. doi:10.1080/14786419.2015.1010165

Ma QG, Tian J, Yang JB, Wang AG, Ji TF, Wang YG, Su YL. 2013. Bioactive carbazole alkaloids from *Murraya koenigii* (L.) Spreng. Fitoterapia. 87:1–6.

Ma QG, Wang YG, Liu WM, Wei RR, Yang JB, Wang AG, Ji TF, Tian J, Su YL. 2014. Hepatoprotective sesquiterpenes and rutinosides from *Murraya koenigii* (L.) Spreng. J Agric Food Chem. 62:4145–4151.

Meng YH, Wang QH, Jiang H, Yang BY, Kuang HX. 2009. Study on chemical constituents of *Herba cirsii*. Zhong Yao Cai. 32:58–61.

Nan ZD, Zeng KW, Shi SP, Zhao MB, Jiang Y, Tu PF. 2013. Phenylethanoid glycosides with anti-inflammatory activities from the stems of *Cistanche deserticola* cultured in Tarim desert. Fitoterapia. 89:167–174.

Sun Z, Li LL, Yuan ZZ, Wang AL, Li JJ, Shang XY. 2012. Isolation, purification and structural elucidation of steroids from *Cirsium setosum* (wild.) MB. Food Sci. 33:124–127.

Yang XH, Cui JH, Ding AW. 2006. Impact of *Herba cirsii* extracts on hemorrhage, blood coagulation and experimental inflammation in rats. J Tradit Chin Med. 24:17–19.

Yin YY, Liu CY. 1997. The structure and calculation of rotation of monosaccharide. Chem Res App. 9:629–636.

Zhao P, Tanaka T, Hirabayashi K, Zhang YJ, Yang CR, Kouno I. 2008. Caffeoyl arbutin and related compounds from the buds of *Vaccinium dunalianum*. Phytochemistry. 69:3087–3094.

Zhu D, Zhang JW. 2007. Study on extraction technology of flavonoids in *Herba cirsii*. J Anhui Agric Sci. 35:1262–1263.