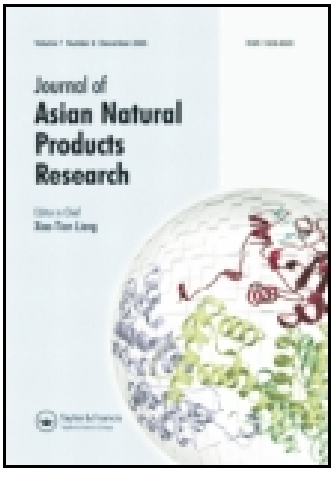

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Two new glycosides from Carduus acanthoides

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Two new glycosides from *Carduus acanthoides*

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Two new glycosides, syringic acid-4-*O*-β-L-arabinopyranoside (**1**) and kaempferol-3-*O*-α-L-rhamnopyranosyl-7-*O*-β-D-glucuronopyranoside (**2**), were isolated from whole plants of *Carduus acanthoides* (Asteraceae), and their structures were elucidated on the basis of spectroscopic analysis.

Keywords: *Carduus acanthoides*; flavonol glycoside; phenolic glycoside; structural elucidation

1. Introduction

The genus *Carduus*, which belongs to the Asteraceae family, comprises about 95 species all over the world, among which three species are widely distributed in China [1]. The whole plants or roots of *Carduus acanthoides* were used in China for the treatment of rheumatism, urethritis, chyluria, hematemesis, epistaxis, hematuria, menorrhagia, furuncle, scald, and edema [2,3]. Recent pharmacological studies demonstrated that ethanol extracts of *Carduus* plants had various activities such as anticancer [4], hepatoprotection [5–7], antioxidation [8], bacteriostasis [9], etc. Chemical studies on flavonoids [10,11], lignans [10], coumarins [12], and alkaloids [13,14] have been reported from the plants of the genus *Carduus*. This paper deals with the isolation and structural elucidation of two new glycosides, syringic acid-4-*O*-β-L-arabinopyranoside (**1**) and kaempferol-3-*O*-α-L-rhamnopyranosyl-7-*O*-β-D-glucuronopyranoside (**2**).

2. Results and discussion

Compound **1** was isolated as a white needle crystal (MeOH). The molecular formula of **1** was determined to be $C_{14}H_{18}O_9$ on the basis of quasi-molecular ion peaks at *m/z* 329.0869 $[M - H]^-$, 353.0844 $[M + Na]^+$ in the negative and positive HR-ESI-MS. The ^{1}H NMR spectrum showed the presence of two aromatic proton signals at δ_H 7.35 (2H, s) as an A_2 spin system and two methoxyl signals at δ_H 3.89 (6H, s). In addition, six proton signals were found in the range of δ_H 3.30–5.40, which suggested the existence of a sugar moiety. The signal at δ_H 5.40 (1H, d, $J = 4$ Hz) was assigned as the anomeric proton. The ^{13}C NMR and DEPT spectra clearly exhibited 14 carbon signals, including a carboxyl group (δ_C 169.4), four sp^2 quaternary carbons (δ_C 154 × 2, 138.6, 128), two sp^2 methines (δ_C 108.2 × 2), two methoxyls (δ_C 56.8 × 2), and a pentosyl residue (δ_C 102.7, 73.0, 71.7, 66.6, 63.5). Except for the pentosyl residue, the signals observed above were quite similar to those of the aglycone moiety of syringic acid-4-*O*-α-L-rhamnopyranoside [15], which suggested that the aglycone moiety of **1** was syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid). The sugar was identified as L-arabinose by gas chromatography (GC)

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analysis, in which the retention times of trimethylsilyl ether derivatives of the sugar residue and authentic sugars were compared, as described in Section 3. According to the coupling constant of the anomeric proton at δ_H 5.40 (1H, d, $J = 4$ Hz), the arabinosyl unit was determined to be β configuration. In HMBC spectrum, the correlation of the anomeric proton signal at δ_H 5.40 (H-1′) with the carbon signal at δ_C 138.6 (C-4) revealed that the arabinopyranose was attached to C-4. The ^{1}H and ^{13}C NMR data were completely assigned on the basis of the analysis of ^{1}H–^{1}H COSY, HSQC, and HMBC spectra. Thus, the structure of **1** was characterized as syringic acid-4-*O*-β-L-arabinopyranoside (Figure 1).

Compound **2** was isolated as a yellow amorphous powder (MeOH). The molecular formula of **2** was determined to be $C_{27}H_{28}O_{16}$ on the basis of quasi-molecular ion peaks at *m/z* 607.1296 $[M - H]^-$ and 609.1461 $[M + H]^+$ in the negative and positive HR-ESI-MS. The UV spectrum with absorption maxima at 261 and 340 nm indicated the presence of flavone. The ^{1}H NMR spectrum displayed six aromatic proton signals, of which four aromatic proton signals at δ_H 7.77 (2H, d, $J = 8.5$ Hz) and 6.93 (2H, d, $J = 8.5$ Hz) belong to H-2′, 6′ and H-3′, 5′ as an AA′BB′ spin system, and the other two proton signals at δ_H 6.78 (1H, d, $J = 1.5$ Hz) and 6.47 (1H, d, $J = 1.5$ Hz) belong to the *meta*-coupled aromatic protons H-6 and H-8, suggesting that **2** was a 5,7,4′-trisubstituted flavone. In addition, a methyl proton signal at δ_H 0.81 (3H, d, $J = 5.4$ Hz) and 10 complex proton signals in the range of δ_H 3.00–5.50 suggested the existence of two sugar moieties, among which the two proton signals at δ_H 5.32 (1H, br s) and 5.22 (1H, d, $J = 7.3$ Hz) were assigned as the anomeric protons of two sugar moieties. The ^{13}C NMR spectrum exhibited 27 carbon signals, among which 15 carbons were attributed to the skeleton of a 5,7,4′-trisubstituted flavone, and 12 carbons were attributed to two sugar moieties. β-Glucuronidase hydrolysis yielded D-glucuronic acid by co-thin layer chromatography (co-TLC) analysis comparing with the authentic sugar (D-glucuronic acid). And further acid hydrolysis of the glucuronidase hydrolysate afforded L-rhamnose, determined by preparation of trimethylsilyl ether derivatives and comparing with the authentic sugar in GC analysis. According to the coupling constant of the anomeric proton at δ_H 5.22 (1H, d, $J = 7.3$ Hz), the glucuronopyranosyl unit was determined to be β configuration. Comparing ^{13}C NMR data of the rhamnopyranosyl to those of α- and β-rhamnopyranose, the rhamnopyranosyl unit was determined to be α configuration [16]. In HMBC spectrum, the correlation of the anomeric proton signal at δ_H 5.32 (H-1‴) with the carbon signal at δ_C 134.5 (C-3) showed that the rhamnopyranosyl unit was linked to C-3, and the correlation of the anomeric proton signal at δ_H 5.22 (H-1″) with the carbon signal at δ_C 162.6 (C-7) showed that the glucuronopyranosyl unit was linked to C-7. The ^{1}H and ^{13}C NMR data were completely assigned on the basis of the analysis of ^{1}H–^{1}H COSY, TOCSY, HSQC, and HMBC spectra. Thus, the structure of **2** was characterized as kaempferol-3-*O*-α-L-rhamnopyranosyl-7-*O*-β-D-glucuronopyranoside (Figure 2).

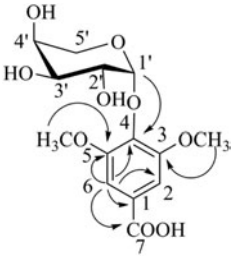

Figure 1. Structure and key HMBC correlations of **1**.

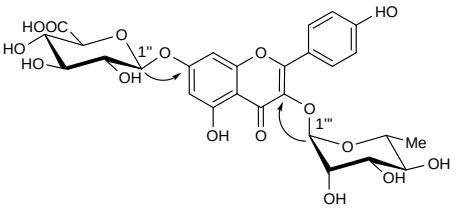

Figure 2. Structure and key HMBC correlations of **2**.

3. Experimental

3.1 General experimental procedures

Melting points (uncorrected) were determined on a X-5 micromelting point apparatus (Cany Precision Instruments Co., Ltd, Shanghai, China). Optical rotations were recorded on a AUTOPOL® automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). UV spectra were obtained on a UV-2201 spectrophotometer (Shimadzu, Kyoto, Japan). HR-ESI-MS was obtained on a Xevo G2 Q-TOF mass spectrometer (Waters, Milford, MA, USA). NMR spectra were recorded on AVANCE III-400 and INOVA-500 spectrometers (Bruker, Faellanden, Switzerland) with TMS (tetramethylsilane) as the internal standard. High-performance liquid chromatography (HPLC) preparation was performed on a Preparative HPLC system (Gilson, Middleton, WI, USA) equipped with two 306 pumps, a 811C dynamic mixer, a 806 manometric module, a 118 UV/Vis detector, and a Grace ODS column (Allsphere ODS-2, 5 μm, 22 mm × 250 mm) with a flow rate of 10 ml/min. HPLC analysis was performed on an analysis HPLC system (Shimadzu, Kyoto, Japan) consisting of a LC-10A*VP* pump, a DGU-14A degasser, a SCL-10A*VP* system controller, a SPD-M10A*VP* diode-array detector, and a Grace ODS column (Allsphere ODS-2, 5 μm, 4.6 mm × 250 mm) with a flow rate of 1 ml/min. Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), Sephadex LH-20 (GE Healthcare, Uppsala, Sweden), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Corp., Kyoto, Japan), HP20 (75–150 μm, Mitsubishi Chemical Corp., Kyoto, Japan), and MDS-5 (75 um, Beijing Medicine Technology Center, Beijing, China) were used for normal pressure column chromatography. Fractions were monitored and analyzed by TLC (Qingdao Marine Chemical, Inc.). L-Arabinose, D-arabinose, D-glucuronic acid, and L-rhamnose were obtained from Sigma-Aldrich Co., Ltd (St Louis, MO, USA). L-Cysteine methyl ester hydrochloride was obtained from TCI Co., Ltd (Shanghai, China). HMDS–TMCS–Pyridine (3:1:9) and β-glucuronidase (Type HP-2 from *Helix pomatia*) were obtained from Sigma-Aldrich Co., Ltd (St Louis, MO, USA). High purity water was provided by Peking University Health Science Center, while reagents used in HPLC were HPLC grade (J&K Scientific Ltd, Beijing, China). Other solvents used were of analytical grade (Beijing Chemical Works, Beijing, China).

3.2 Plant material

The whole plants of *C. acanthoides* were collected in September 2010 in Guide County, Qinghai Province, China. Species identification was confirmed by Associate Prof. Ming-Ying Shang and Associate Prof. Ying-Tao Zhang, School of Pharmaceutical Sciences, Peking University. A voucher specimen (FL2010090901) is maintained in the Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University.

3.3 Extraction and isolation

The air-dried whole plants of *C. acanthoides* (7.5 kg) were crushed into raw powders and successively refluxed with 95% EtOH (3 × 60 liters, 2 h each) and 50% EtOH (3 × 60 liters, 2 h each). The EtOH extract (870 g) was suspended in water and successively partitioned with petroleum ether, EtOAc, and *n*-BuOH, respectively,

to obtain petroleum ether extract (120 g), EtOAc extract (95 g), *n*-BuOH extract (220 g), and water extract (430 g). The dried *n*-BuOH extract was subjected to HP-20 CC (column chromatography) (10 cm × 70 cm, EtOH–H_2O 0:100–95:5, v/v) to give five fractions (Fr. 1–Fr. 5). Fraction 2 (50 g) was further subjected to MCI CC (6 cm × 70 cm, EtOH–H_2O 10:90–70:30, v/v) to yield five subfractions (Fr. 2A–Fr. 2E). Fraction 2A (13 g) was separated on silica gel CC (3 cm × 55 cm, $CHCl_3$–MeOH–H_2O 100:0:0–60:40:10, v/v/v) to obtain five fractions (Fr. 2A-1–Fr. 2A-5). Fraction 2A-4 (1.9 g) was further separated by MCI CC (2 cm × 40 cm, MeOH–H_2O 0:100–40:60, v/v), preparative HPLC (MeOH–H_2O–HCOOH 15:85:0.5, flow rate 10 ml/min, wavelength 260 nm), and then purified by Sephadex LH-20 CC (1.8 cm × 100 cm, MeOH–H_2O 20:80, v/v) to afford **1** (30 mg). Fraction 2B (9 g) was subjected to MDS-5 reversed phase CC (4 cm × 60 cm, MeOH–H_2O 30:70–70:30, v/v), preparative HPLC (MeOH–H_2O–HCOOH 35:65:0.5, flow rate 10 ml/min, wavelength 300 nm), and further purified by Sephadex LH-20 CC (2 cm × 100 cm, MeOH–H_2O 50:50, v/v) to afford compound **2** (20 mg).

*3.3.1 Syringic acid-4-*O*-β-L-arabinopyranoside (**1**)*

White needle crystal (MeOH); melting point (mp) 196–198°C; $[\alpha]_D^{13}$ −64.6° ($c = 0.46$, MeOH); UV (MeOH) λ_{max} (log ε): 229 (2.47), 254 (4.13), and 298 (1.84) nm; for ^{1}H NMR and ^{13}C NMR spectral data see Table 1; HR-ESI-MS *m/z*: 329.0869 $[M - H]^-$ (calcd for $C_{14}H_{17}O_9$, 329.0873), 353.0844 $[M + Na]^+$ (calcd for $C_{14}H_{18}O_9Na$, 353.0849).

*3.3.2 Kaempferol-3-*O*-α-L-rhamnopyranosyl-7-*O*-β-D-glucuronopyranoside (**2**)*

Yellow amorphous powder (MeOH); mp 216–218°C; $[\alpha]_D^{14}$ −143° ($c = 0.44$, MeOH); UV (MeOH) λ_{max} (log ε): 236 (2.56), 261 (4.20), 316 (3.87), and 340 (4.09) nm; for ^{1}H NMR and ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 607.1296 $[M - H]^-$ (calcd for $C_{27}H_{27}O_{16}$, 607.1299), 609.1461 $[M + H]^+$ (calcd for $C_{27}H_{29}O_{16}$, 609.1456).

3.4 Hydrolysis of compounds 1 and 2

Compound **2** (5 mg) was dissolved in 0.5 ml of KH_2PO_4/KOH buffer solution (pH 4.90) before 0.2 ml of β-glucuronidase solution was added, and incubated at 37°C for 5 h. The reaction mixture (about 0.7 ml) was mixed with 3 ml of methanol,

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of **1** in CD_3OD.

Number	δ_H (mult, *J* in Hz)	δ_C	HMBC
1		128.0	
2, 6	7.35, s	108.2	C-1, 3, 4, 6/2, 7
3, 5		154.0	
4		138.6	
7		169.4	
3,5-OCH_3	3.89, s	56.8	C-3/5
1′	5.40, d (4.0)	102.7	C-4, 3′
2′	4.02, dd (5.6, 4.0)	71.7	C-1′, 3′
3′	3.78, dd (5.6, 2.4)	73.0	C-1′, 2′, 4′, 5′
4′	3.90, m	66.6	C-2′, 5′
5′a	3.45, dd (14.8, 7.2)	63.5	C-1′, 3′, 4′
5′b	3.93, m		C-1′, 3′, 4′

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **2** in DMSO-d_6.

Number	δ_H (mult, J in Hz)	δ_C	Number	δ_H (mult, J in Hz)	δ_C
2		157.8	1″	5.22, d (7.3)	99.3
3		134.5	2″	3.29, m	72.8
4		178.0	3″	3.33, m	75.8
5		161.0	4″	3.36, m	71.4
6	6.47, d (1.5)	99.3	5″	3.94, d (9.2)	75.0
7		162.6	6″		170.7
8	6.78, d (1.5)	94.5	1‴	5.32, brs	101.8
9		156.1	2‴	3.99, m	70.1
10		105.9	3‴	3.48, dd (8.4, 2.9)	70.3
1′		120.3	4‴	3.13, m	70.6
2′, 6′	7.77, d (8.5)	130.6	5‴	3.15, m	71.1
3′, 5′	6.93, d (8.5)	115.4	6‴	0.81, d (5.4)	17.4
4′		160.2			

vortexed at 1500 rpm for 5 min, and centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to a clean test tube and dried under a gentle flow of nitrogen gas at 40 °C. The residue was partitioned between EtOAc and H_2O (4 × 1 ml). The water-soluble part was reduced to dryness and then analyzed by co-TLC over silica gel (EtOAc–EtOH–H_2O–HCOOH 6:4:1:1), by comparison with authentic sugar (D-glucuronose), and the EtOAc-soluble part was analyzed by LC-MS^n compared with compound **2** [Agilent 1100 series HPLC system coupled with Finnigan LCQ Advantage ion trap mass spectrometer via ESI interface, Grace Allsphere ODS-2 column (5 μm, 4.6 mm × 250 mm), mobile phase consisted of acetonitrile (A) and water containing 0.1% (v/v) formic acid (B) in the following gradient program: 0 min, 10% A; 60 min, 60% A; flow rate 1 ml/min], which confirmed the presence of D-glucuronic acid.

Compound **1** (2 mg) and compound **2** (the dried EtOAc extract) were hydrolyzed with 1 N HCl (2 ml) for 6 h at 100°C, respectively. After lyopilization, the dry residue was partitioned between Et_2O and H_2O (3 × 2 ml). The dried water extract and 3 mg of L-cysteine methyl ester hydrochloride were dissolved in 0.3 ml of anhydrous pyridine, stirred at 60 °C for 2 h. The reaction mixture was dried under vacuum and was trimethylsilylated with 0.5 ml of HMDS–TMCS–pyridine (3:1:9) at 60 °C for 2 h. The mixture was then concentrated and partitioned between *n*-hexane and H_2O. The *n*-hexane extract was analyzed by GC under the following conditions: Varian CP-3800 GC, DB-5 GC column (30 m × 0.25 mm × 0.25 μm), column temperature increased from 50 to 260 °C at 6°C/min, carrier gas N_2. The t_R values (min) of trimethylsilyl ether derivatives of authentic sugars (L-Ara, D-Ara, and L-Rha) prepared in a similar way were 24.12, 24.51 and 24.96 min, respectively. L-Ara and L-Rha were detected from compounds **1** (t_R 24.13 min) and **2** (t_R 24.92 min).

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