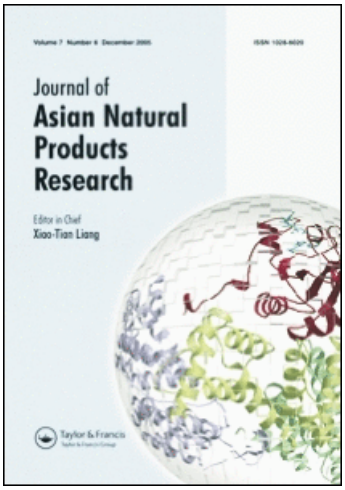

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Coumarin glycosides from *Euphorbia soongarica* (Boiss)

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Two new coumarin glycosides (**1** and **2**), along with six known compounds (**3**–**8**), were isolated from the roots of *Euphorbia soongarica*. The structures of two new compounds were elucidated as aesculetin-6-*O*-(6′-*O*-galloyl)-β-D-galactopyranoside (**1**) and fraxetin-8-*O*-(6′-*O*-galloyl)-β-D-galactopyranoside (**2**) by spectral methods and chemical evidences.

Keywords: *Euphorbia soongarica*; aesculetin-6-*O*-(6′-*O*-galloyl)-β-D-galactopyranoside; fraxetin-8-*O*-(6′-*O*-galloyl)-β-D-galactopyranoside; coumarin glycosides

1. Introduction

The genus *Euphorbia* is the largest one in the family of Euphorbiaceae, comprising about 2000 species. More than 80 of them are distributed in China, many of which have series of applications in either traditional Chinese medicine or folklore herbs. *Euphorbia soongarica* Boiss., a perennial herbaceous plant, with a milky juice in the aerial part and a yellow juice in the roots, is mainly distributed in Xinjiang, Gansu province of China. As a Chinese folk medicine, the roots of this plant have been used as purgative, apocenosis, and discussive [1]. Previous phytochemical investigations on this plant aimed only to the constituents of petroleum ether-soluble extract [2]. In the course of our research for bioactive metabolites from plants, we were interested in the polar constituents [3–5]. As a result, two new coumarin glycosides (**1** and **2**), along with six known compounds (**3**–**8**), were isolated from the roots of *E. soongarica*. Herein, we describe the structure elucidation of the new compounds.

2. Results and discussion

Compound **1**, obtained as a yellowish lamellar crystal (MeOH), was assigned the molecular formula $C_{22}H_{20}O_{13}$ as determined from HR-ESI-MS. Its UV spectrum with λ_{max} 212 and 343 nm was characteristic of a coumarin derivative. The IR spectrum showed the presence of an ester function and an α, β-unsaturated lactone (1680 and 1610 cm^{-1}). Acid hydrolysis of **1** afforded galactose, galloyl acid, and aesculetin by co-TLC with authentic samples. That suggested the presence of a galactyl and galloyl group in the coumarin. Its ESI-MS exhibited fragment ion peaks at *m/z* 491 $[M - H]^-$, 339 $[M - H\text{-}152]^-$, and 177 $[M - H\text{-}152\text{-}162]^-$, which further confirmed the existence of galloyl and galactosyl residues. The ^{13}C NMR spectrum indicated the existence of 1 sugar (δ 101.8, 73.1, 74.1, 70.0, 75.6, 63.5), 2 ester carbonyl groups (δ 160.3, 165.6), and 14 sp^2-carbons. The ^1H NMR spectrum of **1** showed two proton doublets at δ 7.28 and 6.04 ($J = 9.5$ Hz) characteristic for the H-3 and H-4 of coumarin. The presence of a

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

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
2	160.3	–	160.1	–
3	111.6	6.04 (d, 9.5)	112.2	6.07 (d, 9.5)
4	144.1	7.28 (d, 9.5)	144.7	7.26 (d, 9.5)
5	113.7	7.18 (s)	108.6	6.98 (s)
6	142.5	–	143.8	–
7	151.0	–	144.9	–
8	103.1	6.80 (s)	143.1	–
9	150.3	–	134.9	–
10	110.5	–	110.4	–
1′	101.8	4.84 (d, 7.4)	102.2	4.80 (d, 7.3)
2′	73.1	3.37 (m)	73.3	3.36 (m)
3′	74.1	3.78 (m)	74.3	3.78 (m)
4′	70.0	3.30 (m)	70.1	3.30 (m)
5′	75.6	3.50 (m)	75.7	3.50 (m)
6′	63.5	4.32 (m), 4.55 (m)	63.7	4.32 (m), 4.55 (m)
1″	119.4	–	119.6	–
2″, 6″	108.5	7.00 (s)	108.8	7.00 (s)
3″, 5″	145.7	–	145.9	–
4″	138.5	–	138.8	–
7″	165.6	–	165.7	–
$-OCH_3$		–	60.8	3.84 (s)

* Assignments confirmed by $^{1}H-^{1}H$ COSY, HMQC, and HMBC spectra.

further two proton signals at δ 6.80 and 7.18 (each 1H, s) in the ^{1}H NMR spectrum and resonances of carbons bearing oxygen moieties at δ 142.5 and 151.0 indicated that a 6,7-disubstituted coumarin aglycone unit of **1** was identified as aesculetin [6]. The signal of the aromatic protons at δ 7.00 (2H, s) indicated the presence of a 1, 3, 4, 5-*tetra*-substituted benzene ring. Thus, the aromatic acyl unit was identified as a galloy moiety. Additionally, the signal of the anomeric proton at δ 4.84 (1H, d, $J = 7.4$ Hz) showed that galactose was attached in the β-configuration. The assignments of the carbon and proton signals of **1** (Table 1) were fulfilled from $^{1}H-^{1}H$ COSY, HSQC, and HMBC experiments. HMBC correlations between the two protons of the galactose methylene group (δ 4.32 and 4.55) and the ester carbonyl group (δ 165.6) of galloyl moiety, and between the anomeric proton at δ 4.84 and the carbon at δ 142.5 (C-6), defined the galloyl acylation at C-6′ of galactose and the glycosidation at C-6 (Figure 1), respectively. From the above evidences, compound **1** was characterized as aesculetin-6-*O*-(6′-*O*-galloyl)-β-D-galacto-pyranoside.

Compound **2** was obtained as white lamellar crystal (MeOH). The HR-ESI-MS spectrum showed a molecular ion peak at *m*/*z* 545.0969 $[M + Na]^{+}$, corresponding to a molecular formula of $C_{23}H_{22}O_{14}$. A preliminary analysis of its spectral data (Table 1)

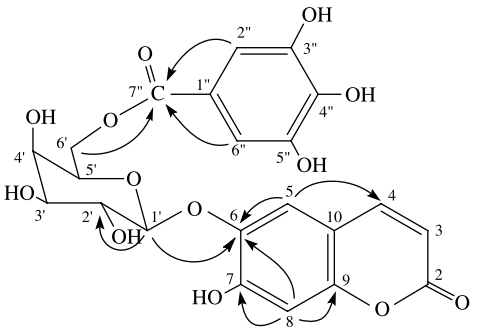

Figure 1. Selected HMBC correlations for **1**.

showed that **2** is similar to compound **1** with a galactosyl and a galloyl groups, only differed in aglycone moiety. In fact, acid hydrolysis of **2** also afforded a galactose and a galloyl acid, except for fraxetin (**3**) as aglycone [7]. The IR spectrum showed the presence of an ester function and an α, β-unsaturated lactone (1690 and 1612 cm^{-1}). Its ESI-MS exhibited fragment ion peaks at *m/z* 521 $[M - H]^-$, 369 $[M - H\text{-}152]^-$, and 207 $[M - H\text{-}152\text{-}162]^-$ indicating the loss of a galloyl unit and a hexose. Similar to **1**, the ^1H NMR spectrum of **2** also showed two proton signals at δ 7.26 and 6.07 (1H, d, $J = 9.5$ Hz), characteristic for the H-3 and H-4 of coumarin, the signal at δ 7.00 (2H, s) assigned to the aromatic protons of a galloy moiety, and the signal of the anomeric proton at δ 4.80 (1H, d, $J = 7.3$ Hz) attributed to β-anomeric proton of galactose. The remaining signals at δ 6.98 (1H, s) and 3.84 (3H, s) suggested the presence of a trisubstituted coumarin with a methoxyl group. HMBC correlation between methoxyl protons (δ 3.84) and C-6 (δ 143.8) (Figure 2) indicated that the methoxyl was substituted at C-6. Thus, the aglycone was identified as fraxetin [7]. Additionally, the $^1H-^{13}C$ long-range correlations between the anomeric proton at δ 4.80 with the carbon signal at δ 143.1 (C-8), and between the two protons of the galactose methylene group (δ 4.32 and 4.55) and the ester carbonyl group (δ 165.7) of galloyl moiety (Figure 2), unambiguously suggested that the galactose was attached to C-8 and the galloy moiety was linked at C-6′ of galactose, respectively. On the basis of the above evidences, the structure of **2** was established as fraxetin-8-*O*-(6′-*O*- galloyl)-β-D-galactopyranoside.

Compounds **3**–**8** were identified as fraxetin (**3**) [7], fraxin (**4**) [7], (+)-isolariciresinol-9-*O*-β-D-xylopyranoside (**5**) [8], 3-(4-*O*-β-D-glucopyranosyloxy-3, 5-dimethoxy)-phenyl-2*E*-propenol (**6**) [9], 3,3′-di-*O*-methylellagic acid 4-*O*-β-D-glucopyranoside (**7**) [10], and 3, 3′-di-*O*-methylellagic acid 4-*O*-β-D-xylopyranoside (**8**) [11] by physicochemical constants and spectral analysis.

3. Experimental

3.1 General experimental procedures

Melting points were measured on an X-4 melting point apparatus and are uncorrected.

Optical rotations were determined on a JASCO P-1020 polarimeter. UV spectra were recorded on a Shimadzu UV-2501 PC spectrophotometer in MeOH solution. IR spectra were obtained on a Nicolet Impact-410 spectrophotometer. 1D- and 2D NMR spectra were recorded on a Bruker ACF-300 or DRX-500 spectrometer using TMS as internal standard. ESI-MS and HR-ESI-MS were measured on an Agilent 1100 Series LC/MSD Trap spectrometer and on an Agilent TOF MSD 1946D spectrometer, respectively. Column chromatography was

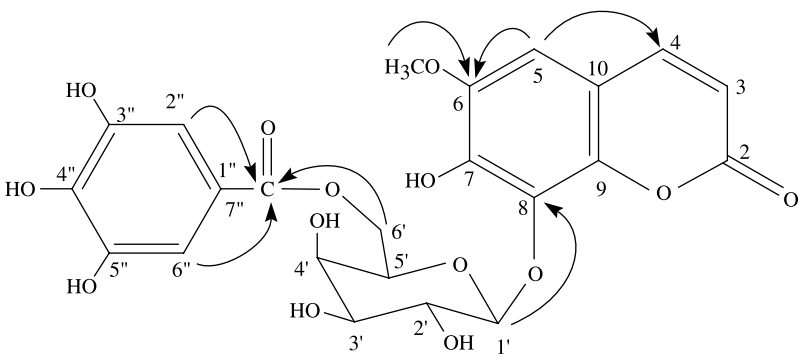

Figure 2. Selected HMBC correlations for **2**.

carried out using silica gel H (Qingdao Marine Chemical Industry, 10–40 μm, Qingdao, China) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden).

3.2 Plant material

Roots of *E. soongarica* were collected in the Yili of Xinjiang province in China and identified by Professor Chang-You Yang, Xinjiang Agricultural University. A voucher specimen (No. 020816) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3 Extraction and isolation

Roots of *E. soongarica* (10 kg) were crushed and extracted with EtOH thrice. After evaporation of the EtOH *in vacuum*, the extract was suspended in water and partitioned with ethyl acetate to obtain 40.0 g of ethyl acetate-soluble residue and 80.0 g of water-soluble fraction. The ethyl acetate fraction was subjected to column chromatography on silica gel H (300 g), eluted with a mixture of $CHCl_3$–Me_2CO with gradually increasing polarity to yield **3** (30 mg) and **6** (20 mg). The water-soluble fraction was subjected to column chromatography over silica gel H (500 g), eluted with a $CHCl_3$/MeOH (99:5–70:30) gradient system to give four subfractions. Subfraction 1 (2.2 g) was subjected to SiO_2 column chromatography, eluted with $CHCl_3$/MeOH (96:4), then purified with preparative TLC to give **4** (42 mg). Subfraction 2 (3.2 g) was subjected to SiO_2 column chromatography with $CHCl_3$/MeOH (92:8) as eluent and purified by Sephadex LH-20 column chromatography with MeOH to provide **1** (24 mg) and **2** (28 mg). Subfraction 3 (2.6 g) was subjected to SiO_2 column chromatography and eluted with $CHCl_3$/MeOH (85:15) to yield **5** (26 mg) and **6** (18 mg). Subfraction 4 (5.3 g) was further chromatrographed on a SiO_2 column with $CHCl_3$/MeOH (68:32) as eluent, afforded **7** (29 mg) and **8** (38 mg).

3.3.1 *Aesculetin-6-O-(6′-O-galloyl)-β-D-galactopyranoside (**1**)*

Yellowish lamellar crystal (MeOH); mp 226–228°C, $[\alpha]_D^{20} = -32.6$ ($c = 0.1$, C_5H_5N); UV λ_{max} (nm): 212, 343 (MeOH); IR ν_{max} (cm^{-1}): 3332, 1680, 1610, 1572, 1508, 1456; HR-ESI-MS *m/z*: 493.1033 $[M + H]^+$ (calcd for $C_{22}H_{21}O_{13}$, 493.0982); ESI-MS *m/z*: 491 $[M - H]^-$, 339, 177; ^1H and ^{13}C NMR spectral data (Table 1).

3.3.2 *Fraxetin-8-O-((6′-O-galloyl)-β-D-galactopyranoside (**2**)*

White lamellar crystal (MeOH); mp 230–232°C; $[\alpha]_D^{20} = -42.8$ ($c = 0.1$, C_5H_5N); UV λ_{max} (nm): 216, 345 (MeOH); IR ν_{max} (cm^{-1}): 3336, 1690, 1612, 1578, 1506, 1466, 1415; HR-ESI-MS *m/z*: 545.0969 $[M + Na]^+$ (calcd for $C_{23}H_{22}O_{14}Na$, 545.0907); ESI-MS *m/z*: 521 $[M - H]^-$, 369, 207. ^1H and ^{13}C NMR spectral data (Table 1).

3.3.3 *Acid hydrolysis of* **1** *and* **2**

Compounds **1** and **2** (each 5 mg) dissolved in 0.5 ml of 2 N HCl was refluxed for 1 h. The reaction mixture was neutralized and extracted with EtOAc to obtain aglycones. The aglycones were identified as aesculetin for **1** and fraxetin for **2** by TLC [$CHCl_3$/MeOH (8:2)] in comparison with authentic samples. The water-soluble residue was analyzed by TLC [*n*-BuOH/HOAc/H_2O (4:1:5), upper layer] giving galactose and galloyl acid in **1** and **2**, identified in comparison with the authentic samples.

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