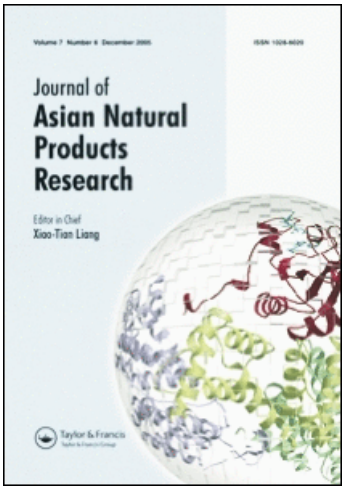

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ORIGINAL ARTICLE

Two new phenolic glycosides from *Inula cappa*

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Two new phenolic glycosides, syringic acid-4-*O*-α-L-rhamnoside (**1**) and (−)-hydnocarpin-7-*O*-β-D-glucoside (**2**), were isolated from the traditional Chinese medicinal herb *Inula cappa*. The structures of the new compounds were elucidated by means of spectroscopic methods such as 1D, 2D NMR, and HR-ESI-MS.

Keywords: *Inula cappa*; Compositae; syringic acid-4-*O*-α-L-rhamnoside; (−)-hydnocarpin-7-*O*-β-D-glucoside

1. Introduction

Inula cappa DC. (Compositae), a traditional Chinese medicinal herb called 'Yang Er Ju', is a widespread plant growing in the south of China. It has been widely used in folklore medicine for the treatment of rheum, rheumatoid arthritis, malaria, dysentery, and hepatitis [1]. Previous phytochemical investigation has reported the isolation and structural elucidation of sesquiterpene lactones, inositol derivatives, flavonoids, organic acids, ceramides, triterpenoids, and sterols [2–8]. In this paper, we report the isolation and structural elucidation of two new phenolic glycosides, syringic acid-4-*O*-α-L-rhamnoside (**1**) and (−)-hydnocarpin-7-*O*-β-D-glucoside (**2**) (Figure 1), from *I. cappa* DC.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{20} - 56$ ($c = 0.07$, MeOH). Its molecular formula was determined to be $C_{15}H_{20}O_9$ by the quasi-molecular ion peak at *m/z* 367.1015 $[M + Na]^+$ in the HR-FAB-MS. The UV spectrum showed two absorption maxima at 213 and 259 nm, consistent with a phenol structure. The IR spectrum of **1** showed absorption bands for hydroxyl (3421 cm^{-1}), carboxyl (3421–2750 and 1695 cm^{-1}), and aromatic (1596, 1503, and 1466 cm^{-1}) groups. Observations of two singlets at δ 7.24 (2H) and 3.81 (6H) in the ^1H NMR spectrum suggested the presence of a 1,4-disubstituted 3,5-dimethoxy benzene ring. Detailed analysis of the carbon resonances for the sp^2 carbons (Table 1) suggested that compound **1** possessed a 4-hydroxy-3,5-dimethoxybenzoic acid (i.e. syringic acid) moiety [9]. HMBC correlations from the singlet at δ 7.24 (H-2/H-6) to the carbon resonances at δ 126.9 (C-1), 137.7 (C-4), 152.8 (C-3/C-5), and 167.1 (C-7) and those from the singlet at δ 3.81 (OCH_3-3/OCH_3-5) to the carbon resonances at δ 152.8 (C-3/C-5) (Figure 2) further supported this assignment. In addition, proton signals at δ 5.16 (1H, s), 4.01 (1H, m), 3.90 (1H, br s), 3.66 (1H, dd, $J = 9.0$, 2.5 Hz), 3.24

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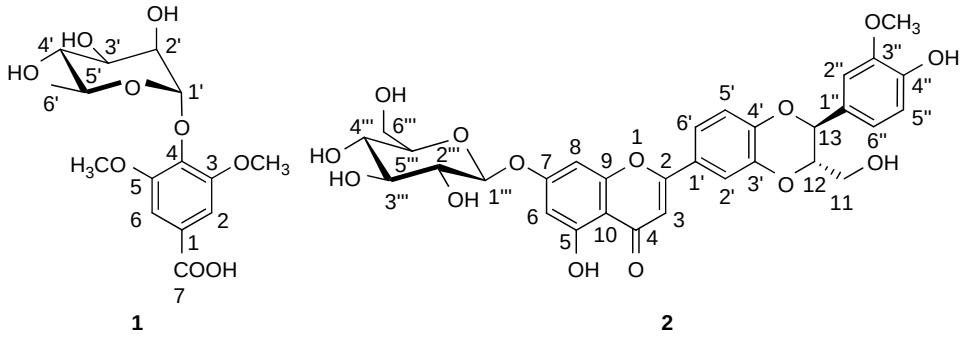

Figure 1. Structures of compounds **1** and **2**.

(1H, dd, $J = 9.7$, 9.0 Hz), 1.07 (3H, d, $J = 5.8$ Hz) in the ^{1}H NMR spectrum (Table 1) were indicative of the presence of an α rhamnose moiety, the presence of which was further confirmed by the ^{13}C NMR spectral data (Table 1). The linkage of the syringic acid and the rhamnose via a C-4—*O*—C-1′ ether bond was further figured out through diagnostic HMBC correlation from the signal at δ 5.16 (H-1′) to the carbon resonance at δ 137.7 (C-4). The structure of compound **1** was thus established as syringic acid-4-*O*-α-L-rhamnoside. Full assignments of the ^{1}H and ^{13}C NMR spectral data were made by a combination of DEPT, HMQC, HMBC, and ^{1}H–^{1}H COSY (Figure 2, Table 1) spectroscopic analysis.

Compound **2** was obtained as a yellow amorphous powder, $[\alpha]_D^{20} - 20$ ($c = 0.2$, MeOH). Its molecular formula $C_{31}H_{30}O_{14}$ was deduced from the HR-FAB-MS peak at *m/z* 627.1711 $[M + H]^+$. The UV absorption maxima at 340 and 271 nm suggested the presence of a flavone chromophore in **2**, while the IR absorptions indicated the existence of aromatic (1613, 1498, and 1444 cm^{-1}), hydroxyl (3423 cm^{-1}), and carbonyl (1658 cm^{-1}) functionalities. In the ^{1}H NMR spectrum (Table 1), two

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

Compound **1**			Compound **2**					
No.	δ_H	δ_C	No.	δ_H	δ_C	No.	δ_H	δ_C
1	–	126.9	2	–	163.5	5′	7.12 d (8.6)	117.6
2	7.24 s	106.4	3	7.01 s	104.2	6′	7.66 br d (8.6)	120.1
3	–	152.8	4	–	182.1	1″	–	126.9
4	–	137.7	5	–	161.1	2″	7.05 br s	111.8
5	–	152.8	6	6.45 br s	99.7	3″	–	147.7
6	7.24 s	106.4	7	–	163.1	4″	–	147.3
7	–	167.1	8	6.90 br s	94.8	5″	6.81 d (8.0)	115.3
1′	5.16 s	102.1	9	–	157.0	6″	6.89 br d (8.0)	120.7
2′	3.90 br s	70.3	10	–	105.4	1‴	5.10 d (7.5)	99.6
3′	3.66 dd (9.0, 2.5)	70.5	11	3.59, 3.36 m	60.1	2‴	3.29 m	73.1
4′	3.24 dd (9.7, 9.0)	71.6	12	4.30 m	78.0	3‴	3.26 m	76.5
5′	4.01 m	70.1	13	5.04 d (8.0)	76.5	4‴	3.18 m	69.5
6′	1.07 d (5.8)	17.8	1′	–	123.6	5‴	3.48 m	77.1
OCH_3	3.81 s	56.1	2′	7.74 br s	115.0	6‴	3.72, 3.48 m	60.6
OCH_3	3.81 s	56.1	3′	–	143.7	OCH_3	3.78 s	55.7
–	–	–	4′	–	147.2	–	–	–

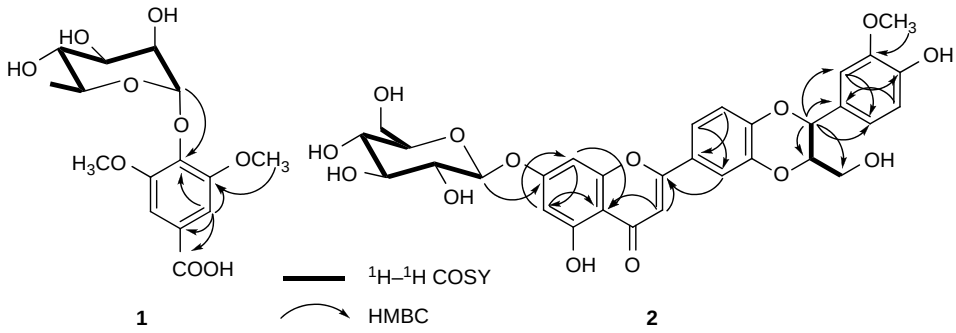

Figure 2. Key HMBC and $^{1}H-^{1}H$ COSY correlations of compounds **1** and **2**.

1,2,4-trisubstituted benzene rings (one at δ 7.74, 7.66, and 7.12, the other at δ 7.05, 6.89, and 6.81) and a 1,2,3,5-tetrasubstituted benzene moiety (δ 6.90 and 6.45) were easily identified. In addition, an olefinic proton at δ 7.01 (1H, s), a methoxyl at δ 3.78 (3H, s), and 11 protons (δ 5.10–3.18) attached to oxygen-containing carbons were also observed (Table 1). Thirty-one carbons were resolved as 21 sp^2 carbons (one carbonyl, three benzenes, and one carbon–carbon double bond) and 10 oxygenated sp^3 carbons (one methoxyl, two methylenes, and seven methines with one appearing at δ 99.6) in the ^{13}C NMR spectrum (Table 1). These NMR spectral data suggested that compound **2** possessed a flavonolignan skeleton as in hydnocarpin [10–12] and a β-glucose moiety ($\delta_{H\text{-}1'''}$ 5.10, 1H, d, $J = 7.5$; $\delta_{C\text{-}1'''}$ 99.6). Detailed analysis of the $^{1}H-^{1}H$ COSY, HMQC, and HMBC spectra (Figure 2) not only confirmed this assignment but also identified the positions of the methoxyl and the glucose moiety. In particular, the diagnostic HMBC correlation from the signal at δ 5.10 (H-1‴) to the carbon resonance at δ 163.1 (C-7) indicated that the glucose was linked at C-7, while the HMBC correlation from the signal at δ 3.78 (CH_3) to the carbon resonance at δ 147.7 (C-3″) placed the methoxyl at C-3″. Connection of the flavonone and the phenylpropane substructure via C-4′—*O*—C-13 and C-3′—*O*—C-12 was determined by comparison of its NMR spectral data with those reported in the literature [10–12], in which the carbon resonances for C-12 and C-13 appeared at ca. 78.0 and 76.3, respectively. The levorotatory property of the aglycone obtained through acid hydrolysis of **2** was consistent with that of (−)-hydnocarpin [10], suggesting that the configurations at C-12 and C-13 were 12*S* and 13*S*, respectively. The structure of **2** was therefore established as (−)-hydnocarpin-7-*O*-β-D-glucoside. Full assignments of the ^{1}H and ^{13}C NMR spectral data were determined by combined analyses of the DEPT, HMQC, HMBC, and $^{1}H-^{1}H$ COSY (Figure 2, Table 1) spectra.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Beijing Taike X-5 micromelting apparatus and are uncorrected. Optical rotations were recorded on a Rudolph Autopol-II automatic polarimeter. The UV spectra were measured on a Shimadzu UV-2401 spectrophotometer. The IR spectra were obtained on a Bruker Vector-22. Mass spectra were obtained on an MS Agilent 1100 series LC/MSD ion trap mass spectrometer (ESI-MS), and the positive-ion HR-FAB-MS was performed on a VG AutoSpec-3000 spectrometer. The NMR spectra were obtained on JEOL ECX-500 spectrometers (500 and 125 MHz, respectively). Column chromatography (CC) was

performed with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). TLC was performed with silica gel GF254 (Marine Chemical Industry Factory).

3.2 Plant material

I. cappa DC. (Compositae) was collected from the suburb of Guiyang, Guizhou Province, China in August 2007, and identified by Prof. Deyuan Chen (Guiyang College of Traditional Chinese Medicine, Guiyang, China). A voucher specimen (No. 20070825) has been deposited at the School of Pharmacy, Guiyang Medical College, China.

3.3 Extraction and isolation

The dried whole plants of *I. cappa* DC. (5 kg) were coarsely powdered, and the obtained powder was extracted with 80% EtOH two times. After filtration and the removal of the solvent under reduced pressure, the residue was partitioned between H_2O and *n*-BuOH. The *n*-BuOH extract (182 g) was subjected to silica gel CC eluted with a gradient of petroleum ether–EtOAc (10:0–0:10). The eluates were monitored by TLC and grouped into four fractions (A–D). Fraction B (35.2 g) was further separated by silica gel CC gradiently eluted with $CHCl_3$–MeOH (10:0–9:1) to obtain five subfractions (B1–B5). Compound **1** (15 mg) was obtained from subfraction B4 by Sephadex LH-20 CC using MeOH as the eluent. Fraction D (49.7 g) was further subjected to silica gel CC gradiently eluted with $CHCl_3$–MeOH (9:0.5–7:3) to yield seven subfractions (D1–D7). Compound **2** (9 mg) was yielded from subfraction D2 by Sephadex LH-20 CC using MeOH as the eluent and semi-preparative HPLC ($t_R = 19.58$ min) using MeOH–0.1% phosphoric acid (58:42) as the eluent.

3.3.1 *Syringic acid-4-O-α-L-rhamnoside (1)*

A white amorphous powder; mp 109–110°C; $[\alpha]_D^{20}$ − 56 ($c = 0.07$, MeOH); UV (MeOH) λ_{max} (log ε): 213 (4.36), 259 (3.81) nm; IR (KBr) ν_{max}: 3421, 3421–2750, 2929, 1695, 1596, 1503, 1466, 1418, 1323, 1221, 1133, 1061, 1018, 974 cm^{-1}; ^1H and ^{13}C NMR spectral data: see Table 1; ESI-MS *m/z*: 343.1 $[M-H]^-$, 367.0 $[M + Na]^+$; HR-FAB-MS *m/z*: 367.1015 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_9Na$, 367.1005).

3.3.2 *(−)-Hydnocarpin-7-O-β-D-glucoside (2)*

A yellow amorphous powder; mp 192–194°C; $[\alpha]_D^{20}$ − 20 ($c = 0.2$, MeOH); UV (MeOH) λ_{max} (log ε): 215 (4.36), 271 (4.09), 340 (4.17) nm; IR (KBr) ν_{max}: 3423, 2924, 1658, 1613, 1498, 1444, 1259, 1175, 1074 cm^{-1}; ^1H and ^{13}C NMR spectral data: see Table 1; ESI-MS *m/z*: 627.2 $[M + H]^+$; HR-FAB-MS *m/z*: 627.1711 $[M + H]^+$ (calcd for $C_{31}H_{31}O_{14}$, 627.1713).

3.3.3 *Acid hydrolysis of 2*

Three milligrams of **2** were dissolved in 5 ml of 5% HCl and the mixture was refluxed for 2 h. The hydrolysate was then extracted with ethyl acetate (3 × 3 ml), concentrated, and purified by preparative TLC (silica gel, $CHCl_3$–acetone, 6:4) to afford 0.9 mg of the aglycone ($[\alpha]_D^{20}$ − 16 ($c = 0.2$, DMSO)).

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