# A new phenylpropanoid glycoside from *Cirsium setosum*

KE Rui[1, 2], ZHU En-yuan[1, 2*], CHOU Gui-xin[1, 2]

(*1. Key Laboratory of Standardization of Chinese Medicines of Ministry of Education, Key Laboratory of New Resources and Quality Evaluation of Chinese Medicines, Key Laboratory of Compound Chinese Medicine of Shanghai, Institute of Chinese Materia Medica of Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China;*
*2. Shanghai R&D Centre for Standardization of Chinese Medicine, Shanghai 201203, China*)

**Abstract**: To study the chemical constituents of *Cirsium setosum* (Willd.) MB., 70% ethanol extract of the aerial parts was subjected to column chromatography. One new phenylpropanoid glycoside, sinapyl alcohol 9-*O*-(*E*)-*p*-coumaroyl-4-*O*-*β*-*D*-glucopyanoside (**1**) was isolated, along with three known compounds: lycoperodine-1 (**2**), apigenin-7-*O*-(6″-(*E*)-*p*-coumaroyl)-*β*-*D*-galactopyranoside (**3**) and quercetin (**4**). The structures were elucidated on the basis of spectral and chemical evidence. Compound **2** was obtained from *Cirsium* genus for the first time, compounds **3** and **4** were obtained from this plant for the first time.

**Key words**: *Cirsium setosum*; phenylpropanoid glycoside; sinapyl alcohol

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# 小蓟中一个新的苯丙素苷类化合物

柯 睿[1, 2], 朱恩圆[1, 2*], 俞桂新[1, 2]

(1. 上海中医药大学中药研究所、教育部中药标准化重点实验室、中药新资源与质量标准综合评价国家中医药管理局重点研究室、上海市复方中药重点实验室, 上海 201203; 2. 上海中药标准化研究中心, 上海 201203)

**摘要**: 为研究小蓟 *Cirsium setosum* (Willd.) MB.地上部分的化学成分, 采用硅胶、树脂和凝胶柱色谱法从其70%乙醇提取物中分离得到 4 个化合物, 并根据理化性质和波谱数据鉴定其结构分别为银槭醇 9-*O*-反式-对-香豆酰基-4-*O*-*β*-*D*-葡萄糖苷 (**1**)、lycoperodine-1 (**2**)、芹菜素-7-*O*-(6"-反式-对-香豆酰基)-*β*-*D*-半乳糖苷 (**3**) 和槲皮素 (**4**)。其中, 化合物 **1** 为新化合物, 化合物 **2** 为首次从该属植物中分离得到, 化合物 **3** 和 **4** 为首次从该植物中分离得到。

**关键词**: 小蓟; 苯丙素苷; 银槭醇

*Cirsium setosum* (Willd.) MB. widely distributes in China. It was reported to possess hemostatic, anti-inflammatory, antimicrobial and anticancer activities in recent studies[1, 2]. Flavonoids, organic acids, sterols and lignanoids had been isolated from this plant[3], and the flavonoids was found to be the active hemostatic and anti-inflammatory component[4]. In this study, a phenylpropanoid glycoside named sinapyl alcohol 9-*O*-(*E*)-*p*-coumaroyl-4-*O*-*β*-*D*-glucopyanoside (**1**) and three known compounds: lycoperodine-1 (**2**), apigenin-7-*O*-(6″-(*E*)-*p*-coumaroyl)-*β*-*D*-galactopyranoside (**3**) and quercetin (**4**) were reported. Compound **2** is an alkaloid and obtained from *Cirsium* genus for the first time. Compound **3** and **4** are flavonoids and obtained from this plant for the first time. The chemical structures of compounds **1** − **4** are shown in Figure 1.

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*Corresponding author Tel: 86-21-51322513, Fax: 86-21-51322519, E-mail: wxzey@163.com

## Results and discussion

Compound **1** was obtained as yellowish needles ($CH_2Cl_2$/$CH_3OH$), melting point (mp) 223−225 ℃ and

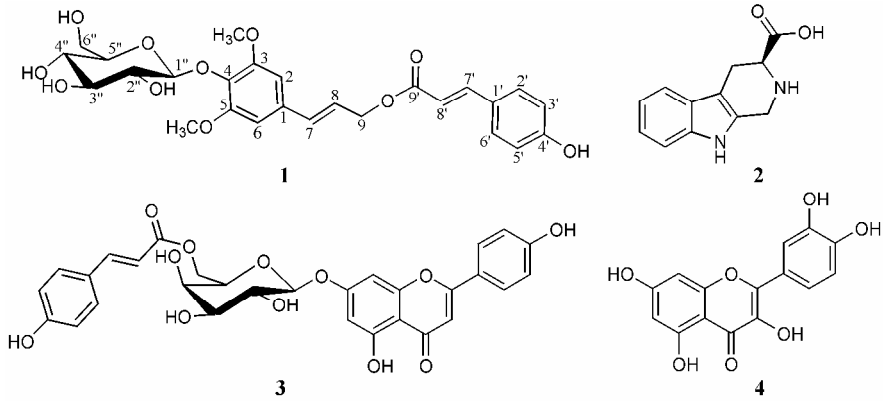

**Figure 1** The chemical structures of compounds **1** − **4**

$[\alpha]_D^{25}$ −111.4 (*c* 0.05, $CH_3OH$). The molecular formula was deduced as $C_{26}H_{30}O_{11}$ from the pseudomolecular ion peak at *m/z* 517.1717 0 $[M-H]^-$ (calcd. 517.1715 4) in HR-ESI-MS. Hydroxyl (3 471 $cm^{-1}$) and carbonyl (1 683 $cm^{-1}$) absorptions were observed in the IR spectrum. The UV spectrum displayed three maximum absorptions at 222, 272 and 312 nm ($CH_3OH$).

The $^1H$ NMR spectrum of **1** (Table 1) showed a pair of symmetrical aromatic protons ($\delta$ 6.69, 1H×2, s, H-2, 6) which revealed the presence of a 1, 3, 4, 5-tetrasubstituted benzene. A methylene attached to oxygen ($\delta$ 4.08, 2H, br s, H-9), two *trans* olefinic protons ($\delta$ 6.41 (1H, d, *J* = 16.0 Hz, H-7) and 6.28 (1H, d, *J* = 16.0 Hz, H-8)), and two methoxyl groups ($\delta$ 3.73, 3H×2, s), suggesting the presence of a phenylpropanoid moiety. The signals belonged to a *p*-coumaroyl group: four aromatic protons ($A_2B_2$ system, $\delta$ 7.49 (2H, d, *J* = 8.5 Hz, H-2′, 6′), 6.79 (2H, d, *J* = 8.5 Hz, H-3′, 5′)) for the symmetrical 1, 4-disubstituted aromatic ring, and two *trans* olefinic protons ($\delta$ 7.44 (1H, d, *J* = 16.0 Hz, H-7′), 6.27 (1H, d, *J* = 16.0 Hz, H-8′)). The signal at $\delta$ 4.86 (1H, d, *J* = 7.0 Hz) assigns to the anomeric proton of glucose. The $^{13}C$ NMR spectrum (Table 1) confirmed **1** contained a phenylpropanoid moiety, a *p*-coumaroyl group and a sugar residue. The $^1H$ NMR and $^{13}C$ NMR spectra of **1** were similar to those of sinapyl alcohol 9-*O*-(*E*)-*p*-coumaroyl[5] except for a sugar residue, which could also be confirmed by the molecular weight. Moreover, the typical NMR chemical shifts[6], the characteristic coupling constant of its anomeric proton (*J* = 7.0 Hz) as well as the hydrolysis of compound **1** suggested the existence of a *β*-*D*-glucopyranoside.

The HMBC spectral analysis of **1** (Figure 2) confirmed the significant correlation peaks between H-1″ of the glucopyranosyl and C-4 of the sinapyl alcohol as well as H-9 of the sinapyl alcohol and C-9′ of the coumaroyl. Therefore, the structure of compound **1** was assigned as sinapyl alcohol 9-*O*-(*E*)-*p*-coumaroyl-4-*O*-*β*-*D*-glucopyanoside.

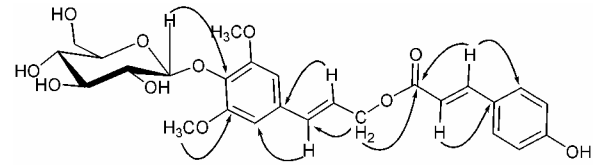

**Figure 2** The key HMBC correlations of compound **1**

## Experimental

### 1 General procedure and reagents

Melting points were measured on a Büchi B-540 apparatus and temperature uncorrected. Optical rotations were measured on a Krüss P8000-T digital polarimeter. UV spectra were measured with a UV-1901 recording spectrophotometer (Beijing Puxi General Instrument Co., Ltd., Beijing, China). IR spectra were recorded on NicoletTM-380 spectrophotometer from Thermo Electron. NMR spectra were recorded on Bruker AV-500 with TMS as internal reference. Electron impact-mass spectrum (EI-MS) and ESI-MS spectra were taken on Trace DSQ and LCQ DECAXP mass spectrometer (Thermo) respectively. HR-ESI-MS were obtained on Bruker APEXIII 7.0 TESLA FTMS.

Column chromatography (CC): silica gel (200−300 mesh; Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (GE-Healthcare Bio-Sciences AB, Uppsala, Sweden), macroporous resin (HPD-600; Shanghai Mosu Science Equipment Co., Ltd., Shanghai, China), microporous resin (MCI) (75−150 μm; Mitsubishi Chemical Corporation, Tokyo, Japan) and octadecylsilyl (ODS) (SepaxGP-C18; 40−60 μm; Sepax Technologies Inc.). All other chemicals were of analytical grade.

**Table 1** The NMR data for compound **1** (dimethyl sulphoxide-$d_6$)

| Position | $^1$H (500 MHz) | $^{13}$C (125 MHz) | HMBC (H-C) |
|---|---|---|---|
| 1 | | 133.1 | |
| 2, 6 | 6.69 (s) | 104.5 | C-2, C-3, C-4, C-6, C-7 |
| 3, 5 | | 153.1 | |
| 4 | | 133.8 | |
| 7 | 6.41 (d, *J* = 16.0 Hz) | 128.7 | C-1, C-2, C-6, C-8, C-9 |
| 8 | 6.28 (d, *J* = 16.0 Hz) | 130.4 | C-1, C-9 |
| 9 | 4.08 (br s) | 61.7 | C-7, C-8, C-9′ |
| 3, 5-$OCH_3$ | 3.73 (s) | 56.5 | C-3, C-5 |
| 1′ | | 125.3 | |
| 2′, 6′ | 7.49 (d, *J* = 8.5 Hz) | 130.5 | C-2′, C-4′, C-6′, C-7′ |
| 3′, 5′ | 6.79 (d, *J* = 8.5 Hz) | 116.0 | C-1′, C-3′, C-4′, C-5′ |
| 4′ | | 160.1 | |
| 7′ | 7.44 (d, *J* = 16.0 Hz) | 144.9 | C-1′, C-2′, C-6′, C-8′, C-9′ |
| 8′ | 6.27 (d, *J* = 16.0 Hz) | 114.2 | C-1′, C-9′ |
| 9′ | | 166.6 | |
| 1″ | 4.86 (d, *J* = 7.0 Hz) | 102.9 | C-4 |
| 2″ | 3.21−3.36 (m) | 74.2 | |
| 3″ | 3.21−3.36 (m) | 74.3 | |
| 4″ | 3.21−3.36 (m) | 70.3 | |
| 5″ | 3.21−3.36 (m) | 76.6 | |
| 6″ | 4.31 (d, *J* = 11.0 Hz) | 63.7 | |
| | 4.12 (dd, *J* = 11.0, 6.5 Hz) | | |

## 2 Plant material

Aerial parts of *C. setosum* were collected in Shanghai, China, in October 2008 and were identified by Prof. Wu Li-hong (Shanghai University of Traditional Chinese Medicine). A voucher specimen (No. 20081024) was deposited at Shanghai R&D Center for Standardization of Traditional Chinese Medicines.

## 3 Extraction and isolation

Dry and crushed aerial parts of *C. setosum* (5.0 kg) were extracted three times with 70% ethanol (EtOH) 50 L and concentrated *in vacuo*. The residue was suspended in water and partitioned with petroleum ether, ethyl acetate and *n*-butanol successively. The ethyl acetate residue (31 g) was subjected to silica gel column chromatography eluted with a gradient mixture of $CH_2Cl_2$-$CH_3OH$ (100∶1 to 1∶1, *v/v*) to yield ten fractions (A−J). Fr. I was further subjected to a silica gel column eluted with $CH_2Cl_2$-$CH_3OH$ (20∶1 to 5∶1, *v/v*) to yield three fractions ($I_1$−$I_3$). Repeated chromatography by Sephadex LH-20 column ($CH_2Cl_2$-$CH_3OH$ 1∶1, *v/v*) afforded compounds **4** (5 mg), **1** (37 mg) and **3** (2 mg) from Fr. G, $I_2$ and $I_3$ respectively. The *n*-butanol residue (105 g) was subjected to a macroporous resin column using a gradient eluent of EtOH-$H_2O$ (from 100% $H_2O$ to 95% EtOH) and yielded four fractions ($H_2O$, 30% EtOH, 60% EtOH and 95% EtOH). The Fr. 30% EtOH (15 g) was successively subject to ODS column ($CH_3OH$-$H_2O$ from 5% to 70% $CH_3OH$), MCI column ($CH_3OH$-$H_2O$ from 5% to 40% $CH_3OH$) and Sephadex LH-20 column eluted with $CH_3OH$ to afford compound **2** (10 mg).

## 4 Structure identification

**Compound 1** yellowish needles ($CH_2Cl_2$/$CH_3OH$), mp 223−225 ℃ and $[\alpha]_D^{25}$ −111.4 (*c* 0.05, $CH_3OH$). UV ($CH_3OH$) $\lambda_{max}$: 222, 272, 312 nm. IR (KBr) $\nu$ cm$^{-1}$: 3 471, 3 259, 1 683, 1 635, 1 604, 1 587, 1 421, 1 338, 1 224, 1 137, 833. HR-ESI-MS: *m/z* 517.1717 0 $[M-H]^-$ (calcd. 517.1715 4). $^1$H NMR and $^{13}$C NMR data were shown in Table 1.

**Compound 2** yellowish needles ($CH_3OH$). EI-MS: *m/z* 216 $[M]^+$. $^1$H NMR (dimethyl sulphoxide-$d_6$) $\delta$: 10.88 (1H, s, 1-NH), 7.44 (1H, d, *J* = 8.0 Hz, H-4), 7.32 (1H, d, *J* = 8.0 Hz, H-7), 7.07 (1H, t, *J* = 7.5 Hz, H-6), 6.99 (1H, t, *J* = 7.5 Hz, H-5), 4.22 (1H, d, *J* = 15.5 Hz, H-11b), 4.16 (1H, d, *J* = 15.0 Hz, H-11a), 3.62 (1H, dd, *J* = 10.5, 5.0 Hz, H-9), 3.13 (1H, dd, *J* = 16.0, 4.5 Hz, H-8b), 2.82 (1H, dd, *J* = 16.0, 10.5 Hz, H-8a). $^{13}$C NMR (dimethyl sulphoxide-$d_6$) $\delta$: 169.3 (9-COOH),

136.2 (C-6a), 127.7 (C-2), 126.2 (C-3a), 121.2 (C-6), 118.7 (C-5), 117.8 (C-4), 111.1 (C-7), 106.6 (C-3), 56.6 (C-9), 40.4 (C-11), 22.9 (C-8). The $^1$H and $^{13}$C NMR data are in accordance with those in literature[7], so compound **2** was identified as lycoperodine-1.

**Compound 3** yellowish powder ($CH_3OH$). ESI-MS: *m/z* 577 $[M-H]^-$. $^1$H NMR (dimethyl sulphoxide-$d_6$) $\delta$: 12.96 (1H, s, 5-OH), 7.93 (2H, d, $J$ = 8.5 Hz, H-2′, 6′), 7.48 (1H, d, $J$ = 16.0 Hz, H-7‴), 7.36 (2H, d, $J$ = 8.5 Hz, H-2‴, 6‴), 6.91 (2H, d, $J$ = 8.5 Hz, H-3′, 5′), 6.82 (1H, s, H-3), 6.81 (1H, s, H-8), 6.66 (2H, d, $J$ = 8.5 Hz, H-3‴, 5‴), 6.47 (1H, s, H-6), 6.32 (1H, d, $J$ = 16.0 Hz, H-8‴), 5.16 (1H, d, $J$ = 7.5 Hz, H-1″), 4.46 (1H, d, $J$ = 11.0 Hz, H-6a″), 4.15 (1H, dd, $J$ = 11.5, 7.0 Hz, H-6b″), 3.30−3.80 (H-2″~5″). $^{13}$C NMR (dimethyl sulphoxide-$d_6$) $\delta$: 182.7 (C-4), 166.9 (C-9‴), 164.5 (C-2), 163.2 (C-7), 162.3 (C-5), 160.2 (C-4′), 159.3 (C-4‴), 157.5 (C-9), 145.5 (C-7‴), 130.5 (C-2‴, 6‴), 129.0 (C-2′, 6′), 124.2 (C-1‴), 121.0 (C-1′), 116.3 (C-3′, 5′), 115.9 (C-3‴, 5‴), 114.2 (C-8‴), 105.6 (C-10), 103.3 (C-3), 99.9 (C-1″), 99.4 (C-6), 95.1 (C-8), 76.3 (C-5″), 74.2 (C-3″), 73.2 (C-2″), 70.5 (C-4″), 63.9 (C-6″). The $^1$H and $^{13}$C NMR data are consistent with those in literature[8], and then compound **3** was deduced as apigenin-7-*O*-(6″-(*E*)-*p*-coumaroyl)-*β*-*D*-galactopyranoside.

**Compound 4** yellow powder ($CH_3OH$). ESI-MS: *m/z* 301 $[M-H]^-$. $^1$H NMR (dimethyl sulphoxide-$d_6$) $\delta$: 12.48 (1H, s, 5-OH), 7.67 (1H, d, $J$ = 2.0 Hz, H-2′), 7.53 (1H, dd, $J$ = 8.5, 2.5 Hz, H-6′), 6.88 (1H, d, $J$ = 8.5 Hz, H-5′), 6.40 (1H, d, $J$ = 2.0 Hz, H-8), 6.18 (1H, d, $J$ = 2.0 Hz, H-6). $^{13}$C NMR (dimethyl sulphoxide-$d_6$) $\delta$: 176.1 (C-4), 164.1 (C-7), 160.9 (C-5), 156.4 (C-9), 147.9 (C-4′), 147.1 (C-2), 145.3 (C-3′), 135.9 (C-3), 122.2 (C-1′), 120.2 (C-6′), 115.8 (C-2′), 115.3 (C-5′), 103.3 (C-10), 98.4 (C-6), 93.6 (C-8). The $^1$H and $^{13}$C NMR data are in agreement with those in literature[9], and the structure of **4** was identified as quercetin.

## 5 Acid hydrolysis of compound 1: determination of the sugar

Compound **1** (1 mg) and 2 mol·L$^{-1}$ trifluoroacetic acid (2 mL) was added in an ampoule and sealed. The mixture was heated at 120 ℃ for 2 h, then cooled to room temperature. To 100 μL of the mixture, 2 mol·L$^{-1}$ NaOH (100 μL), 0.5 mol·L$^{-1}$ $NaBH_4$ in DMSO (1 mL) were added and reacted at 40 ℃ for 1.5 h. Further reaction was followed by adding acetic acid (100 μL), 1-methylimidazole (200 μL) and acetic anhydride (1 mL) at the same temperature for another 10 min. The reaction mixture was extracted with chloroform (2 mL × 3), washed with 0.5 mol·L$^{-1}$ $NaHCO_3$ (2 mL × 3) and diluted water (2 mL × 3). Organic layer was dried by anhydrous $Na_2SO_4$ and subjected to GC-MS (Thermo TR-5MS column (60 mm × 0.25 mm × 2.5 μm); carrier gas helium; flow rate 1 mL·min$^{-1}$; oven-temp. gradient: 140 ℃ → 198 ℃ (2 ℃·min$^{-1}$, 4 min), 198 ℃ → 214 ℃ (40 ℃·min$^{-1}$), 214 ℃ → 217 ℃ (1 ℃·min$^{-1}$, 4 min), 217 ℃ → 250 ℃ (3 ℃·min$^{-1}$, 5 min) )[10]. GC-MS analysis result showed the sugar was glucose (the same retention time compared to the reference glucose derivative).

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