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Coumarin Glycosides from the Roots of *Angelica dahurica*

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Abstract □ From the roots of *Angelica dahurica* Bentham et Hooker (Umbelliferae), five known coumarin glucosides together with adenosine have been isolated and identified as nodakenin, 3′-hydroxymarmesinin, tert.-*O*-β-D-glucopyranosyl-byakangelicin, sec.-*O*-β-D-glucopyranosyl-byakangelicin and scopolin. This is the first report of the occurrence of these compounds in this plant.

Keywords □ *Angelica dahurica*, Umbelliferae, furanocoumarin glucoside, nodakenin, 3′-hydroxymarmesinin, tert.-*O*-β-D-glucopyranosyl-byakangelicin, sec.-*O*-β-D-glucopyranosyl-byakangelicin, scopolin, adenosine.

The crude drug *Angelicae Dahuricae 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 swollen face[1,2]. 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]. Recently Kim and coworkers[6] reported that the polar fractions from this crude drug were found to be showed significant antimicrobial and liver protective activities as well as restoring activity against dexamethasone-induced disorders. However, no chemical work has been done on these polar fractions. This paper deals with the isolation and structure elucidation of coumarin glucosides together with adenosine from the polar BuOH fraction of this crude drug.

EXPERIMENTAL METHODS

General experimental procedures

Melting points were determined on a Fisher/Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Rudolph Autopol III automatic polarimeter. IR spectra were obtained on a Perkin-Elmer 281B spectrophotometer. ^{1}H-NMR spectra were obtained either a Bruker AMX-500 (500 MHz) or a Bruker AM-300 (300 MHz) spectrometer using TMS as an internal standard. Mass spectra were obtained with Hewlett Packard 5985B GC/MS System. HPLC was carried out on a Shimadzu LC-9A instrument. LC columns were from Shimadzu [Shimpack CLC(M) ODS reverse phase column (4.5×250)] and Merck [Lichroprep. RP-8 (size A & B) Lobar column].

Plant material

The roots of *A. dahurica* were collected from the cultivated land around Jinbu, Kangweon Province in 1990 and authenticated by one of us (K.C.M.). A voucher specimen was deposited at Department of Pharmacognosy, College of Pharmacy, Kangweon National University.

Extraction and isolation

The dried and chopped roots (3.8 kg) were refluxed with MeOH (3 times, 5h for each time) on a water bath. The extracts were combined and concentrated *in vacuo* to afford a residue, which was suspended in H_2O and successively partitioned with

benzene, EtOAc and then BuOH. The BuOH fraction was subjected to silica gel column chromatography with $CHCl_3$-MeOH-H_2O (70:25:5) to give subfractions A, B and C on the basis of TLC characteristics. Subfraction C gave compound **6**. Subfraction B was rechromatographed over SiO_2 with $CHCl_3$-MeOH (8:1) to yield B_1, B_2 and B_3 fractions. Subfraction B_2 was recrystallized from MeOH to give compound **1**. Subfraction B_1 was rechromatographed on SiO_2 eluting with EtOAc-MeOH-H_2O (10:1:0.5) followed by preparative LC on a Lobar column (RP-8A) with MeOH-H_2O (1:1) to give compounds **3-5**. Subfraction B_3 was purified by SiO_2 column chromatography with $CHCl_3$-MeOH (6:1) followed by preparative LC on a Lobar column (RP-8A) with MeOH-H_2O (4:6) to afford compound

Compound 1

Crystallized from MeOH to give pure compound **1** as colorless needles. mp. 217-218℃; $[\alpha]_D^{18}$ = + 104.4℃ (c, 0.16, EtOH); IR ν_{max} (KBr) 3370 (OH), 1728 (α-pyrone C=O), 1635, 1578, 1495 (aromatic C=C), 1270, 1140, 1110, 1080, 1040 (C-O) cm^{-1}; UV λ_{max} (MeOH) 224.5, 248 (sh.), 258.5, 298 (sh.), 335.5 nm; ^{1}H-NMR: see Table I; MS *m/z* (rel. int., %) 408 [M^+] (0.7), 275 (0.4), 247 (4.7), 246 [M-162] (27.1), 231 [M-162-CH_3] (1.4), 230 (2.3), 228 [M-162-H_2O] (10.1), 213 [M-162-H_2O-CH_3] (21.3), 188 [M-162-C_3H_6O] (65.7), 187 [M-162-C_3H_7O] (100), 175 (13.1), 160 [188-CO] (23.0), 159 [187-CO] (11.4), 158 (6.1), 147 (3.8), 131 [187-2CO] (22.7), 59 [$(CH_3)_2C{=}O^+H$] (68.8).

Compound 2

Crystallized from MeOH to yield compound **2** as colorless needles. mp. 262-263℃; $[\alpha]_D^{15}$ = − 11℃ (c, 0.1, pyridine); IR ν_{max} (KBr) 3460 (OH), 1720 (α-pyrone C=O), 1630, 1488 (aromatic C=C), 1140, 1070, 1040 (C-O) cm^{-1}; UV λ_{max} (MeOH) 222, 257 (sh.), 283.5 (sh.), 297 (sh.), 326.5 nm; ^{1}H-NMR: see Table I; MS *m/z* (rel. int., %) 424 [M^+] (5.3), 406 [M-H_2O] (0.6), 262 [M-162] (2.2), 246 (2.9), 245 (10.5), 229 [M-162-H_2O-CH_3] (6.9), 228 (10.7), 227 (17.7), 226 [M-162-2H_2O] (9.1), 213 (8.6), 204 (14.5), 203 [M-162-C_3H_7O] (8.6), 189 (8.5), 188 (19.5), 187 (100), 186 (31.4), 175 [203-CO] (3.4), 158 (11.4), 131 (3.5), 59 [$(CH_3)_2C{=}O^+H$] (14.5).

Compound 3

Crystallized from EtOAc-MeOH to afford compound **3** as pale yellowish needles. mp. 160-163℃; UV λ_{max} (MeOH) 224, 240 (sh.), 249 (sh.), 271, 312 nm; ^{1}H-NMR: see Table I; MS *m/z* (rel. int., %) 496 [M^+] (0.4), 372 (0.4), 275 [M-162-$(CH_3)_2C{=}O^+H$] (0.6), 245 [M-162-$C_4H_9O_2$] (2.2), 233 (21.1), 232 [M-162-$C_5H_{10}O_2$] (100), 231 (19.0), 217 [232-CH_3] (66.2), 203 [231-CO] (2.1), 189 [232-CH_3-CO] (14.9), 175 [231-2CO] (2.6), 161 [189-CO] (7.6), 103 [$C_5H_{11}O_2$] (4.7), 89 [$C_4H_9O_2$] (4.3), 59 [$(CH_3)_2C{=}O^+H$] (13.1).

Compound 4

Crystallized from EtOAc-MeOH to give compound **4** as pale yellowish needles. mp. 88-91℃; UV λ_{max} (MeOH) 230, 242 (sh.), 253 (sh.), 274, 311 nm; ^{1}H-NMR: see Table I; MS *m/z* (rel. int., %) 496 [M^+] (0.3), 275 (0.5), 245 (1.5), 233 (21.2), 232 (100), 231 (17.6), 217 (45.1), 203 (1.5), 189 (5.4), 175 (1.7), 161 (2.8), 103 (1.3), 89 (1.6), 59 (10.0).

Compound 5

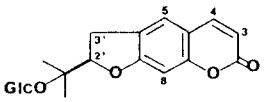
Colorless needles from MeOH, mp. 218-219℃; IR ν_{max} (KBr) 3460 (OH), 1720 (α-pyrone C=O), 1615, 1570, 1515 (aromatic C=C), 1120, 1080, 1040 (C-O); UV λ_{max} (MeOH) 227, 254, 288, 340 nm; ^{1}H-NMR: see Table I; MS *m/z* (rel. int., %) 264 (0.2), 234 (0.2), 218 (1.4), 202 (1.5), 193 (15.0), 192 [M-glucose] (100), 191 (2.4), 177 [192-CH_3] (42.0), 164 [192-CO] (20.2), 149 [192-CH_3-CO] (33.5), 121 [192-CH_3-2CO] (14.4), 85 (5), 73 (22.6), 69 (32.2).

Compound 6

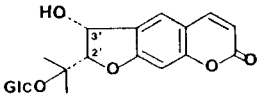
Colorless needles from MeOH, mp. 235-236℃; IR ν_{max} (KBr) 3300 (NH_2), 3150 (OH), 1650, 1610 (N-H), 1110 (C-N), 1075, 1040 (C-O); UV λ_{max} (MeOH) 214, 260 nm; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.55 (1H, ddd, J=3.6, 6.7, 12.2 Hz, H-5'), 3.66 (1H, dd, *J*=3.6, 12.2 Hz, H-5'), 3.97 (1H, dd, *J*=3.4, 6.7 Hz, H-4'), 4.14 (1H, dd, *J*=3.1, 5.0 Hz, H-3'), 4.59 (1H, dd, *J*=5.3, 5.8, H-2'), 5.87 (1H, d, *J*=6.2 Hz, H-1'), 8.13 (1H, s, H-2), 8.32 (1H, s, H-8); MS *m/z* (rel. int., %) 267 [M^+] (2.1), 237 (12.7), 178 (42.3), 164 (100), 136 (86.9), 135 (96.0), 119 (10.2), 108 (32.7).

Acid hydrolysis of compounds 1-5[7]

Compounds **1-5** were applied on silica gel G plate and left in an c-HCl atmosphere at room temperature for 1h. HCl vapors were eliminated under



1



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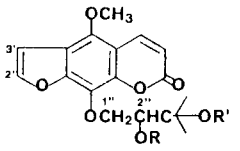
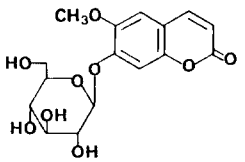


3 R=H R'=Glc

4 R=Glc R'=H



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hot ventilation and then authentic sugars were applied to the plate. The plate was developed ($CHCl_3$-MeOH-H_2O=26:14:5) and spots were detected by spraying with 20% H_2SO_4 solution followed by heating. The sugars were identified as glucose.

RESULTS AND DISCUSSION

The BuOH soluble fraction of the MeOH extract of the roots on repeated column chromatography followed by recrystallizations gave six compounds. All the compounds isolated gave a positive Molisch test for sugars. Among them, compounds **1-4** liberated D-glucose only on acid hydrolysis and showed UV absorption characteristics of linear furocoumarins[8]. The IR spectrum of **1** showed the presence of hydroxyl, α-pyrone and aromatic rings. The mass spectrum of **1** gave a molecular ion at *m/z* 408 and other important fragment ions at *m/z* 246, 213, 188, 187, 175, 160 and 59. The base peak at *m/z* 187 was derived from the aglycone (*m/z* 246) by the principal fragmentation pathway of hydroxyisopropyl dihydrofuranocoumarins[9,10]. The ^{1}H-NMR spectrum of **1** exhibited a pair of doublets at δ 6.33 and 8.10 (J=9.5 Hz), which are characteristic signals for H-3 and H-4 of α-pyrone ring system, and two singlets at δ 6.92 and 7.60 indicate the presence of two *para* aromatic protons of the coumarin ring. Two methyl singlets at δ 1.35 and 1.37 supported the presence of hydroxyisopropyl moiety[11]. A doublet at δ 4.52 with large coupling constant (J=8 Hz) indicated that the glucosidic linkage is β. All the spectral data as described above are very similar to those of nodakenin[9,12] or its epimer, marmesinin [13,14]. However, the optical rotation of **1** shows a postive value, supporting the former structure for compound **1**. Nodakenin (**1**) has been isolated from umbelliferous plants[8,10,12,15,16] and reported as a strong inhibitor on human platelet aggregation induced by ADP[9,17].

Compound **2** showed quite similar spectral data to those of **1** with a slight difference. The mass spectrum of **2** exhibited a molecular ion at *m/z* 424, which was larger by 16 mass units than that of **1**. The further fragments observed allowed us to support the hydroxyisopropyl dihydrofuranocoumarin structure with an additional oxygen function for the compound[9,10]. The multiplicities and chemical shifts due to the dihydrofuran ring protons in **1** were replaced by a pair of doublets at δ 4.66 and 5.36 (J=6.5 Hz) in **2**. Therefore, **2** must possess an additional hydroxyl group at C-3′ of dihydrofuran moiety. The *cis*-configuration of dihydrofuran protons in **2** was inferred from their large coupling constant[11,13,18]. The physical and spectral data of **2** were in full agreement with the literature data[13]. Therefore, the structure of **2** was characterized as 3′-hydroxy marmesinin which was isolated from *A. archangelica* subsp. *litoralis* and *A. silvestris*[13].

Compound **3** exhibited the presence of a 5,8-dioxygenated furanocoumarin skeleton[19] in its UV spectrum. This result was further supported by the fact that the methoxy and H-4 signals in **3** were found at δ 4.26 and 8.31, respectively, in its ^{1}H-NMR spectra[11,20]. Thus, compound **3** should be a linear 5-methoxy furanocoumarin glucoside which was also supported by the presence of a molecular ion at *m/z* 496 and the base peak at *m/z* 232, due to the removal of a glucose unit and 3-methyl-2,3-dihydroxybutyl moiety[21,23,24] from the molecular ion. The presence of 3-methyl-2,3-dihydroxybutyl moiety at C-8 was inferred from the characteristic ABX patterns together with *gem*-dimethyl singlets in the ^{1}H-NMR spectra (see Table I)[11,22] and the prominent fragment ions at *m/z* 103, 89 and 59 in its mass spectrum[21,23,24]. Based on this evidence **3** seems to be byakangelicin glucoside.

The UV, mass and ^{1}H-NMR spectral patterns of the compound **4** are very similar to those of **3**. The mass spectrum of **4** exhibits a fragmentation pattern almost identical with that of **3** with only slight difference in certain peak intensities. In the ^{1}H-NMR spectrum of **4**, the chemical shifts attributable to

Table I. ^{1}H-NMR spectral data for compounds 1-5 in DMSO-d_6*

Proton	1	2	3	4	5
H-3	6.33 d(9.5)	6.39 d(9.5)	6.45 d(9.8)	6.46 d(9.8)	6.32 d(9.5)
H-4	8.10 d(9.5)	8.15 d(9.5)	8.31 d(9.8)	8.31 d(9.8)	7.96 d(9.5)
H-5	7.60 s	7.81 s			7.29 s
H-8	6.92 s	7.05 s			7.16 s
H-2′	4.96 t(9.0)	4.66 d(6.5)	8.13 d(2.2)	8.20 d(2.3)	
H-3′	overlap	5.36 d(6.5)	7.43 d(2.2)	7.50 d(2.3)	
H-1″			4.30 dd(7.6, 10.2)	4.42 dd(6.0, 10.8)	
			4.59 dd(2.1, 10.2)	4.75 dd(3.1, 10.8)	
H-2″			3.95 dd(2.1, 7.6)	4.02 dd(3.1, 6.0)	
Gem-CH_3	1.35 s	1.59 s	1.29 s	1.22 s	
	1.37 s	1.59 s	1.31 s	1.30 s	
OCH_3			4.27 s	4.25 s	3.82 s
Glc H-1	4.52 d(8.0)	4.65 d(8.0)	4.49 d(7.8)	4.78 d(7.8)	5.02 d(7.2)

*Measured with 500 MHz.
Multiplicities underlined are for D_2O exchanged spectra and figures in parentheses are coupling constants in Hz.

the protons of 3-methyl-2,3-dihydroxybutyloxy moiety were slight different from the ^{1}H-NMR spectrum of **3**. These results coupled with the mass spectral data suggested that the difference between **3** and **4** could only involve the position of attachment of glucose residue linked to one of the hydroxyl group of 3-methyl-2,3-dihydroxybutyloxy moiety. The point of attachment of glucose was deduced from the deuterium substitution of the hydroxyl groups in the ^{1}H-NMR spectra. Removal of the protons in the ^{1}H-NMR spectrum of compound **3** by exchange with D_2O gave rise to the disappearance of the doublet (J=5 Hz) signal at δ 5.05 and also a simplification of the H-2″ multiplet at δ 3.95 to the double doublets (J=2.1 and 7.6 Hz). In the light of the above observations a glucose unit was linked at tertiary hydroxyl group of the 3-methyl-2,3-dihydroxybutyloxy moiety. Therefore, the structure of compound **3** was determined to be tert.-*O*-β-D-glucopyranosyl-byakangelicin. Analogous results could be obtained from the ^{1}H-NMR spectrum of compound **4**. The disappearance of the singlet signal for tertiary hydroxyl proton at δ 4.48 and no collapsing the splitting pattern of H-2″ proton could be observed by deuterium substitution. Based on the above data the structure of compound **4** was established as sec.-*O*-β-D-glucopyranosyl-byakangelicin. The two glucosides **3** and **4** were previously isolated from the same genus *A. archangelica* subsp. *litoralis*[25].

Compound **5** was identified as a simple coumarin glucoside, scopolin on the basis of spectral analysis as well as comparisons of physical constants with those reported in the literature[14,26].

Compound **6** was characterized as adenosine by direct comparison with an authentic sample[27].

Although all the compounds isolated were the known ones, but this is the first report of the occurrence of the five coumarin glucosides together with adenosine in *A. dahurica*.

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