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COUMARINS FROM *PEUCEDANUM PRAERUPTORUM*

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Key Word Index—*Peucedanum praeruptorum*; Umbelliferae; qianhucoumarin H; angular dihydropyranocoumarins.

Abstract—A new angular dihydropyranocoumarin named qianhucoumarin H and four known coumarins, peucedanocoumarin I, 5,8-dimethoxypsoralen, isoscopoletin, umbelliferone along with anchoic acid were isolated from the root of *Peucedanum praeruptorum*. By spectral analysis and chemical evidence, the structure of qianhucoumarin H was elucidated as 3′(*S*)-angeloyloxy-4′(*R*)-isovaleryloxy-3′,4′-dihydroseselin and the absolute configurations were established by chemical correlations with known compounds.

INTRODUCTION

The root of *Peucedanum praeruptorum* Dunn is known as a traditional Chinese medicine (Qianhu). It has the action of expelling wind-evil and clearing away heat as well as lowering the adverse-rising energy and dissipating phlegm. Qianhu can be used to cure some diseases such as cough caused by pathogenic wind-heat, accumulation of phlegm and heat in the lung. The major constituents of the plant are angular dihydropyranocoumarins; some of these compounds have calcium antagonistic activity [1]. We have described the structural analysis of several new coumarins [2, 3]. In the present paper, we report the isolation and elucidation of six compounds, qianhucoumarin H (**1**), peucedanocoumarin I (**2**), 5,8-dimethoxypsoralen (**3**), isoscopoletin (**4**), umbelliferone (**5**) and anchoic acid (**6**). By combination of spectral analysis and chemical evidence, the structure of **1** was elucidated as 3′(*S*)-angeloyloxy-4′(*R*)-isovaleryloxy-3′,4′-dihydroseselin and the absolute configurations were established by chemical correlation with known compounds.

RESULTS AND DISCUSSION

Compound **1**, isolated as needles, had the $[M]^+$ peak at *m/z* 428.4835 in the high resolution electron ionization mass spectrum which indicated the molecular formula was $C_{24}H_{28}O_7$. The absorption bands of a carbonyl (1722 cm^{-1}) and an aromatic system (1612 cm^{-1}, 1492 cm^{-1}) in the IR spectrum are characteristic of a coumarin skeleton. The ^1H NMR spectrum in the aromatic proton region of **1** contained two pairs of doublets at δ6.23 (1H, *d*, J = 9.5 Hz), 7.61 (1H, *d*, J = 9.5 Hz) and at δ7.37 (1H, *d*, J = 8.6 Hz), 6.82 (1H, *d*, J = 8.6 Hz), which are in agreement with the H-3 and H-4 signals of the α-pyrone ring system and signals of H-5 and H-6 of the benzene ring, indicating that **1** is a coumarin substituted at the C-7 and C-8 positions. A pair of doublets at δ5.38 (1H, *d*, J = 3.5 Hz) and 6.27 (1H, *d*, J = 3.5 Hz) was assigned to the methine protons at H-3′ and H-4′ attached to two ester groups, showing C-7 and C-8 of **1** formed a dihydropyran ring. The signals at δ6.12 (1H, *m*), 1.97 (3H, *br d*), 1.88 (3H, *br s*) are due to an angeloyl group and at δ2.21 (2H, *m*), 1.11 (1H, *m*), 0.98 (3H, *d*, J = 6.3 Hz), 0.96 (3H, *d*, J = 6.2 Hz) are due to an isovaleryl group.

On partial alkaline hydrolysis, compound **1** produced another new coumarin, identified as 3′(*S*)-angeloyloxy-4′(*R*)-hydroxy-3′,4′-dihydroseselin (**7**). From this evidence it was deduced that in compound **1** the angeloyloxy group is linked to the C-3′ and the isovaleryloxy is linked to C-4′.

The molecular structure of **1** contained two chiral carbon atoms, and the relative configuration was determined from the ^1H NMR spectrum. The configuration at C-3′ and C-4′ was *trans* on the basis of the coupling constant of H-3′ and H-4′ (3.5 Hz) and the difference (0.08 ppm) in the methyl proton signals at δ1.46 and 1.38 of the 2′-gem dimethyl group [4–6].

Compound **1** is an optically active compound and its absolute configurations were determined by chemical correlation with known compounds. On total alkaline hydrolysis, compound **1** gave a mixture of two products

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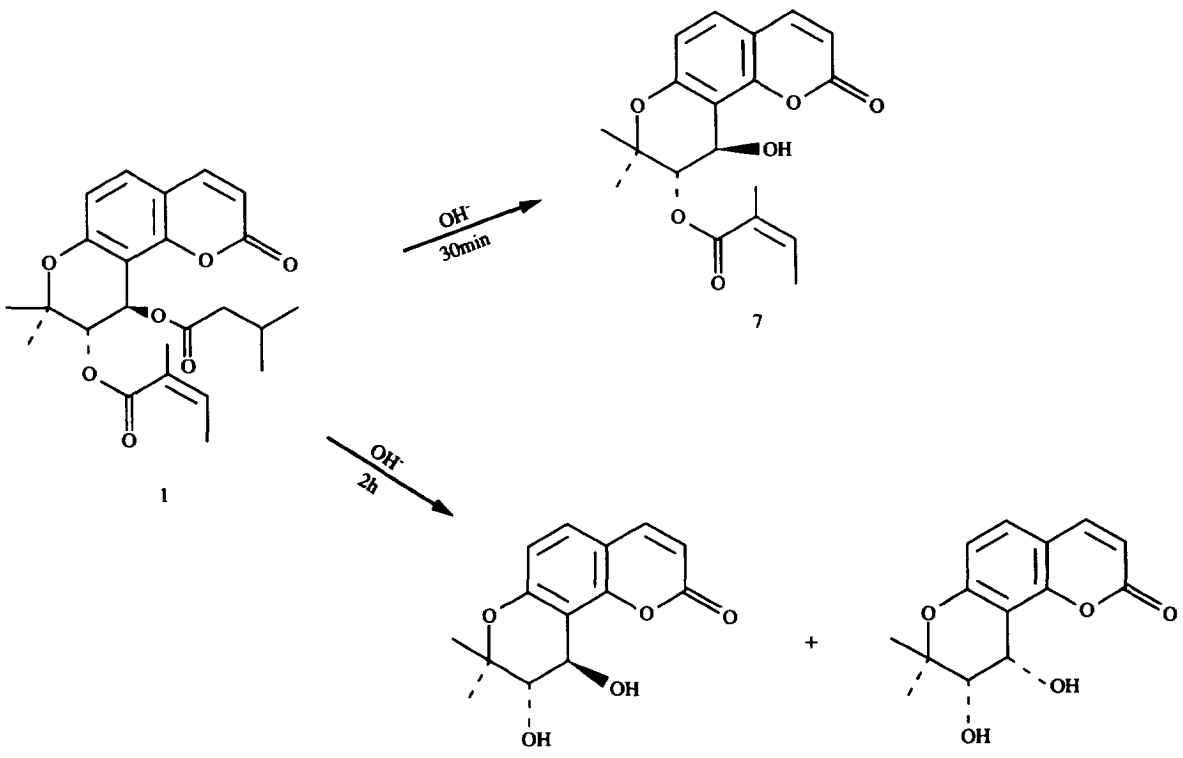

1 7

(+)-*trans*-Khellactone (-)-*cis*-Khellactone

8 9

after HPLC separation. By spectral analysis and optical activity these compounds were identified as (+)-*trans*-khellactone (**8**) and (−)-*cis*-khellactone (**9**). The latter occurs as a minor artefact resulting from epimerization at C-4′ because of the benzyl effect and SN_2 reaction mechanism. The absolute configurations of **8** and **9** were described previously as 3′*S*,4′*R* and 3′*S*,4′*S* by chemical correlation with known compounds and X-ray diffraction analysis [7, 8]. Therefore, the absolute configurations of **1** were established as 3′*S*,4′*R*. The chemical structure was finally elucidated as 3′(*S*)-angeloyloxy-4′(*R*)-isovaleryloxy-3′,4′-dihydroseselin.

EXPERIMENTAL

Mps were determined on a Yanaco MP-S micro-melting point apparatus, the themometer was uncorr. UV spectra were obtained on a Shimadzu UV-260 spectrophotometer in MeOH soln. IR spectra were recorded on a Perkin-Elmer 599B spectrometer. ^{1}H NMR and ^{13}C NMR spectra were taken on a Varian XL-400 spectrometer using tetramethylsilane as an internal standard, EI-MS were measured on a JEOL-DX-300 mass spectrometer. $[\alpha]_D$ values were determined on a Perkin-Elmer 241 automatic polarimeter at 20°.

Silica gel H (10–40 μ) was used for CC. Petrol refers to that fraction having bp 60–90°. Prep. HPLC was carried out on a Shimadzu Liquid Chromatograph LC-6A equipped with a UV detector, using a Shim-Pack PREP-SIL column (10 mm × 250 mm, Shimadzu): detector wavelength: 320 nm; flow rate: 2.0 ml min^{-1}; mobile phase: cyclohexane–EtOAc (85:15) for purification of natural compound; cyclohexane–EtOAc (50:50) for sepn of products of partial alkaline hydrolysis, and $CHCl_3$–MeOH (98:2) for sepn of products of total alkaline hydrolysis.

Plant material. Roots of *P. praeruptorum* Dunn were collected in Wuyi County, Zhejiang Province, China in September, 1990 and identified by Prof. Chunquan Xu, Shenyang Pharmaceutical University, China.

Isolation and identification. The root material was extracted with petrol, the concd extract was applied to silica gel CC and eluted by petrol–EtOAc mixtures of increasing polarities to yield six major frs (I–VI). Pure compounds **1** (53 mg) and **2** (16 mg) were obtained from fr. I by further HPLC separation. Compound **3** (27 mg) was isolated from fraction II CC on a small column using petrol–EtOAc (90:10) as eluent. Compounds **4** (38 mg) and **5** (65 mg) were individually sepd from fraction IV by repeated silica gel CC eluted by petrol–Me_2CO (85:15). Compound **6** (41 mg) was purified also using a small silica gel column eluted by petrol–Me_2CO (75:25).

Qianhucoumarin H (**1**). White needles, mp 158.0–160.0° (petrol–EtOAc). $[\alpha]_D^{20}$ + 15.6° (*c* 0.1, $CHCl_3$). HR-MS: M_r Calcd. 428.4867 for $C_{24}H_{28}O_7$, Obs. 428.4835. EI-MS *m/z* (rel. int.): 428 $[M]^+$ (8.5), 328 [M − angelic acid]$^+$ (14.6), 327 [M − isovaleryloxy]$^+$ (2.6), 244 (42.8), 229 (85.6), 213 (11.2), 191 (6.5), 85 (28.6), 83 (100), 57 (42.1), 55 (57.4). UV λ_{max}^{MeOH} nm: 224.0, 256.2, 325.8. IR ν_{max} cm^{-1}: 2974, 2926, 2878, 1745, 1722, 1612, 1492, 1385, 1296, 1283, 1254, 1235, 1124, 1018, 842. ^1H NMR (400 Hz, $CDCl_3$): δ6.23 (1H, *d*, *J* = 9.5 Hz, H-3), 7.61 (1H, *d*, *J* = 9.5 Hz, H-4), 7.37 (1H, *d*, *J* = 8.6 Hz, H-5), 6.82 (1H, *d*, *J* = 8.6 Hz, H-6), 5.38 (1H, *d*, *J* = 3.5 Hz, H-3′), 6.27 (1H, *d*, *J* = 3.5 Hz, H-4′), 1.46 (3H, *s*, *C*-2′–CH_3), 1.38 (3H, *s*, C-2′–CH_3), 6.12 (1H, *m*, H-3″), 1.97 (3H, *br d*, H-4″), 1.88 (3H, *br s*, H-5″), 2.21 (2H, *m*, H-2‴), 1.11 (1H, *m*, H-3‴), 0.98 (3H, *d*, *J* = 6.3 Hz, H-4‴), 0.96 (3H, *d*, *J* = 6.2 Hz, H-5‴). ^{13}C NMR (100 MHz, $CDCl_3$): δ159.71 (C-2), 113.35 (C-3), 143.26 (C-4), 128.98 (C-5), 114.38 (C-6), 156.61 (C-7), 107.52 (C-8), 154.16 (C-9), 112.27 (C-10), 77.12 (C-2′), 72.14 (C-3′), 63.42 (C-4′), 23.86 (C-2′–CH_3), 23.58 (C-2′–CH_3), 166.41 (C-1″), 126.82 (C-2″), 139.81 (C-3″), 15.46 (C-4″), 20.62 (C-5″), 171.43 (C-1‴), 43.26 (C-2‴), 25.59 (C-3‴), 22.51 (C-4‴), 22.18 (C-5‴).

Partial alkaline hydrolysis of **1**. A soln of compound **1** (10 mg) in dioxane (1 ml) containing 0.5 N KOH (0.5 ml) was stirred at room temp. for 30 min. The reaction mixture was acidified with 10% H_2SO_4, extracted with $CHCl_3$, and evapd, to yield a major product, which was subjected to HPLC. The resultant white needles (3 mg) were 3′(*S*)-angeloyloxy-4′(*R*)-hydroxy-3′,4′-dihydroseselin (**7**). Mp 130.5 − 132.0° (petrol–EtOAc). $[\alpha]_D^{20}$ + 11.4° (*c* 0.05, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ6.26 (1H, *d*, *J* = 9.5 Hz, H-3), 7.64 (1H, *d*, *J* = 9.5 Hz, H-4), 7.34 (1H, *d*, *J* = 8.6 Hz, H-5), 6.82 (1H, *d*, *J* = 8.6 Hz, H-6), 5.27 (1H, *d*, *J* = 3.7 Hz, H-3′), 5.09 (1H, *d*, *J* = 3.7 Hz, H-4′), 1.50 (3H, *s*, C-2′–CH_3), 1.41 (3H, *s*, C-2′–CH_3), 6.10 (1H, *m*, H-3″), 1.92 (3H, *br d*, H-4″), 1.87 (3H, *br s*, H-5″). EI-MS *m/z*: 344 $[M]^+$, 244 [M − angelic acid]$^+$, 230, 229 (100), 191, 170, 153, 83, 55.

Total alkaline hydrolysis of **1**. Compound **1** (20 mg) dissolved in dioxane (1.5 ml) was added to 0.5 N KOH (3 ml) and the reaction mixt. was stirred at 60° for 2 hr. The soln was neutralized with 10% H_2SO_4, extracted with $CHCl_3$, washed with 10% $NaHCO_3$, dried with Na_2SO_4, and evapd; the residue was subjected to HPLC to yield two products. The first eluant (3 mg), which contained the epimerization artefact at C-4′, gave (−)-*cis*-khellactone (**9**): white needles, mp 171.0–172.5° (cyclohexane–EtOAc), $[\alpha]_D^{20}$ − 74.1° (*c* 0.05, $CHCl_3$). ^1H NMR (500 MHz, CD_3OD): δ6.25 (1H, *d*, *J* = 9.5 Hz, H-3), 7.88 (1H, *d*, *J* = 9.5 Hz, H-4), 7.46 (1H, *d*, *J* = 8.6 Hz, H-5), 6.77 (1H, *d*, *J* = 8.6 Hz, H-6), 3.76 (1H, *d*, *J* = 4.9 Hz, H-3′), 5.10 (1H, *d*, *J* = 4.9 Hz, H-4′), 1.43 (3H, *s*, C-2′-CH_3), 1.42 (3H, *s*, C-2′–CH_3), 3.35 (1H, *s*, OH), 2.15 (1H, *s*, OH). EI-MS *m/z*; 262 $[M]^+$, 191 (100), 162, 134, 72. The second eluant (5 mg) gave (+)-*trans*-khellactone (**8**): white needles, mp 183.0–184.5° (cyclohexane–EtOAc), $[\alpha]_D^{20}$ +17.8° (*c* 0.05, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ6.24 (1H, *d*, *J* = 9.5 Hz, H-3), 7.65 (1H, *d*, *J* = 9.5 Hz, H-4), 7.31 (1H, *d*, *J* = 8.6 Hz, H-5), 6.78 (1H, *d*, *J* = 8.6 Hz, H-6), 3.84 (1H, *d*, *J* = 6.7 Hz, H-3′), 4.99 (1H, *d*, *J* = 6.7 Hz, H-4′), 1.52 (3H, *s*, C-2′–CH_3), 1.30 (3H, *s*, C-2′–CH_3), 1.25 (2H, *s*, 2OH). EI-MS *m/z*: 262 $[M]^+$, 191 (100), 162, 134, 72.

Peucedanocoumarin I (**2**). Needles, mp 151.5–153.0° (petrol–EtOAc). The spectral data of UV, IR, ^1H NMR, ^{13}C NMR and EI-MS are identical to literature [9].

5,8-Dimethoxypsoralen (**3**). Crystals, mp 152.0–154.0° (petrol–EtOAc). IR ν_{max} cm^{-1}: 3025, 2960, 1725, 1624, 1612, 1545, 1466, 1380, 1354, 1262, 1145, 1120, 1072, 1025, 982. ^1H NMR (400 MHz, $CDCl_3$): δ6.24 (1H, *d*, *J* = 9.8 Hz, H-3), 8.10 (1H, *d*, *J* = 9.8 Hz, H-4), 7.59 (1H, *d*, *J* = 2.5 Hz, H-2′), 6.95 (1H, *d*, *J* = 2.5 Hz, H-3′), 4.20 (3H, *s*, OCH_3), 4.25 (3H, *s*, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ160.91 (C-2), 113.54 (C-3), 140.15 (C-4), 144.68 (C-5), 115.20 (C-6), 150.34 (C-7), 127.97 (C-8), 143.12 (C-9), 108.11 (C-10), 145.18 (C-2′), 105.62 (C-3′), 62.31 (OCH_3), 62.55 (OCH_3). EI-MS *m/z*: 246 $[M]^+$, 231 (100), 203, 188, 175, 160, 147, 104.

Isoscopoletin (**4**). Yellow crystals, mp 182.0–184.0° (petrol–Me_2CO), IR ν_{max} cm^{-1}: 3320, 2965, 2920, 1705, 1628, 1612, 1575, 1464, 1430, 1281, 1204, 1135, 1014, 845. ^1H NMR (400 MHz, DMSO): δ6.11 (1H, *d*, *J* = 9.5 Hz, H-3), 7.78 (1H, *d*, *J* = 9.5 Hz, H-4), 6.91 (1H, *s*, H-5), 6.98 (1H, *s*, H-8), 3.82 (3H, *s*, OCH_3). EI-MS *m/z*: 192 $[M]^+$ (100), 191, 164, 163, 135, 69.

Umbelliferone (**5**). Crystals, mp 224.5–226.5° (petrol–acetone). UV λ_{max}^{MeOH} nm: 244.3, 257.6, 320.6, 324.2. IR ν_{max} cm^{-1}: 3175, 1690, 1628, 1605, 1568, 1512, 1413, 1384, 1320, 1236, 1204, 1133, ^1H NMR (400 MHz, DMSO): δ6.10 (1H, *d*, *J* = 9.5 Hz, H-3), 7.79 (1H, *d*, *J* = 9.5 Hz, H-4), 7.42 (1H, *d*, *J* = 8.4 Hz, H-5), 6.77 (1H, *dd*, *J* = 8.4, 2.2 Hz, H-6), 6.71 (1H, *d*, *J* = 2.2 Hz, H-8). ^{13}C NMR (100 MHz, DMSO): δ160.82 (C-2), 112.23 (C-3), 144.42 (C-4), 129.73 (C-5), 113.37 (C-6), 162.02 (C-7), 102.84 (C-8), 156.31 (C-9), 112.23 (C-10). EI-MS *m/z*: 162 $[M]^+$ (100), 134, 106, 105, 78, 77, 51.

Anchoic acid (**6**). Crystals, mp 107.0–108.0° ($CHCl_3$). IR ν_{max} cm^{-1}: 3200 to around 2800, 1688, 1463, 1430, 1350, 1338, 1260, 1244, 1220, 1200, 1185, 1121, 1089, 998, 915. ^1H NMR (400 MHz, CD_3OD): δ2.28 (4H, *t*, *J* = 7.4 Hz, $2CH_2$), 1.60 (4H, *t*, *J* = 6.8 Hz, $2CH_2$), 1.35 (6H, *s*, $3CH_2$). ^{13}C NMR (100 MHz, CD_3OD): δ177.70 (*s*, $2CO_2H$), 35.00 (*t*, $2CH_2$), 30.10 (*t*, $2CH_2$), 26.08 (*t*, $3CH_2$). EI-MS *m/z*: 152 [M − $2H_2O$] $^+$, 124, 111, 110, 98, 97, 84, 83, 73, 69, 60, 55 (100), 41.

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