TWO NEW COUMARIN BIOSIDES FROM *Angelica dahurica*

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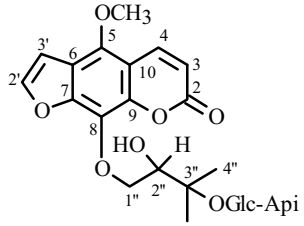
Two new coumarin biosides, tert-O-β-*D-apiofuranosyl-(1→6)*-O-β-*D-glucopyranosyl-byakangelicin* (***1***) *and 2′*-O-β-*D-apiofuranosyl-(1→6)*-β-*D-glucopyranosyl-peucedanol* (***2***), *were isolated from the fresh roots of* Angelica dahurica. *The structures of the new compounds were elucidated on the basis of spectral analysis*.

Key words: *Angelica dahurica*, coumarin glycosides, *tert-O-β*-D-apiofuranosyl-(1→6)-*O-β*-D-glucopyranosyl-byakangelicin, 2′-*O-β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranosyl-peucedanol, structure elucidation.

The roots of *Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f. ex Franch. et Sav. cv. Hangbaizhi (Umbelliferae) are important Chinese traditional medicines. They have been widely used for the treatment of headache caused by cold, toothache, coryza, vitiligo, acne, freckle, etc. Previous phytochemical studies on this plant led only to the isolation of about 20 coumarins and 3 coumarin glycosides [1–7]. Our research was focused on the water-soluble constituents from fresh materials of this plant and led to the isolation of 29 glycosides. We have already reported the isolation of three new linear coumarin glycosides and a new neolignan glycoside from this plant in our previous articles. Herein, we describe the isolation and structure elucidation of two new coumarin biosides.



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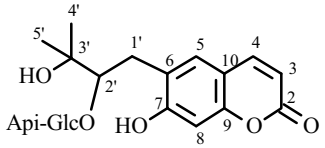

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Compound **1** was obtained as a yellowish amorphous powder, $[\alpha]_D^{21.6}$ –20.8° (*c* 0.15 MeOH–H_2O 40:60). Its molecular formula $C_{27}H_{34}O_{15}$ was determined on the basis of its ESI-MS (621 $[M+Na]^+$), ^1H NMR and ^{13}C NMR data. Detailed analysis of its ^1H NMR, ^{13}C NMR, COSY, HSQC and HMBC spectra indicated that compound **1** was a very similar in structure to compounds we isolated previously [*sec-O-β*-D-glucopyranosyl-(*R*)-heraclenol, *tert-O-β*-D-apiofuranosyl-(1→6)-*O-β*-D-glucopyranosyl-oxypeucedanin hydrate, *sec-O-β*-D-apiofuranosyl-(1→6)-*O-β*-D-glucopyranosyl-oxypeucedanin hydrate; see previous articles published in the same journal]. So we use the same way to discuss its structure.

Firstly, by analysis of its spectra, we see the presence of the linear furanocoumarin glucoside and 2,3-dioxygenated isopentyloxy structural unit; see Table 1.

The ^1H NMR spectrum of **1** was measured in pyridine-d_5. In the aromatic proton region, there was a pair of doublets at δ 6.31 and 8.02 ppm (d, J = 9.7 Hz), which was identified as the signals of H-3 and H-4 of the *α*-pyrone ring system. Another pair of doublets at δ 7.79 and 7.05 ppm (d, J = 2.0 Hz), which was identical to the signals of H-2′ and H-3′, indicated that **1** was a linear furanocoumarin. A distinct singlet at δ 4.03 ppm showed the presence of a methoxyl group. Acid hydrolysis (view Experimental) of **1** together with two doublets at δ 5.10 ppm (d, J = 7.7 Hz) and 5.66 ppm (d, J = 2.2 Hz) in the ^1H NMR spectra as well as signals in the ^{13}C NMR spectra indicated the presence of a *β*-D-glucose and a *β*-D-apiose.

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TABLE 1. ^{1}H NMR, ^{13}C NMR, and HMBC Spectral Data for Compounds **1** and **2** in C_5D_5N (δ, ppm, J/Hz)*

C atom	**1**			C atom	**2**		
	δ_H	δ_C	HMBC		δ_H	δ_C	HMBC
2		160.51		2		159.52	
3	6.31 (d, J = 9.7)	112.96	2, 10	3	6.20 (d, J = 9.4)	113.59	2, 10
4	8.02 (d, J = 9.7)	139.68	2, 5, 8, 9	4	7.60 (d, J = 9.4)	143.88	2, 5, 8, 9, 10
5		145.58		5	7.54 (s)	131.51	4, 6, 7, 9, 1′
6		115.26		6		126.34	
7		149.60		7		160.97	
8		128.17		8	6.90 (s)	102.28	6, 7, 9
9		144.54		9		154.69	
10		107.87		10		113.39	
2′	7.79 (d, J = 2.0)	145.95	6, 7, 3′	1a′	4.08 (dd, J = 2.0, 10.2)	29.96	5, 6, 2′, 3′
3′	7.05 (d, J = 2.0)	105.52	6, 7, 2′	1b′	3.56 (dd, J = 9.5, 10.3)		
1a″	5.03 (dd, J = 2.6, 9.7)	77.88	8, 3″	2′	3.95 (dd, J = 2.0, 9.5)	78.50	1′, 4′, 5′
1b″	4.80 (dd, J = 5.0, 9.7)			3′		68.94	
2″	4.75 (dd, J = 2.4, 5.0)	76.47	4″, 5″	4′	1.60 (s)	25.74	1′, 2′, 3′
3″		79.81		5′	1.59 (s)	24.23	1′, 2′, 3′
4″	1.65 (s)	23.86	1″, 2″, 3″	G-1	4.97 (d, J = 7.8)	102.31	2′, g-2
5″	1.63 (s)	22.48	1″, 2″, 3″	2	3.90 (m)	74.94	g-1, g-3
OCH_3	4.03 (s)	60.78	5	3	4.20 (m)	78.49	g-4, g-5
G-1	5.10 (d, J = 7.7)	98.66	3″, g-3	4	4.09 (m)	71.23	g-3, g-5
2	3.97 (m)	75.19	g-1, g-3	5	4.05 (m)	78.16	g-4, g-6
3	4.21 (m)	78.59	g-4	6a	4.60 (dd, J = 2.2, 11.5)	69.08	g-5, a-1
4	4.20 (m)	71.99	g-3, g-5	6b	4.15 (dd, J = 5.1, 11.5)		
5	3.91 (m)	76.93	g-4, g-6	A-1	5.76 (d, J = 2.6)	111.43	a-2, g-6
6a	4.46 (dd, J = 2.2, 11.6)	69.10	g-5, a-1	2	4.81 (d, J = 2.6)	77.41	a-1, a-3
6b	4.31 (dd, J = 5.2, 11.5)			3		80.37	
A-1	5.66 (d, J = 2.2)	111.15	a-2, g-6	4a	4.68 (d, J = 6.8)	75.17	a-3
2	4.37 (m)	76.47	a-1, a-3	4b	4.33 (d, J = 6.8)		
3		80.43		5	4.24 (m)	65.70	a-3
4a	4.32 (m)	75.11	a-3				
4b	4.55 (m)						
5	4.18 (m)	68.52	a-3				

*^{1}H NMR data of compound **1** were measured with a Bruker AV 300 spectrometer; ^{1}H NMR data of compound **2**, ^{13}C NMR, and 2D NMR spectra data of compounds **1** and **2** were measured with a Bruker AV 500 spectrometer.

In the aromatic proton region of the ^{13}C NMR spectrum, the signal at δ 160.51 can be easily assigned to C-2. We can also see the following structural segments: 6 signals of a glucoside, 5 signals of an apioside, and 5 signals of the 2,3-dioxygenated isopentyloxy structural unit.

We can then confirm all the chemical shifts of carbons that were connected with the hydrogen proton through HSQC, including C-3, C-4, C-2′, C-3′ carbons in the 2,3-dioxygenated isopentyloxy structural unit, and Api-C-1 and Glc-C-1 in the sugar skeletons.

By analysis of the above spectra, the chemical shifts of C-2, 3, 4 in the α-pyrone ring system was ascertained. During our investigation of HMBC, such relations were cofirmed: δ 107.87 ppm was assigned to C-10 from the correlations of H-3 to C-2 and δ 107.87 ppm; the position of the glucosyl unit was ascribed to C-3″ from the correlation between the glucoside-1-H and C-3″ signal. The apiosyl unit was assigned to glucosyl-6-C from the chemical downshift of glucosyl-6-C and the observed correlation between the apiosyl anomeric proton signal and the glucosyl-6-C in the HMBC spectrum. The 2,3-dioxygenated isopentyloxy structural unit was assigned to C-8 from the weak correlations between C-8 and H-1″. The methoxyl group was assigned to C-5 from the correlations between C-5 and the hydrogen signal of the methoxyl group.

Therefore, compound **1** was finally characterized as the new compound *tert*-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl-byakangelicin.

Compound **2** was obtained as a white amorphous powder. Its molecular formula $C_{25}H_{34}O_{14}$ was determined on the basis of its ESI-MS (581 $[M+Na]^+$) and confirmed by ^1H NMR and ^{13}C-NMR data.

Detailed analysis of its ^1H NMR, ^{13}C NMR, COSY, HSQC, and HMBC spectra indicated the presence of a simple coumarin glucoside skeleton, a 2,3-dioxygenated isopentyloxy structural unit, a β-D-glucose, and a β-D-apiose; see Table 1. Using the same method described previously, we ascertained the following connections: the apiofuranosyl-(1→6)-glucopyranosyl unit was connected with C-2′, and the 2,3-dioxygenated isopentyloxy group was connected with C-6.

Therefore, compound **2** was characterized as the new compound 2′-*O*-β-D-apiofuranosyl-(1→6)-β-D-gluco-pyranosyl-peucedanol.

EXPERIMENTAL

General Methods. ESI-MS was measured with an Agilent 1100 LC/MSD SL; optical rotation value was measured with a JASCO P-1020 optical rotation apparatus.

Plant Material. Fresh roots, collected from Jiangsu province of PR China in 2004, were taxonomically identified by Prof. Chang-Qi Yuan. A voucher specimen was deposited in the Herbarium of Nanjing Botanical Garden Mem. Sun Yat-Sen, Nanjing, Jiangsu, China.

Extraction and Purification. The fresh roots (38.0 kg) were extracted with ethanol at room temperature. After removal of ethanol, the water suspension was re-extracted with petroleum ether and EtOAc. The obtained aqueous portion was subjected to HP-20 (H_2O→MeOH). The methanol eluate (70.0 g) was chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (10:1:0.0→17:3:0.2→4:1:0.1→7:3:0.5)] to furnish four fractions (frs.1 to 4). Fraction 2 was subjected to ODS column and then Sephadex LH-20 to afford compound **1** (9.6 mg) and **2** (9.0 mg).

Acid Hydrolysis of Compound 1 and 2. Samples (5 mg each) were refluxed with 2N H_2SO_4 (5 mL) at 80° for 4 hours. After neutralization with $Ba(OH)_2$ and extraction with $CHCl_3$, the aqueous supernatant separated from the $CHCl_3$ layer was dried and dissolved in DMSO (2 mL), and then extracted with *n*-hexane (2 mL). Reaction of the solution with a hexamethyl disilazane: trimethylchlorosilane (2:1) mixture under shaking for 15 minutes yielded the corresponding derivatives. After deposition for 1 hour, the upper solution was detected by GC under the following conditions.

Samples and authentic glucides were analyzed by a Shimadzu GC-2010 using Shimadzu GC solution software. A Phenomenex ZB-WAX column (30 m × 0.25 mm, 0.25 μm film thickness) was used with nitrogen as carrier gas (50.0 mL/min). GC oven temperature was kept at 160° for 2 min and programmed to 190° at a rate of 2.5° per min. The injector temperature was at 225°C. Split ratio was adjusted at 50:1. The FID temperature was at 250°C.

Authentic glucide samples (glucoside and apioside) were analyzed respectively by GC under such conditions, and then compared with the chromatograms of the samples. The existence of glucoside and apioside was detected in both the two samples.

Compound 1, *tert*-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl- byakangelicin, yellowish amorphous powder, $[\alpha]_D^{21.6}$ –20.8° (*c* 0.15 MeOH–H_2O 40:60).

ESI-MS *m/z*: 621 $[M+Na]^+$, indicating that the molecular weight is 598, and combined with the data of ^1H NMR and ^{13}C NMR, the molecular formula can be deduced to $C_{27}H_{34}O_{15}$.

Compound 2, 2′-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl-peucedanol, white amorphous powder; ESI-MS *m/z*: 581 $[M+Na]^+$, indicating that the molecular weight is 558, and combined with the data of ^1H NMR and ^{13}C NMR, the molecular formula can be deduced to $C_{25}H_{34}O_{14}$.

For ^1H NMR and ^{13}C NMR, as well as HMBC spectral data, see Table 1.

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