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A New Coumarin from the Stem of *Angelica dahurica*

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One new and three known coumarins were isolated from the $CHCl_3$ soluble fraction of *Angelica dahurica* stem. On the basis of spectral data, the structures of the isolated compounds were determined to be scopoletin, angelol I, angelol H and 6-[(1S), 2(R)-2, 3-dihydroxy-1-methoxy-3-methylbutyl]-7-methoxycoumarin; the latter being isolated for the first time from a plant source.

Key words: *Angelica dahurica*, Umbelliferae, coumarins, 6-[(1S), 2(R)-2, 3-dihydroxy-1-methoxy-3-methylbutyl]-7-methoxycoumarin, NMR

INTRODUCTION

The *Angelica dahurica*(umbelliferae) root has been used korean traditional medicine as an analgesic (Kim *et al.* 1998). To date, over twenty coumarins have been isolated from this plant (Fujiwara *et al.* 1980; Kim *et al.* 1992; Kwon *et al.* 1997). We were interested in the chemical constituents of *A. dahurica* stem. Repeated column chromatography of the $CHCl_3$ soluble fraction led to the isolation of four compounds. This paper deals with their isolation and the elucidation of their structure.

MATERIALS AND METHOD

Instruments and reagents

Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. Optical rotations were measured with a JASCO DIP-1000 instrument. Nuclear magnetic resonances (^{13}C-NMR and ^{1}H-NMR spectra taken at 50, 125, 200 and 500MHz) were recorded on a Varian Gemini 200 and Buruker AMX-500 spectrometers using deuterated solvents as the internal standard. The EI/MS (70 eV) and CI/MS (methane) spectra were determined using an Autospec Micromass, UV spectra using a Hitachi U-2000, and IR spectra in a KBr disk using a Bio-Rad FTS-7. TLC work was carried out using plates coated with silica gel 60 F254 (Merck Co.). All solvents were routinely distilled prior to use. Silica gel column chromatography was performed on Merck silica gel 60 (70-230 mesh and 230-400 mesh). Other reagents were commercial grade without purification.

Plant material

The stem of *A. dahurica* was collected at Mt. Samak, Korea in September 2000 and identified taxonomically with respect to morphology. A voucher specimen of the plant was deposited at the College of Pharmacy, Kangwon National University.

Extraction and isolation

The air-dried stems (1.2 kg) were ground and extracted three times with hot MeOH over a total 4 h period. The resultant extracts were combined and concentrated under reduced pressure to afford 160 g of the residue. This MeOH extract was suspended in 10 volumes of water and then partitioned successively with equal volumes of n-hexane, $CHCl_3$, and *n*-BuOH, leaving a residual water soluble fraction. Each fraction was evaporated in vaccuo to yield the residues of *n*-hexane fraction(fr.), (63 g), $CHCl_3$ fr., (5.3 g), and *n*-BuOH fr., (12 g).

The $CHCl_3$ soluble fraction (5.3 g) was column chromatographed on a silica gel (250 g, 70-230 mesh, 15 × 50 cm) using stepwise gradient elution with the solvents benzene-EtOAc (4:1, 2:1, 1:1, v/v) to divide the fraction into four sub-fractions (Fr.1-Fr.4).

Sub-fraction 3 was re-chromatographed on ODS (70 g,

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YMC gel, ODS-A, S-150 μm) and a Sephadex LH-20 (50 g, Pharmacia) column by elution with 50% MeOH to produce compound **1** (8 mg) and compound **2** (130 mg). Subfraction 4 was re-chromatographed on a silica gel column (80 g, 70-230 mesh, 2 × 50 cm) to produce compound **3** (23 mg) and compound **4** (27 mg).

Compound 1 (scopoletin); mp : 204-205°; UV λ_{max}: 211, 226.5, 252, 297, 341 nm (MeOH); IR ν_{max} (KBr)cm^{-1}: 3420 (OH), 1685 (C=O), 1620, 1560 (aromatic C=C); ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 7.60 (1H, d, J=9.4Hz, H-4), 6.92 (1H, s, H-5), 6.85 (1H, s, H-8), 6.19 (1H, d, J=9.4Hz, H-3), 3.95 (3H, s, -OCH_3).

Compound 2 (angelol I); $[\alpha]_D^{19}$ -124.04 (c 1.0, MeOH); UV λ_{max}: 222.5, 242.5(sh), 250.5, 294, 327 nm (MeOH); IR ν_{max} (KBr) cm^{-1}: 3465(OH), 1740 and 1706(C=O), 1619, 1562(aromatic C=C); EI-MS m/z (rel. int.): 378 (M+, 6.14), 289 (39.52), 218 (70.14), 205 (73.24), 189 (41.75), 175 (17.47), 85 (100), 57 (71.29); ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 7.70 (1H, d, J=9.4Hz, H-4), 7.69 (1H, s, H-5), 6.82 (1H, s, H-8), 6.30 (1H, d, J=9.4Hz, H-3), 5.68 (1H, br. s, H-11), 5.17 (1H, br. s, H-12), 4.76 (1H, br. s, -OH), 3.99 (3H, s, -OCH_3), 2.08~1.85 (3H, m, acyl protons), 1.30 (3H, s, -CH_3), 1.02 (3H, s, -CH_3), 0.79 (3H, d, J=6.4Hz, acyl-CH_3), 0.69 (3H, d, J=6.4Hz, acyl-CH_3); ^{13}C-NMR ($CDCl_3$) δ_C (ppm): 71.26 (C-1'), 160.77 (C-2), 158.47 (C-7), 154.66 (C-10), 143.05 (C-4), 125.81 (C-3 and C-6), 112.49 (C-5), 111.37 (C-9), 97.95 (C-8), 74.97 (C-13), 73.88 (C-11), 66.96 (C-12), 55.57 (-O$\underline{C}H_3$), 42.15 (C-2'), 27.31 (-$\underline{C}H_3$), 25.66 (C-3'), 24.59 (-$\underline{C}H_3$), 21.50 (C-4' and C-5').

Compound 3 (angelol H); $[\alpha]_D^{19}$ -76.71(c 1.0, MeOH); UV λ_{max}: 208.5, 220.5, 243.5(sh), 253.5(sh), 296, 326 nm (MeOH); IR ν_{max} (KBr) cm^{-1}: 3363 (OH), 1737 and 1704 (C=O), 1619, 1562 (aromatic C=C); EI-MS m/z (rel. int.): 378 (M^+, 4.82), 289 (27.61), 218 (64.38), 205 (100), 189(45.21), 175 (15.43), 85 (70.36); ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 7.59 (1H, d, J=9.4Hz, H-4), 7.52 (1H, s, H-5), 6.76 (1H, s, H-8), 6.22 (1H, d, J=5.8Hz, H-11), 6.18 (1H, d, J=9.4Hz, H-3), 3.92 (3H, s, -OCH_3), 3.81 (1H, d, J=5.8Hz, H-12), 2.73 (1H, br. s, -OH), 2.36 (1H, br. s, -OH), 2.19~1.88 (2H, m, acyl protons), 1.26 (3H, s, -CH_3), 1.21 (3H, s, -CH_3), 0.87 (6H, d, J=6.4Hz, acyl-$(CH_3)_2$); ^{13}C-NMR ($CDCl_3$) δ_C (ppm): 171.19 (C-1'), 160.51 (C-2), 159.64 (C-7), 154.84 (C-10), 143.05 (C-4), 127.95 (C-3), 123.91 (C-6), 112.73 (C-5), 111.58 (C-9), 98.52 (C-8), 78.09 (C-11), 71.91 (C-13), 68.88 (C-12), 55.76 (-O$\underline{C}H_3$), 42.86 (C-2'), 26.33 (-$\underline{C}H_3$), 24.98 (C-3'), 23.93 (-$\underline{C}H_3$), 21.72 (C-4'), 21.68 (C-5').

Compound 4 (6-[(1S, 2R)-2, 3- dihydroxy-1-methoxy-3-methylbutyl]-7-methoxycoumarin); $[\alpha]_D^{19.5}$ + 105(c 1.0, MeOH); UV λ_{max}: 213, 220.5, 243(sh), 252.5(sh), 293.5, 326 nm (MeOH); IR ν_{max} (KBr) cm^{-1}: 3447(OH), 1732(C=O), 1619, 1562(aromatic C=C); CI-MS m/z (rel. int.): 309 [$(M+1)^+$, 100], 219(56.53), 205(3.24), 177(3.12), 59(17.09); ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 7.62 (1H, d, J=9.4Hz, H-4), 7.42 (1H, s, H-5), 6.75 (1H, s, H-8), 6.19 (1H, d, J=9.4Hz, H-3), 4.88(1H, d, J=1.2Hz, H-11), 3.82 (3H, s, -OCH_3), 3.28 (1H, dd, J=7.9 and 1.2Hz, H-12), 3.24 (3H, s, -OCH_3), 3.14 (1H, br. s, OH), 2.72 (1H, d, J=7.9Hz, OH), 1.32 (3H, s, -CH_3), 1.20 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$) c (ppm): 161.65 (C-2), 160.04 (C-7), 155.68 (C-10), 144.30 (C-4), 127.64 (C-5), 124.29 (C-6), 113.63 (C-3), 112.61 (C-9), 99.23 (C-8), 77.35 (C-11), 77.19 (C-12), 73.61 (C-13), 57.33 (-O$\underline{C}H_3$), 56.44 (-O$\underline{C}H_3$), 27.05 (-$\underline{C}H_3$), 26.90 (-$\underline{C}H_3$); ORD (MeOH, c 0.1) $[\alpha]^{20}$ (nm): +39.1(360), +68.9 (354), +73.2(337), +46.6(330), +48.4(276), +45.3(272), +42.2(258).

RESULTS AND DISCUSSION

The $CHCl_3$-soluble fraction was separated using various chromatography modes to produce compounds **1-4**. Compound **1** was obtained as white needles by a variety of chromatography modes followed by re-crystallization (in *n*-hexane-EtOAc). The IR and UV spectra of compound **1** showed typical coumarin skeletons (Goodwin and Pollock, 1954). The ^{1}H-NMR spectrum of compound **1** exhibited signals due to protons of the C-3 and C-4 position of the coumarin ring at 6.19 (1H, d, J=9.4Hz) and 7.60 (1H, d, J=9.4Hz), due to the protons of the C-5 and C-8 positions at 6.92 (1H, s) and 6.85 (1H, s), and due to the methoxyl group at 3.95 (3H, s). Based on these results and on values previously reported in the literature (Steck and Mazurek, 1972), compound **1** was identified as scopoletin.

Compound **2** was obtained as white powder, $[\alpha]_D^{19}$ -124.04. The IR and UV spectra of compound **2** showed typical coumarin skeletons (Goodwin and Pollock, 1954). The ^{1}H-NMR spectrum of compound **2** exhibited signals due to protons of the C-3 and C-4 position of the

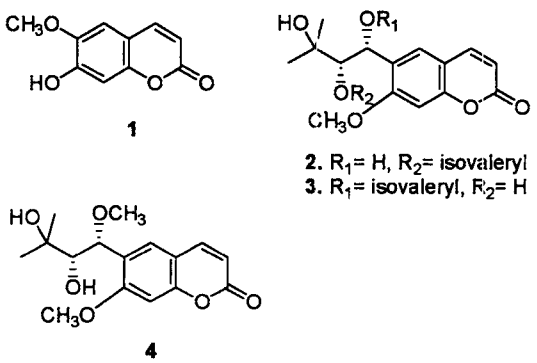

Fig. 1. The structures of compound **1**, **2**, **3** and **4**

coumarin ring at 6.30 (1H, d, J=9.4Hz) and 7.70 (1H, d, J=9.4Hz), due to the protons of the C-5 and C-8 positions at 7.69 (1H, s) and 6.82 (1H, s), due to the methoxyl group at 3.99 (3H, s) and due to a trioxyisopentyl group at 5.68 (1H, br s), 5.17 (1H, br s), 4.76 (1H, br s), 2.08 (1H, br s), 1.30 (3H, s) and 1.02 (3H, s). Furthermore, the ^{1}H-NMR spectrum of compound **2** showed signals arising from an isovaleryl group at 2.00 (2H, m), 1.85 (1H, m), 0.79 (3H, d, J=6.4Hz) and 0.69 (3H, d, J=6.4Hz) (Steck and Mazurek, 1972). In the MS spectrum of compound **2**, the fragmentation ion peaks at m/z 205, 175 and 85 also demonstrated that compound **2** possesses a trioxyisopentyl group and an isovaleryl group. These spectral data indicated that compound **2** was 7-methoxy-6-trihydroxyisopentylcoumarin, an angelol-type coumarin, and that one of the hydroxyl groups on the isopentyl group was esterified with isovaleric acid. In the ^{1}H-NMR spectrum of compound **2**, the signals arising from the two methine protons of the C-11 and C-12 positions were observed at 5.68 and 5.17, which suggested that the isovaleryl group of compound **2** was attached to C-12 position (Kozawa *et al.*, 1983). Based on these results and on values previously reported in the literature (Baba *et al.*, 1982; Kozawa *et al.*, 1983), compound **2** was identified as Angelol I.

Compound **3** was obtained as white power, $[\alpha]_D^{19}$ - 76.71. The IR and UV spectra of compound **3** showed coumarin skeletons. The ^{1}H-NMR spectrum of compound **3** exhibited signals arising from a trioxyisopentyl group at 6.22 (1H, d, J=5.8Hz), 3.81 (1H, d, J=5.8Hz), 2.19~1.88 (2H, m), 1.26 (3H, s) and 1.21 (3H, s) in addition to those from a 6, 7-disubstituted coumarin ring, a methoxyl group and an isovaleryl group. Therefore, it was initially assumed that compound **3** was the ester derivative of 7-methoxy-6-trihydroxyisopentylcoumarin having an isovaleryl group as the acyl moiety (Steck and Mazurek, 1972). But, the ^{1}H-NMR spectrum of compound **3** differed from that of compound **2**, and signals due to a *gem*-dimethyl group were seen at 1.26, 1.21 (each 3H, s) and due to two methine protons at 6.22, 3.81 (each 1H, d, J=5.8Hz). These spectral data suggested that an acyl moiety of compound **3** was attached to the C-11 position (Kozawa *et al.*, 1983). Based on these results and on values previously reported in the literature (Baba *et al.*, 1982; Kozawa *et al.*, 1983), compound **3** was identified as Angelol H.

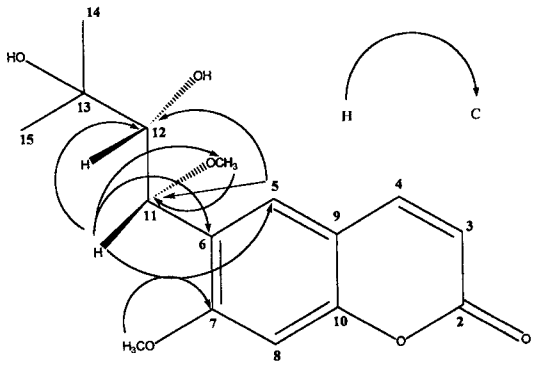

Fig. 2. The Structure and important HMBC correlations of compound **4**

Compound **4**, on the other hand, appeared to be a new compound, $[\alpha]_D^{19.5}$ + 105. The CI-MS spectrum of compound **4** showed a pseudo molecular ion peak at m/z 309 $[M+1]^+$. The UV, and IR spectra of compound **4** showed coumarin skeletons. The ^{1}H-NMR features of this compound were similar to those of Buntansin C (Wu *et al.*,

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound 4 in $CDCl_3$ (δ values in ppm)

C	δ_C	δ_H	Cross peaks(c) in HMBC spectrum
2	161.65		
3	113.63	6.19	161.65(2), 144.30(4), 112.61(9)
4	144.30	7.62	161.65(2), 113.63(3), 127.64(5), 112.61(9), 155.68(10)
5	127.64	7.42	144.30(4), 124.29(6), 160.04(7), 99.23(8), 112.61(9), 155.68(10), 77.35(11), 77.19(12)
6	124.29		
7	160.04		
8	99.23	6.75	124.29(6), 160.04(7), 112.61(9), 155.68(10)
9	112.61		
10	155.68		
11	77.35	4.88	127.64(5), 124.29(6), 160.04(7), 77.19(12), 56.44(OCH_3)
12	77.19	3.28	77.35(11)
13	73.61		
14	27.05	1.32	77.19(12), 73.61(13), 26.90(15)
15	26.90	1.20	77.19(12), 73.61(13), 27.05(14)
OCH_3	57.33	3.82	160.04(7)
OCH_3	56.44	3.24	77.35(11)

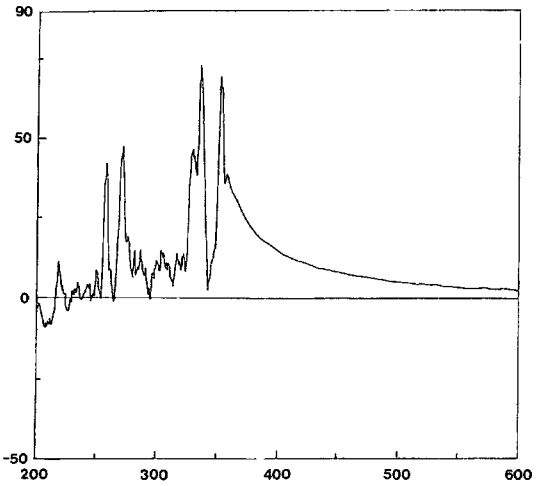

Fig. 3. ORD specturm of compound **4** in MeOH

1994), except for the presence of another methoxyl signal at 3.24 ppm. The presence of mass fragment ions at *m/z* 277[M+1-OCH_3]$^+$ and *m/z* 219[M-CH(OCH_3)-C(OH)-C($Me)_2$ OH]$^+$ also suggested that compound **4** has two methoxyl groups, which were placed on C-7 and C-11 based on HMBC correlations (Fig. 2 and Table I) observed between the methoxyl group at 3.82 and C-7, and between the methoxyl group at 3.24 and C-11, respectively. On the other hand, the neighboring methine protons at C-11 and C-12 were shown to be in a *cis* configuration from the small vicinal coupling constant (*J*=1.2Hz) (Pereda-Miranda *et al.*, 1993). Finally, the ORD spectrum (Fig. 3) of compound **4** exhibited positive value, which suggested that the relative configuration of C-11 and C-12 were 11S and 12R, respectively (Liu *et al.*, 1995). From these spectral data, compound **4** was tentatively identified as 6-[(1S, 2R)-2,3-dihydroxy-1-methoxy-3-methylbutyl]-7-methoxycoumarin.

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