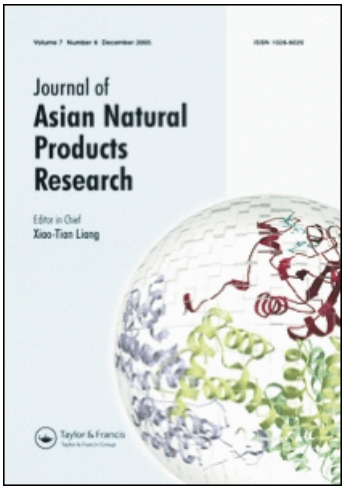

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Two new coumarin glycosides from *Peucedanum praeruptorum*

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Two new coumarin glycosides, praerosides VI (**1**) and VII (**2**), were isolated from the roots of *Peucedanum praeruptorum* Dunn. Their structures were elucidated by chemical reaction and NMR spectroscopic methods.

Keywords: *Peucedanum praeruptorum*; coumarin glycosides; praeroside VI; praeroside VII

1. Introduction

A literature survey revealed that a number of coumarins were isolated from the traditional Chinese medicine "Bai-Hua Qian-Hu", the root of *Peucedanum praeruptorum* Dunn.[1–9] Some biochemical studies on these coumarin compounds were performed to find a calcium antagonistic action in Pd-Ia,[10] as well as the effect of Qian-Hu coumarins on histamine release and calcium influx into mast cells,[11] their inhibitory effects on human platelet aggregation induced by ADP,[12] and anti-tumor-promoting activity of Pd-II.[13] The present paper describes the isolation and structural elucidation of two new coumarin glycosides, praerosides VI (**1**) and VII (**2**) (Figure 1).

2. Results and discussion

Praeroside VI (**1**) was obtained as a white amorphous powder, and it exhibited an $[M + H]^+$ peak at *m/z* 557.1878 in HRF-ABMS (positive), indicating the molecular formula to be $C_{25}H_{32}O_{14}$. The IR spectrum showed absorptions for hydroxyl groups (3380 cm^{-1}), α-pyrone ring (1715 cm^{-1}), and aromatic ring (1600, 1585, and 1500 cm^{-1}). Acid hydrolysis of **1** with ethanolic 2 N H_2SO_4 afforded an aglycone, which was identified by comparing with an authentic sample of (+)-*cis*-khellactone (**6**) and two sugars. In the ^1H NMR spectrum of **1** (Table 1), two pairs of doublets at δ 6.25 and 7.87 (each 1H, d, $J = 9.6$ Hz) and at δ 7.48 and 6.78 (each 1H, d, $J = 8.8$ Hz) are in agreement with the H-3 and H-4 signals of the α-pyrone ring system and significant *ortho*-coupling signals due to H-5 and H-6 on the angular coumarin ring, respectively. A pair of doublets at δ 4.02 and 5.35 (each 1H, d, $J = 4.4$ Hz) was assigned to the methine protons at C-3′-H and C-4′-H with oxygen-bearing groups. The complex signals appearing at δ 3.3–4.0 corresponded to the protons of two sugar moieties. Two doublets at δ 4.64 (1H, d, $J = 7.6$ Hz) and 5.00 (1H, d, $J = 2.4$ Hz) were assigned to two anomeric protons. As reported in the literature,[2,14] two close singlets at δ 1.48 and 1.49 ($\Delta\delta = 0.01$)

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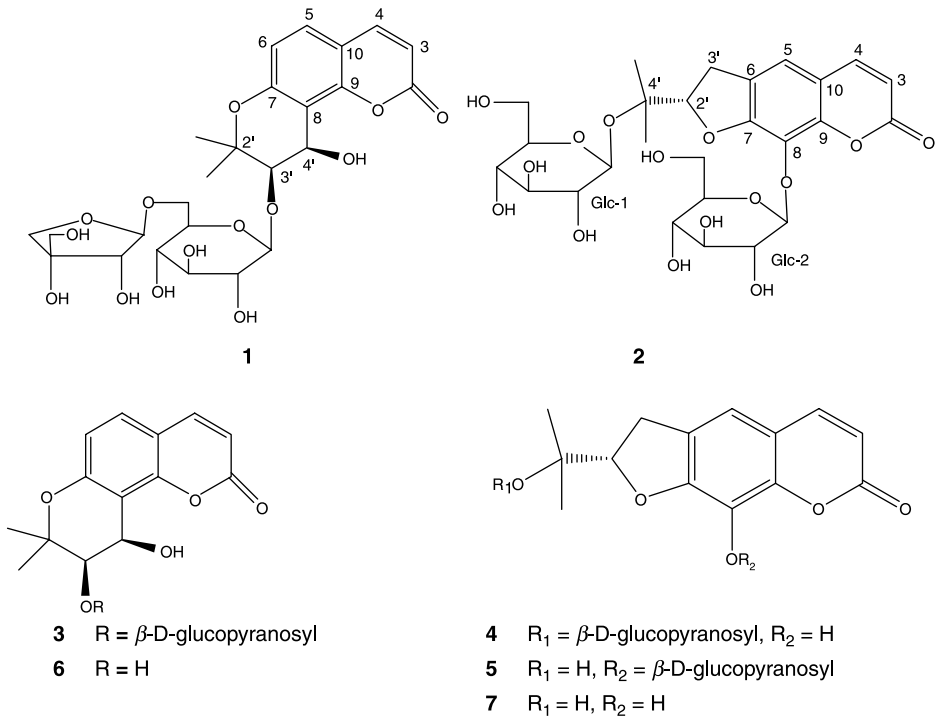

Figure 1. Structures of compounds **1**–**7**.

due to the 2′-*gem*-dimethyl groups of a dihydropyran ring indicate a *cis*-configuration at H-3′ and H-4′. Moreover, this assignment is also supported by the large chemical shift difference in the *gem*-methyl carbon signals ($\Delta\delta = 4.4$).[6]

The ^{13}C NMR spectrum of **1** (Table 1) showed 14 carbon signals due to the pyranocoumarin nucleus,[3] which were assigned by comparing with those of praeroside II (**3**),[3] except for 11 carbon signals due to the sugar moieties. Thus, compound **1** was shown to be a khellactone glycoside having a disaccharide moiety. After acid hydrolysis, the sugar constituents of **1** were treated with a trimethylsilylation reagent to give the corresponding trimethylsilyl (TMS) ethers, which were identified by gas chromatography (GC) with authentic samples of glucose-TMS and apiose-TMS, respectively. The anomeric carbon signals of sugar moieties of **1** appeared at δ 102.7 and 110.6, and the upfield shift of the former was assigned to the anomeric carbon of D-glucopyranoside and the latter was assigned to the anomeric carbon of D-apiofuranoside. The signals of glucose moiety of **1** were almost identical with those of **3** with the exception of a signal at δ 68.4 (approx. $\Delta\delta + 7.3$), which must be attributed to C-6 of glucose. Therefore, C-6 of glucose is joined to C-1 of apiose. Both the anomeric configurations of glucose and apiose were determined to be β from the coupling constants of the anomeric proton signals in the ^{1}H NMR spectrum of **1**. In the above-mentioned evidence, the structure of **1** was determined as (+)-*cis*-khellactone-3′-*O*-β-D-apiofuranosyl (1 → 6)-β-D-glucopyranoside.

Praeroside VII (**2**) was obtained as a white amorphous powder with $[\alpha]_D^{20} = -42.4$ ($c = 1.0$, H_2O). Its molecular formula of $C_{26}H_{34}O_{15}$ was established by HRFABMS (positive), which exhibited a pseudomolecular ion peak $[M + H]^+$ at *m/z* 587.1967. The IR spectrum of **2** showed the presence of a coumarin skeleton with the absorptions at 3380

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** in CD_3OD (500 and 125 MHz, respectively, *J* in Hz).

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
2	–	162.8	–	163.0
3	6.25 (d, $J = 9.6$)	112.6	6.20 (d, $J = 9.2$)	112.2
4	7.87 (d, $J = 9.6$)	145.7	7.82 (d, $J = 9.2$)	146.1
5	7.48 (d, $J = 8.8$)	130.1	7.13 (s)	118.8
6	6.78 (d, $J = 8.8$)	115.4	–	128.2
7	–	157.4	–	154.2
8	–	111.4	–	128.6
9	–	155.4	–	147.7
10	–	113.5	–	114.7
2′	–	80.5	4.94 (t, $J = 9.2$)	92.6
3′	4.02 (d, $J = 4.4$)	79.9	overlapped	31.0
4′	5.35 (d, $J = 4.4$)	60.1	–	79.1
gem-$(Me)_2$	1.48 (s)	22.3	1.37 (s)	23.3
	1.49 (s)	26.7	1.38 (s)	22.5
Glucose-1				
1	4.64 (d, $J = 7.6$)	102.7	4.68 (d, $J = 7.6$)	98.8
2	3.31 (m)	75.4	3.12 (t, $J = 6.8$)	75.2
3	3.42 (d, $J = 9.2$)	77.2	3.22–3.34 (m) [a]	78.2
4	3.33 (m)	71.4	3.35–3.40 (m) [a]	71.3
5	3.43 (m)	77.7	3.17 (m) [a]	77.8
6-α	3.62 (dd, $J = 6.1, 11.3$)	68.4	3.49–3.53 (m) [a]	62.3
6-β	3.98 (dd, $J = 1.8, 11.3$)		3.80 (m) [a]	
Glucose-2				
1			5.41 (d, $J = 7.6$)	103.1
2			3.30 (m) [a]	75.1
3			3.22–3.34 (m) [a]	77.7
4			3.35–3.40 (m) [a]	71.1
5			3.17 (m) [a]	77.8
6-α			3.49–3.53 (m) [a]	62.3
6-β			3.80 (m) [a]	
Apiose				
1	5.00 (d, $J = 2.4$)	110.6		
2	3.86 (d, $J = 2.4$)	78.1		
3	–	79.3		
4	3.73 (d, $J = 9.4$)	74.8		
	3.92 (d, $J = 9.4$)			
5	3.53 (brs)	65.5		

[a] Overlapping signals.

(OH), 1720 (α-pyrone), 1615, 1560, and 1510 cm^{-1} (aromatic ring). On acid hydrolysis, **2** afforded rutaretin (**7**) as an aglycone and D-glucose as a sugar, which was identified by GC comparing with authentic sample of D-glucose-TMS derivative. The ^{1}H NMR spectrum (Table 1) in the aromatic proton region of **2** showed a pair of doublets at δ 6.20 and 7.82 (each 1H, d, $J = 9.2$ Hz), which are identical with the signals of H-3 and H-4 of α-pyrone ring system, and a distinct singlet at δ 7.13 (1H, s), which is ascribable to H-5 of an aromatic proton in the coumarin ring. In addition, two methyl singlets at δ 1.37 and 1.38 demonstrated the existence of a hydroxyl-isopropyl moiety, and a characteristic signal at δ 4.94 (1H, d, $J = 9.2$ Hz) was assigned to a methine proton at C-2′ attached to the hydroxyl-isopropyl group. In the sugar proton region, complex signals appeared at δ 3.1–3.8 corresponding to the

protons of D-glucose moieties. Two doublet signals at δ 4.68 (1H, d, $J = 7.6$ Hz) and 5.41 (1H, d, $J = 7.6$ Hz) were assigned to two anomeric protons, and these large coupling constants indicated a diaxial coupling of H-1 and H-2 of β-D-glucopyranoside, respectively. The ^{13}C NMR spectrum of **2** showed 14 carbon signals of an aglycone and 12 carbon signals of sugar moieties (Table 1). The signals of the aglycone moiety in **2** were easily assigned by comparing with those of isorutarin[4] (**4**) and rutarin (**5**).[4] The signal at δ 79.1 in the ^{13}C NMR spectrum of **2** assigned to C-4′ was low-field shifted by approximately $\Delta\delta$ 10 due to glycosylation in comparison with the corresponding signal of **5**, indicating that a glucose is linked at C-4′. This was further confirmed by the HMBC experiment, which showed long-range correlations between the proton signal at δ 4.68 (H-1 of glucose-1) and the carbon signal at δ 79.1 (C-4′), and between the proton signal at δ 5.41 (H-1 of glucose-2) and the carbon signal at δ 128.6 (C-8). On the basis of the above evidence, the structure of **2** has been established as rutaretin-4′-*O*-β-D-glucopyranosyl-8-*O*-β-D-glucopyranoside.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X-4 digital micro-melting point apparatus and are uncorrected. The UV spectra were recorded on a Shimadzu UV-2401 spectrometer and IR spectra (KBr disks) on a NICOLET AVATER-360 spectrophotometer. Optical rotations were obtained on a Perkin–Elmer 243B digital polarimeter. HRFABMS spectra were obtained on a Bruker APEX II FT-ICRMS spectrometer. Semipreparative HPLC were carried out on a Waters model 600 instrument (Waters column Prep. NovaPak HR C_{18} 300 × 10 mm i.d. 6 μ; flow rate: 2.5 ml/min) with a 2487 dual λ absorbance detector (detection wavelength 320 nm). Column chromatography was carried out using silica gel (Qingdao Marine Chemical Industry, 200–300 mesh) and Sephadex LH-20 (Pharmacia). GC analysis was carried out on an Agilent 6890N gas chromatograph using an HP-5 capillary column (28 m × 0.32 mm i.d.): detection, FID; detector temperature, 260°C; column temperature, 180°C; carrier gas, N_2; flow rate, 40 ml/min.

3.2 Plant material

The roots of *P. praeruptorum* were collected in Lin'an City, Zhejiang Province, China, in August 2000, and identified by Professor Toru Okuyama of Meiji Pharmaceutical University. A voucher specimen (QH20000816) has been deposited at the Herbarium of Modern Research Center for Traditional Chinese Medicine, Peking University Health Science Center, Beijing, China.

3.3 Extraction and isolation

The air-dried roots of *P. praeruptorum* (1.6 kg) were extracted with MeOH (15 l) three times under reflux. After the removal of the solvent under reduced pressure at 60°C, the residue (155 g) was suspended in water (1 l) and defatted with petroleum ether (1 l). The aqueous layer was further extracted with ethyl acetate (2 l) and *n*-butanol (4 l) successively to obtain the EtOAc extract (25 g) and *n*-butanol extract (20 g). The *n*-butanol extract was subjected to a silica gel column (200–300 mesh, 200 g) and eluted with a gradient of $CHCl_3$–MeOH (from 10: 1 to 1: 1, v/v) to afford 12 fractions (Fr. 1–12) on the basis of TLC analyses. Fraction 3 was subjected to semipreparative HPLC [CH_3CN–H_2O (2: 5)] to afford **1** (25.2 mg). Fraction 6 was chromatographed on a Sephadex LH-20 column eluting with MeOH–H_2O (1: 1) to give **2** (35.2 mg).

3.3.1 Praeroside VI (*1*)

A white amorphous powder (MeOH); mp 109–111°C; $[\alpha]_D^{20} = -46.5$ ($c = 0.85$, MeOH); UV (CH_3OH) λ_{max} (log ε): 327 (3.04) nm; IR (KBr) ν_{max} (cm^{-1}): 3380, 1715, 1600, 1585, 1500; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectral data, see Table 1; HRFABMS

m/z 557.1878 $[M + H]^+$ (calcd for $C_{25}H_{33}O_{14}$, 557.1870).

3.3.2 *Praeroside VII (2)*

A white amorphous powder (MeOH); mp 146–148°C; $[\alpha]_D^{20} = -42.4$ ($c = 1.0$, H_2O); UV (CH_3OH) λ_{max} (log ε) 332 (2.69) nm; IR (KBr) ν_{max} (cm^{-1}): 3380, 1720, 1615, 1560, 1510; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectral data, see Table 1; HRFABMS *m/z* 587.1969 $[M + H]^+$ (calcd for $C_{26}H_{35}O_{15}$, 587.1976).

3.3.3 *Acid hydrolysis of praerosides VI (1) and VII (2)*

Compound **1** (10 mg) was refluxed with ethanolic 2 N H_2SO_4 (4 ml) for 2 h. After cooling, the reaction mixture was neutralized with Amberlite resin IRA-47, extracted with $CHCl_3$ (3 ml × 3), and dried over anhydrous Na_2SO_4. The $CHCl_3$ fraction was concentrated *in vacuo*, and the residue was subjected to HPLC [column: Waters, MeOH–H_2O (65: 35)] to obtain the aglycone (4 mg), mp 172.5–174.0°C, $[\alpha]_D = +61.5$ ($CHCl_3$), which was identified by comparison with (+)-*cis*-khellactone (**6**).

The aqueous layer was evaporated, dissolved in anhydrous pyridine (100 μl), 0.1 M L-cysteine methyl ester hydrochloride (200 μl) was added, and the mixture was warmed at 60°C for 1 h. Then, the trimethylsilylation reagent HMDS–TMCS (hexamethyl disilazane–trimethylchlorosilane–pyridine, 2: 1: 10) was added and warmed at 60°C for 30 min. The supernatant was subjected to GC for sugar identification. D-Apiose ($t_R = 5.12$ min) and D-glucose ($t_R = 12.50$ min) were identified with the authentic samples.

Compound **2** (20 mg) was treated under the same conditions as applied for **1**. The reaction mixture was worked up in the above-mentioned way to give an aglycone (6 mg), mp 192.0–193.0°C, $[\alpha]_D = -32.5$ ($CHCl_3$), identical with an authentic sample of rutaretin (**7**) by comparison of mixed fusion and co-TLC on direct comparison. D-Glucose was confirmed by the comparison of its retention time ($t_R = 12.50$ min) with that of the authentic standard.

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