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Isolation and Structure Determination of Coumarin Derivatives from the Roots of *Angelica dahurica*

Ji Yeon Kim*, Jong Keun Son[1] and Dong Keun Song[2]

National Institute of Food and Drug Safety Evaluation, Seoul 122-704, Korea

[1]College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea

[2]College Department of Pharmacology, Hallym University College of Medicine, Institute of Natural Medicine, Chunchon 200-702, Korea

ABSTRACT

From the roots of Angelica dahurica Bentham et Hooker (Umbelliferae), three known coumarin derivatives have been isolated and identified as 8-(2-hydroxy-3-methoxy-3-methylbutyloxy) psoralen, 5,8-di(2,3-dihydroxy-3-methylbutyloxy) psoralen, 9-[3-(β-D-glucopyranosyloxy)-2-hydroxy-3-methylbutoxy] -7H-furo[3,2-g][1]benzopyran-7-one. This is the first report of the occurrence of these compounds in this plant.

These three compounds were tested for activity in septic shock model. Among these compounds, 2 showed relatively strong preventive activity against septic shock.

Key words : Angelica dahurica, Umbelliferae, Coumarin derivatives, Septic shock

Introduction

Angelica dahurica Bentham et Hooker(Umbelliferae) is a pernnial herb, mainly grown in Korea and Japan, the roots of which are most frequency prescribed as a sedative and an analgesic in Chinese medicine under the local name of "Baik-Chi", while two variaties such as *A. dahurica* Benth. et Hook. var. formosana Yen and *A. dahurica* Benth. et Hook. var. Pai-Chi Kimura hata et Yen are occasionally prescribed as the same name and the same use.[1-3)]

The crude drug *Angelica dahurica* Radix, the dried roots of *Angelica dahurica* bentham et Hooker(Umbelliferae), is one of the most important herbal drugs in Korea and has been used to relieve headache caused by cold, toothache, hematuria, gonorrhea, boils, itching skin, liver trouble, and

* Correspondence: Herbal Medicine Research Division, Pharmaceuticals and Medical Devices Research Department, National Institute of Food and Drug Safety Evaluation, 194 Tongil-ro, Eunpyung-gu, Seoul 122-704, Korea. TEL: +82-2-380-1391~2; E-mail: smile75@korea.kr

swollen face.

It has been also used as an anodyne. very effective in relieving neuralgic pain. and a stypic to stop nosebleed. To date. over twenty coumarins have been isolated from this crude drug[3-5].

To date, over twenty coumarins including byakangelicol, xanthotoxin, marmesin, byakgelicin, phellopterin, oxypeucedanin, imperatorin, isoimperatorin, oxypsoralen, anhydrobyakangelicin, neobyakangelicol, angelic acid, angelicotoxin, marmesin, bergapten, xanthotioxin, scopoletin have been isolated and reported from this crude drug.[6]

It has in vitro inhibitory effect against various species of *Shigella* and *Salmonella*. It uses in ophthalmology as a burn ointment, which includes ground *Angelica dahurica* Radix, has been effective in promoting healing and avoiding deleterious sequelae from corneal ulcers secondary to flash burns. It uses in otolaryngology as a powder made up of ground *Angelica dahurica* Radix and Borneol, when inhaled through the nostrils, has been effective for headache and toothache. It also appears to be of use in trigeminal neuralgia.[7-8]

This paper deals with the isolation and structure elucidation of coumarin derivatives from the polar BuOH fraction of this crude drug.

Material and Methods

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 FAB-MS spectra were measured by VG TRIO 2A mass spectrometer. Silica 60 (70-230 and 270-400 mesh, Merck) and Lichroprep RP-18 (40-63 mesh, Merck) were used for column chromatography. TLC plates (Silica 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 roots of *Angelica dahurica* were purchased from a traditional medicine market "Yak-ryong-si" in Daegu. A voucher specimen (YNS-97-01) has been preserved at the College of Pharmacy, Yeungnam University.

Animals

Male ICR mice weighing 25-30 g, supplied by Myung Jin (Seoul, Korea), were used for all the experiments. The animals were housed five per cage in a room maintained at 22 ± 1 ℃ with an alternating 12 hr light-dark cycle. Food and water were available *ad libitum*.

Female ICR mice (16-20 g) and BALB/c mice (16-20 g) were obtained from Hyochang Science (Daegu, Korea) and fed with laboratory chow (Purina, Seoul, Korea) and water *ad libitum*. Animals were acclimatized in a specific pathogen-free animal facility under the conditions of 20 - 22 ℃, 40 - 60% relative humidity, and 12 hr light-dark cycle at least for 7 d.

Extraction and isolation

The dried roots of *Angelica 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 (31 L). The resulting H_2O layer was extracted with EtOAc (3 × 1 L) and BuOH (3 × 1 L) successively.

The BuOH extract (110 g) adsorbed on silica (No. 7734, 70-230 mesh, Merck) was chromatographed on a silica column (No. 9385, 230-400 mesh, Merck, 60 × 9 cm) with mixture of 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) as eluents in a stepwise gradient mode. The fractions (500 mL in each flask) were grouped and combined on the basis of silica TLC and 36 subfractions (F1-F36) were obtained. The subfraction F7 (650 mg) was further rechromatographed on a reversed-phase column (15 × 4.0 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 2:8 to 100% MeOH) to give compound 1 (47.9 mg). The subfraction F16 (600 mg) was further rechromatographed on a reversed -phase column (15 × 4.0 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 1:9 to 100% H_2O) to give compound 2 (25.8 mg). The subfraction F23 (1,000mg) was further rechromatographed on a reversed-phase column (15 × 4.0 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 1:9 to 100% H_2O) to give compound 3 (54.7 mg).

Compound 1. 8-(2-Hydroxy-3-methoxy-3-methylbutyloxy)psoralen

^{1}H-NMR (CD_3OD, 250 MHz) δ 8.02 (1H, d, J = 9.6 Hz, H-4), 7.88 (1H, d, J= 2.1 Hz, H-10), 7.55 (1H, s, H-5), 6.95 (1H, d, J = 2.1 Hz, H-9), 6.37 (1H, d, J = 9.6 Hz, H-3), 4.70 (1H, dd, J = 10.3, 2.5 Hz, H-1'a), 4.43 (1H, dd, J = 10.3, 8.2 Hz, H-1'b), 3.95 (1H, dd, J = 8.2, 2.5 Hz, H-2'), 3.24 (3H, s, 3'-OCH_3), 1.27 (3H, s, H-5'), 1.21 (3H, s, H-4'); ^{13}C-NMR (CD_3OD, 62.9 MHz) δ 162.8 (C-2), 149.2 (C-7), 148.5 (C-10), 146.8 (C-4), 144.3 (C-8a), 133.2 (C-8), 127.9 (C-6), 118.0 (C-4a), 115.0 (C-5), 114.9 (C-3), 108.0 (C-9), 77.7 (C-2'), 76.9 (C-1'), 76.4 (C-3'), 49.7 (3'-OCH_3), 22.3 (C-5'), 20.6 (C-4') Positive FABMS m/z 317 $[M]^+$.

Compound 2. 5,8-Di(2,3-dihydroxy-3-methylbutyloxy) psoralen, Yellow amorphous powder,

^{1}H-NMR (CD_3OD, 250 MHz) δ 8.35 (1H, d, J = 9.8 Hz, H-4), 7.80 (1H, d, J = 2.3 Hz, H-10), 7.16 (1H, d, J = 2.3 Hz, H-9), 6.25 (1H, d, J= 9.8 Hz, H-3), 4.64 (1H, dd, J = 9.9, 2.2 Hz, H-1'a), 4.55 (1H, dd, J = 10.1, 2.7 Hz, H-1"a), 4.27 (1H, dd, J = 9.9, 8.4 Hz, H-1'b), 4.26 (1H, dd, J= 10.1, 8.4 Hz, H-1"b), 3.82 (1H, m, H-2'), 3.79 (1H, m, H-2"), 1.27 (3H, s, H-5"), 1.26 (3H, s, H-4"), 1.23 (3H, s, H-5'), 1.21 (3H, s, H-4'); ^{13}C-NMR (CD_3OD, 62.9 MHz) δ 162.8 (C-2), 151.2 (C-7), 147.0 (C-5), 145.3 (C-10), 144.6 (C-8a), 142.0 (C-4),128.6 (C-8), 117.3 (C-6), 113.1 (C-3), 109.4 (C-4a), 106.4 (C-9), 78.2 (C-2' or C-2"), 78.1 (C-2' or C-2"), 76.7 (C-1' or C-1"), 76.4 (C-1' or C-1"), 72.7 (C-3' or C-3"), 72.7 (C-1"), 27.2 (C-4' or C-4"), 26.7 (C-4' or C-4"), 25.1 (C-5' or C-5"), 24.8 (C-5' or C-5") Positive ESIMS m/z 423 $[M+H]^+$.

Compound 3. 9-[3-(β-D-glucopyranosyloxy)-2-hydroxy -3 methylbutoxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one

^{1}H-NMR (CD_3OD, 250 MHz) δ 8.35 (1H, d, J = 9.7 Hz, H-4), 7.82 (1H, d, J = 2.1 Hz, H-10), 7.27 (1H, d, J = 2.0 Hz, H-9), 7.11 (1H, s, H-8), 6.10

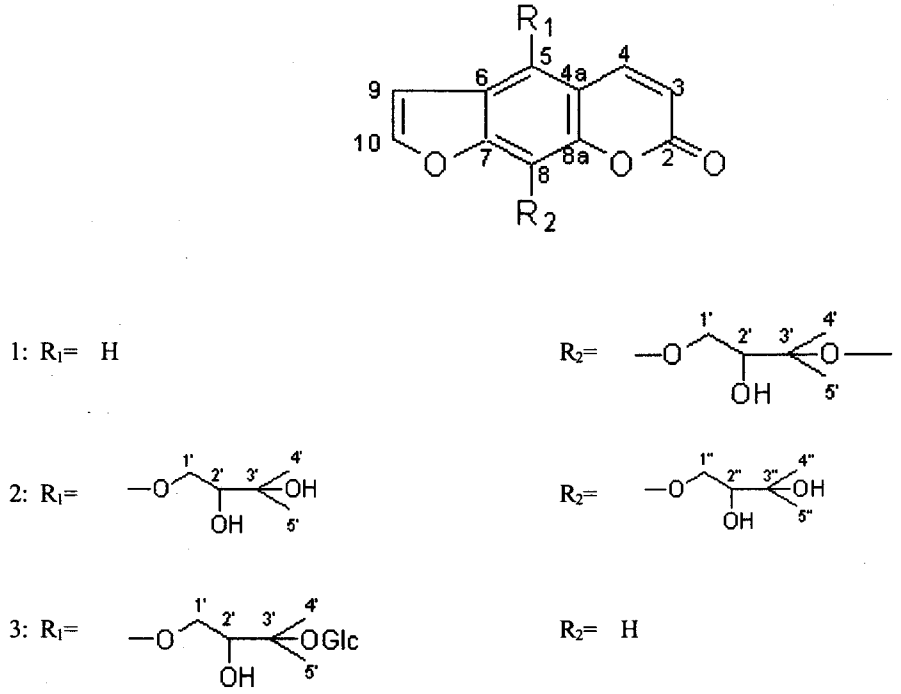

Fig. 1. Structures of compounds 1-3

(1H, d, J = 9.7 Hz, H-3), 4.78 (1H, dd, J = 7.6, 3.1 Hz, H-1'a), 4.53 (1H, m, H-1'b), 3.94 (1H, dd, J = 7.9, 2.0 Hz, H-2'), 1.33 (3H, s, H-4'), 1.30 (3H, s, H-5'), 4.51 (1H, d, J = 7.6 Hz, H-1G), 3.79 (1H, m, H-6aG), 3.55 (1H, m, H-6bG), 3.29 (3H, m, H-3G,5G,4G), 3.16 (1H, t, J = 7.9 Hz, H-2G) Positive FABMS m/z 466 $[M]^+$.

Septic shock model

Male ICR mice weighing 23-28 g were housed 3-5 per cage in a room maintained at 22 ± 1 ℃ with an alternating 12 hr 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 ug/L and stored at -80 ℃ until use. D-GalN (ICN, USA) was dissolved in PBS at 0.16 g/mL and added to 7.2 L of LPS solution. Each mouse received LPS/D-GalN (LPS 36 ug/kg, D-GalN 0.8 g/kg) intra-peritoneally at volume of 1 mL/100 g of body weight. Isolating compounds 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.

Results and Discussion

The methanol extract of the roots of *Angelica dahurica* was partitioned between H_2O and hexane and the resulting H_2O layer was extracted with EtOAc and BuOH, respectively. The BuOH extract was chromatographed on silica column. Then, BuOH extract, which showed strong inhibitory

activity on anti-inflammatory (Table 1), was repeatedly subjected to both silica and reverse-phase silica to afford compounds. Their structures were characterized as 8-(2-hydroxy-3-methoxy-3-methylbutyloxy)psoralen, 5,8-di(2,3-dihydroxy-3-methylbutyloxy)psoralen, 9-[3-(β-D-glucopyranosyloxy)-2-hydroxy-3-methylbutoxy]-7*H*- furo [3,2-*g*][1]benzopyran-7-one.

This is the first report of the occurrence of these compounds in this plant.

Table 1. Inhibitory effects of solvent extracts from the roots of *Angelica dahurica* on septic shock induced by LPS at 30 mg/g.

	Control	n-Hexane	EtOAc	BuOH	DW
Survival rate[a]	0/3	0/3	2/3	2/3	1/3

[a]Number of live mice/number of total mice

Mice were injected i.p. with a dose of 100 mg/kg of solvent extracts of the plant or vehicle 30 min before i.p. injection of LPS/D-GalN. Survival rate was observed once daily and then recorded after 3 days.

Compound 1

In ^{1}H-NMR spectrum, four proton peaks due to aromatic group (δ 6.35 -8.04, H-3, H-4, H-9 and H-10) were recognized. The proton peaks due to methylene proton peak attached to oxygen appeared further downfield (δ 4.38 - 4.72, H-1′). Among aromatic proton peaks, the proton peaks due to methyl group appeared further downfield (δ 1.20 - 1.27, H-4′ and H-5′). The proton peaks due to OH group appeared further downfield (δ 3.92 - 3.97). Their ^{1}H- and ^{13}C-NMR spectra were also in excellent accordance with those reported in the literature.[9,10] Thus compound 1 was characterized as 8-(2-hydroxy-3-methoxy-3-methylbutyloxy)psoralen.

Compound 2

In ^{1}H-NMR spectrum, four proton peaks due to aromatic group (δ 6.23 - 8.39, H-3, H-4, H-9 and H-10) were recognized. The proton peaks due to methylene proton peak attached to oxygen appeared further downfield (δ 4.23 - 4.69, H-1′, H-1″ and H-2′). Among aromatic proton peaks, paired proton peaks due to methyl group appeared further downfield (δ 1.21 - 1.36, H-4′, H-5′, H-4″ and H-5″). The proton peaks due to OH group appeared further downfield (δ 3.76 - 3.85). Their ^{1}H- and ^{13}C-NMR spectra were also in excellent accordance with those reported in the literature.[11] Thus compound 2 was characterized as 5,8-di(2,3-dihydroxy-3-methylbutyloxy)psoralen.

Compound 3

In ^{1}H-NMR spectrum, five proton peaks due to aromatic group (δ 6.10 -8.35, H-3, H-4, H-8, H-9 and H-10) were recognized. The proton peaks due to methylene proton peak attached to oxygen appeared further downfield (δ 4.53 - 4.78, H-1′a and H-1′b). Among aromatic proton peaks, the proton peaks due to methyl group appeared further downfield (δ 1.30 - 1.33, H-4′ and H-5′). The proton peaks due to OH group appeared further downfield (δ 3.94, H-2′). A noticeable signal at δ 4.51 (1H, J=7.6 Hz) was attributed to the anomeric H. By consideration of the coupling constants, the sugar moiety should be β-linkage. Complex signals appearing at δ 3.16 - 3.79 corresponded to protons of the glucose. Their ^{1}H- and ^{13}C-NMR

spectra were also in excellent accordance with those reported in the literature[12-15]. Thus compound 3 was characterized as 9-[3-(β-D-glucopyranosyloxy)-2-hydroxy-3-methyl butoxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one.

Activity in septic shock model

Among solvent extracts from the roots of *Angelica dahurica,* BuOH extract showed protective effect against lethality induced by LPS/D-GalN (Table 1).

These purified seven compounds were tested for activity in septic shock model. Among these compounds, 2 showed protective effect against lethality induced by LPS/D-GalN (Table 2). However, the protective effect of compound 2 was lower than that of dexamethasone, positive control.

Table 2. Effects of the isolated compounds on LPS/D-GalN-induced lethality in mice.

Compounds	Survival ratea		
	1 day	2 day	3 day
control	1/5	1/5	1/5
1	2/5	2/5	2/5
2	3/5	3/5	3/5
3	1/5	1/5	1/5
Dexamethasoneb	4/5	4/5	4/5

[a] Number of live mice/number of total mice; b positive control; Mice were injected intraperitoneally with a dose of 20 mg/kg of the purified compounds from the plant or vehicle 30 min before intraperitoneal injection of LPS/D-GalN. The survival rate was recorded once daily for up to 3 d.

Conclusion

In the screening for anti-inflammatory activities from Korean medicinal plant, three compounds (1-3) from the roots of *Angelica dahurica* was isolated by activity-guide isolation using silica column chromatography, RP-C18 column chromatography, and preparative TLC. Their structures were elucidated on the basis of spectroscopic studies. The isolated compounds were 8-(2-hydroxy-3-methoxy-3-methylbutyloxy)psoralen, 5,8-di(2,3-dihydroxy-3-methylbutyloxy)psoralen, 9-[3-(β-D-glucopyranosyloxy)-2-hydroxy-3-methyl butoxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one. This is the first report of the occurrence of these compounds in this plant.

These three compounds were tested for activity in septic shock model. Among these compounds, 2 showed relatively strong preventive activity against septic shock.

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