Two New Furanocoumarins from the Roots of *Angelica dahurica*

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Studies to find preventive agents against sepsis have been reported.[1,2] In our previous studies, we have screened more than one hundred of Korean medicinal plants based on *in vivo* sepsis model induced by LPS/D-galactosamine (GalN). *Angelica dahurica* Benth. *et* Hook. (Umbelliferae) was selected as one of the active plants. The roots of *A. dahurica* have been used for the treatment of colds, headaches and toothache in Korean traditional medicine.[3] Some coumarins and furanocoumarins have been reported from this plant.[3-9] Two new furanocoumarins (**1** and **2**) were isolated from the *n*-BuOH extract of the roots of *A. dahurica*. The structure elucidation and biological activity of the compounds are described herein.

The MeOH extract of the roots of *A. dahurica* was partitioned between H_2O and hexane, and the resulting H_2O layer was extracted with EtOAc and *n*-BuOH, respectively. The *n*-BuOH extract was chromatographed on Silica-gel column. The two major fractions were separately rechromatographed on a reverse-phase column, which afforded compounds **1** and **2**.

Compound **1** has the molecular formula $C_{21}H_{24}O_7$ as determined by HRFABMS, ^{13}C-NMR, and DEPT spectral data. ^{1}H- and ^{13}C-NMR data of **1** were similar to those of the reported psoralen from *Angelica officinalis*, which has the same furanocoumarin backbone as **1** but has two 2-hydroxy-isopentyl groups substituted at C-5 and C-8 position.[10] The

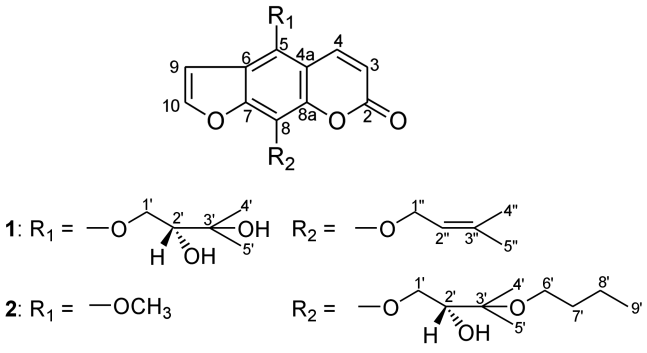

Figure 1. Structures of compounds **1** and **2**.

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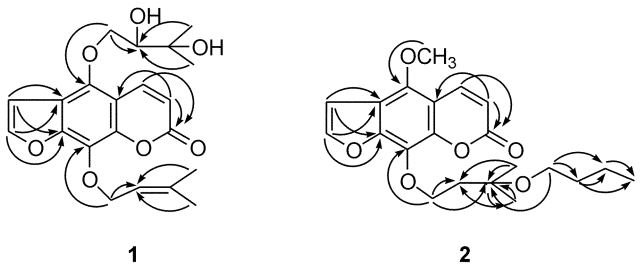

Figure 2. Selective HMBC correlations for compound **1** and **2**.

connectivity among carbons of **1** was determined mainly by analysis of the HMBC spectrum of **1** (Figure 2). Key evidences from ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H, ^{1}H- COSY, HMQC and HMBC spectral data were as follows. In the ^{1}H-NMR spectrum of **1**, two doublet signals (1H, J = 9.9 Hz) at δ 8.36 and 6.25 were assigned as the protons of pyrone ring. Two doublet signals (1H, J = 2.4 Hz) at δ 7.90 and 7.26 were assigned as the protons of furan ring.[9] The ^{1}H-NMR spectrum of **1** showed the presence of a 5,8-disubstitued furanocoumarin moiety, a 2,3-dihydroxy-3-methylbutyloxy moiety [δ 4.74 (1H, dd, J = 9.9, 2.4 Hz), 4.31 (1H, dd, J = 9.9, 8.4 Hz), 3.87 (1H, dd, J = 8.4, 2.2 Hz), 1.26 (3H, s), 1.23 (3H, s)], and a 3-methylbut-2-enyloxy moiety [δ 5.55 (1H, t, J = 7.2 Hz), 4.82 (1H, d, J = 7.2 Hz), 1.70 (3H, s), 1.66 (3H, s)]. In the HMBC spectrum of **1**, the location of 3-methylbut-2-enyloxy moiety was established by a long-range correlation between C-8 with H-1", and the position of a 2',3'-dihydroxy-3'-methylbutyloxy group was determined by both a long-range correlation between C-5 with H-1' and a positive NOE effect between H-9 and H-1' in the 1D-NOE difference spectrum of **1**.[11,12] The absolute stereochemistry of the chiral center in **1** was determined by using Moshers ester based on the differences between the ^{1}H-NMR chemical shifts of (*S*)- and (*R*)-MTPA ester derivatives.[13-15] ^{1}H-NMR data were assigned based on the ^{1}H-^{1}H COSY spectra of $\mathbf{1}_S$ and $\mathbf{1}_R$ (Table 1). For **1**, the positive value of $\Delta\delta_H$ (δ_S-δ_R) at H-1' and the positive value of $\Delta\delta_H$ (δ_S-δ_R) at H-4' and H-5' suggested an *R* configuration at C-2'.

Compound **2** had the molecular formula $C_{21}H_{26}O_7$ as determined by HRFABMS, ^{13}C-NMR, and DEPT spectral data. ^{1}H- and ^{13}C-NMR spectra showed not only signals very similar to those of byakangelicin,[16] but also signals due to one butoxyl moiety. The ^{13}C-NMR signal of C-3' at δ 77.2,

Table 1. Characteristic ^{1}H-NMR data of Mosher's esters of **1** and **2**

Position	$\mathbf{1}_S$ δ_S	$\mathbf{1}_R$ δ_R	$\Delta\delta$ δ_S-δ_R	Position	$\mathbf{2}_S$ δ_S	$\mathbf{2}_R$ δ_R	$\Delta\delta$ δ_S-δ_R
1'	4.93	4.82	+0.11	**1**'	4.89	4.80	+0.09
	4.80	4.64	+0.16		4.64	4.51	+0.13
2'	5.60	5.56	*R*	**2**'	5.47	5.48	*R*
4'	1.34	1.28	+0.06	**4**'	1.25	1.26	–0.01
5'	1.39	1.36	+0.03	**5**'	1.19	1.22	–0.03

Table 2. Effect of the compound **1** on LPS/D-GalN-induced lethality in mice

	Control	10 (mg/kg)	30 (mg/kg)	100 (mg/kg)
Compound **1**	1/5[a]	1/5	2/5	3/5
Dexamethasone[b]	1/5	4/5	ND[c]	ND

[a]Number of live mice/number of total mice; [b]positive control; [c]not determined. Mice were injected i.p. with various doses of compound **1** or vehicle 30 min before injection of LPS/D-GalN. Survival rate was observed once daily for up to 3 days.

which is 5.7 ppm lower than that of free byakagelicin, suggested that butoxyl moiety is linked to C-3' position of **2**.[17] The location of butoxyl moiety was established by the HMBC long-range correlation between C-3' and H-6' (Figure 2), and positive NOE effects from H-6' to H-2', H-4', and H-5' in the 1D-NOE difference spectrum and comparison of the NMR spectral data with those of 9-(2-hydroxy-3-methoxy-3-methylbutoxy)-bergapten.[18] To determine the absolute configuration of the hydroxyl group at C-2', Moshers esters ($\mathbf{2}_R$ and $\mathbf{2}_S$) of **2** were prepared, and ^{1}H-NMR data were also assigned based on the ^{1}H, ^{1}H-COSY spectra (Table 1). For **2**, the positive value of $\Delta\delta_H$ (δ_S-δ_R) at H-1' and the negative value of $\Delta\delta_H$ (δ_S-δ_R) at H-4' and H-5' suggested an *R* configuration at C-2'.

Of two purified compounds, only **1** showed protective effect against lethality induced by LPS/D-GalN (Table 2). Pretreatments of mice with **1** at doses of 10, 30, and 100 mg/kg increased survival rates to 20%, 40%, and 60%, respectively, while the control showed 20% increase of survival rate. However, the protective effect of **1** was lower than that of dexamethasone.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. IR spectra were recorded on a JASCO FT-IR 300E spectrophotometer, and UV spectra were recorded on a JASCO V-550 spectrophotometer. NMR spectra were recorded on Bruker 250 MHz (DMX 250) spectrometer using Bruker's standard pulse program. Samples were dissolved in either CD_3OD or acetone-d_6, and chemical shifts were reported in δ (ppm) downfield from TMS. The FABMS spectra were measured by VG TRIO 2A mass spectrometer. Silica-gel 60 (70-230 and 270-400 mesh, Merck) and Lichroprep RP-18 gel (40-63 mm, Merck) were used for column chromatography. TLC plates (Silica-gel 60 F_{254} and RP-18 F_{254}) were purchased from EM Scientific. Spots were detected under UV radiation and by spraying with 10% H_2SO_4, followed by heating. All other chemicals and solvents were analytical grade and used without further purification.

Plant Material. Dried *A. dahurica* roots were purchased in November 1997 from a traditional medicine market "Yak-ryong-si" in Daegu, and the material was confirmed taxonomically by Professor Gi-Hwan Bae, of Chungnam National University in Taejeon, Republic of Korea. A voucher specimen (YNS-97-01) has been preserved at the College of Pharmacy, Yeungnam University.

Isolation. The dried roots of *A. dahurica* (10 kg) were extracted twice with 70% MeOH (20 L) under reflux for 12 h. The MeOH solution was evaporated to dryness (3 kg) and the residue was partitioned between H_2O (1 L) and hexane (3 × 1 L). The resulting H_2O layer was extracted with EtOAc (3 × 1 L) and *n*-BuOH (3 × 1 L) successively. The *n*-BuOH extract (110 g) was chromatographed on a silica gel column (230-400 mesh, 60 × 9 cm) with CH_2Cl_2-MeOH-H_2O (100 : 1:0.1, 50:1:0.1, 30:1:0.1, 20:1:0.1, 10:1:0.1, 9:2:0.1, 9:4:0.1, 3:8:0.1, 100% MeOH) in a stepwise gradient mode. The fractions (500 mL in each flask) were grouped and combined on the basis of silica-gel TLC and 36 subfractions (F1-F36) were obtained. The subfraction F2 (450 mg) from the column was further purified on a reverse-phase column (75 × 2.6 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 1 : 9 to 2 : 8), affording **1** (34.6 mg). The subfraction F7 (650 mg) was further rechromatographed on a reversed-phase column (70 × 3.0 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 2 : 8 to 100% MeOH) to give **2** (25.6 mg).

5-(2',3'-Dihydroxy-3'-methylbutyloxy)-8-(3''-methylbut-2''-enyloxy)psoralen (1): Brown amorphous powder; $[\alpha]_D^{20}$ -38.1° (*c* 0.18, acetone); UV (MeOH) λ_{max} (log ε) 222.0 (5.69), 249.0 (5.41), 269.0 (5.46), 313.0 (5.32); IR (KBr) ν_{max} 3422, 2927, 1722, 1591, 1474 and 1149 cm^{-1}; ^{1}H-NMR (acetone-d_6, 250 MHz) δ 8.36 (1H, d, *J* = 9.9 Hz, H-4), 7.90 (1H, d, *J* = 2.4 Hz, H-10), 7.26 (1H, d, *J* = 2.4 Hz, H-9), 6.25 (1H, d, *J* = 9.9 Hz, H-3), 5.55 (1H, t, *J* = 7.2 Hz, H-2''), 4.82 (1H, d, *J* = 7.2 Hz, H-1''), 4.74 (1H, dd, *J* = 9.9, 2.4 Hz, H-1'a), 4.31 (1H, dd, *J* = 9.9, 8.4 Hz, H-1'b), 3.87 (1H, dd, *J* = 8.4, 2.2 Hz, H-2'), 1.70 (3H, s, H-5''), 1.66 (3H, s, H-4''), 1.26 (3H, s, H-5'), 1.23 (3H, s, H-4'); ^{13}C-NMR (acetone-d_6, 62.9 MHz) δ 160.5 (C-2), 151.5 (C-7), 146.7 (C-10), 145.4 (C-5), 145.2 (C-8a), 140.8 (C-4), 139.7 (C-8), 127.6 (C-3''), 121.1 (C-2''), 116.6 (C-6), 113.3 (C-3), 109.1 (C-4a), 106.4 (C-9), 77.9 (C-2'), 76.6 (C-1'), 71.9 (C-3'), 70.6 (C-1''), 27.1 (C-5'), 25.9 (C-5''), 25.4 (C-4'), 18.1 (C-4''); HRFABMS *m/z* 389.1603 (calcd. for $C_{21}H_{25}O_7$ [M + H]$^+$, 389.1600).

5-Methoxy-8-(2'-hydroxy-3'-buthoxy-3'-methylbutyl-oxy)psoralen (2): Yellow amorphous solid, $[\alpha]_D^{20}$ +11.1° (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log e) 223.0 (4.40), 241.0 (4.13), 249.0 (4.11), 272.0 (4.22), 313.0 (4.04); IR (KBr) ν_{max} 3423, 2954, 1724, 1592, 1481, 1350, 1144, 1063, 821 and 756 cm^{-1}; ^{1}H-NMR (CD_3OD, 250 MHz) δ 8.15 (1H, d, *J* = 9.8 Hz, H-4), 7.78 (1H, d, *J* = 2.3 Hz, H-10), 7.18 (1H, d, *J* = 2.3 Hz, H-9), 6.22 (1H, d, *J* = 9.8 Hz, H-3), 4.56 (1H, dd, *J*

= 10.3, 2.2 Hz, H-1'a), 4.23 (1H, dd, *J* = 10.3, 8.3 Hz, H-1'b), 4.17 (3H, s, 5-OCH_3), 3.89 (1H, dd, *J* = 8.3, 2.2 Hz, H-2'), 3.37 (2H, m, H-6'), 1.42 (2H, m, H-7'), 1.30 (2H, m, H-8'), 1.27 (3H, s, H-4' or H-5'), 1.16 (3H, s, H-4' or H-5'), 0.85 (3H, t, *J* = 7.2 Hz, H-9'); ^{13}C-NMR (CD_3OD, 62.9 MHz) δ 162.6 (C-2), 151.6 (C-7), 146.8 (C-10), 145.9 (C-5), 144.8 (C-8a), 141.4 (C-4), 128.3 (C-8), 116.0 (C-6), 113.0 (C-3), 108.4 (C-4a), 106.4 (C-9), 77.2 (C-3'), 77.1 (C-2'), 76.9 (C-1'), 62.1 (C-6'), 61.3 (5-OCH_3), 33.6 (C-7'), 23.1 (C-5'), 21.0 (C-4'), 20.4 (C-8'), 14.3 (C-9'); HRFABMS *m/z* 391.1766 (calcd. for $C_{21}H_{27}O_7$ $[M + H]^+$, 391.1757).

Preparation of Mosher's Esters. A previously described method was used.[13-15] To each 1 mg of compounds **1** and **2** in 0.5 mL of CH_2Cl_2 were added sequentially 0.2 mL of pyridine, 0.5 mg of 4-(dimethylamino)pyridine, and 12.5 mg of (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl [(*R*)-MTPA] chloride, separately. The mixture was left at room temperature overnight and purified over a microcolumn (0.6 × 6 cm) of silica gel (230-400 mesh) eluted with 3-4 mL of hexane-CH_2Cl_2 (1 : 2). The elute was dried, CH_2Cl_2 (5 mL) was added, and the CH_2Cl_2 was washed using 1% $NaHCO_3$ (2 × 5 mL) and H_2O (2 × 5 mL). The washed elute was dried *in vacuo* to give the *S*-Mosher esters ($\mathbf{1}_S$ and $\mathbf{2}_S$) of compounds **1** and **2**, respectively. Using (*S*)-MTPA chloride afforded the *R*-Mosher esters ($\mathbf{1}_R$ and $\mathbf{2}_R$) of compounds **1** and **2**, respectively. Their ^{1}H-NMR chemical shifts are given in Table 1.

Animals and LPS/D-GalN-Induced Lethality. Male ICR mice weighing 23-28 g were housed 5 per cage in a room maintained at 22 ± 1 °C with an alternating 12 hours light-dark cycle. Food and water were available *ad libitum*. LPS (*Escherichia coli* 055:B5, Sigma, USA) was dissolved in phosphate-buffer saline (PBS, pH 7.2) at 1 μg/μL and stored at –80 °C until use. D-GalN (ICN, USA) was dissolved in PBS at 0.16 g/mL and added to 7.2 μL of LPS solution. Each mouse received LPS/D-GalN (LPS 36 μg/kg, D-GalN 0.8 g/kg) intra-peritoneally at volume of 1 mL/100 g of body weight. Compounds **1** and **2** were dissolved in 10% DMSO and injected to mice by i.p. administration before LPS/D-GalN injection. Survival rate was observed once daily for up to 3 days.

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