

Furanocoumarins from the Root of *Angelica dahurica*

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Five furanocoumarins including a new one were isolated from the root of *Angelica dahurica* by repeated silica gel column chromatography. Their chemical structures were determined to be isoimperatorin (**1**), oxypeucedanin hydrate-3"-butyl ether (**2**), imperatorin (**3**), knidilin (**4**), and oxypeucedanin hydrate (**5**). This represents the first study in which the compound **2** has been isolated and identified. The long-range coupling (5J) in the $^1\text{H-NMR}$ spectrum observed in the linear furanocoumarin skeleton was also investigated in detail.

Key words: *Angelica dahurica*, Linear furanocoumarin, Long-range coupling (5J), Imperatorin, Isoimperatorin, Oxypeucedanin hydrate, Oxypeucedanin hydrate-3"-butyl ether, Knidilin

INTRODUCTION

Angelicae dahuricae Radix has been frequently prescribed as a sedative and an analgesic in Chinese medicine (Soka, 1985). Several furanocoumarins of *Angelica dahurica* have been extensively studied (Kozawa *et al.*, 1981; Kim *et al.*, 1992; Qiao *et al.*, 1996; Shin *et al.*, 1991), but little has been reported about the antispasmodic principles, so far.

Therefore, in the course of search for anticonvulsant components from the root of *Angelica dahurica* Benth. et Hook, five linear furanocoumarins were isolated by repeated silica gel column chromatographies of EtOAc fraction obtained from MeOH extracts. The chemical structures of five furanocoumarins were determined by means of spectroscopic studies. One of the components was a new furanocoumarin.

Meanwhile, linear furanocoumarin skeleton is known as one of rare examples showing long-range coupling through five bond in the $^1\text{H-NMR}$ spectrum (Lee and Woo, 1982), the coupling constants of which is sensitively affected by the variation of stereochemistry (Pavia *et al.*, 1996).

This paper describes structural determination of the new furanocoumarin and variations in the 5J long-range coupling pattern according to difference of chemical structure.

MATERIALS AND METHODS

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Instrumentation

Melting points were determined on a Fisher Scientific melting point apparatus and uncorrected. Optical rotations were measured on a Rudolph Research Autopol III-7214. UV and IR spectra were obtained on a Shimadzu UV-Vis spectrophotometer UV-1601 and Perkin Elmer Model 599B, respectively. Mass spectra were taken with JEOL JMS-AX505WA. ^1H and $^{13}\text{C-NMR}$ spectra were obtained on a JEOL JNM-LA400 (400 MHz).

Plant materials

The root of *Angelica dahurica* Benth. et Hook was purchased at the market in Seoul and identified by Dr. Hyung-Kyu Lee, Korea Research Institute of Bioscience and Biotechnology, Taejon. The reference specimen has been deposited in the laboratory of natural products chemistry, Kyunghee University, Suwon.

Isolation of furanocoumarins

Coarsely powdered plant material (1 kg) was extracted with 80% aqueous MeOH (4 L \times 2) overnight at room temperature. The extract was evaporated under reduced pressure and partitioned between EtOAc (700 mL \times 2) and H₂O (500 mL). The EtOAc extracts (36 g) obtained by removal of the solvent were chromatographed on a silica gel column. Gradient elution with *n*-hexane-EtOAc with increasing proportions of the EtOAc gave eleven subfractions (ADE-1 ADE-11). Silica gel column chromatography of subfraction ADE-5 (1.89 g) eluting with *n*-hexane-CHCl₃-EtOH (20:20:1) afforded compound **1** (isoimperatorin, 628 mg).

Isoimperatorin (1): colorless prisms (CHCl₃-EtOH), mp

109-110°C; ¹H-NMR: Table I; ¹³C-NMR: Table II.

ADE-7 (2.75 g) was subjected to silica gel column eluting with *n*-hexane-CHCl₃-EtOH (30:10:1) to give four subfractions (ADE-7-1~ADE-7-4) and the third one (ADE-7-3) was chromatographed on a silica gel column eluting with *n*-hexane-CHCl₃-EtOH (28:12:1) to yield compound **2**, (oxypeucedanin hydrate-3^{''}-butyl ether, 143 mg).

Oxypeucedanin hydrate-3^{''}-butyl ether (2): Pale brownish prisms (CHCl₃-EtOH), mp 102-103°C; [α]_D+1.1° (c=1.1, CHCl₃); UV λ_{max} nm (MeOH, logε): 252 (3.5), 269 (3.6), 315 (3.9); EI MS *m/z*: 360 (M⁺), 345, 315, 303, 185, 174; HREIMS *m/z* 360.1577 (calcd for C₂₀H₂₄O₆, 360.1573); IR ν_{max} CHCl₃ (cm⁻¹): 3465, 3015, 1725, 1620, 1520; ¹H NMR: Table I; ¹³C-NMR: Table II.

Compounds **3** (imperatorin, 212 mg) and **4** (knidilin, 154 mg) were obtained by silica gel column chromatography of ADE-8 (4.0 g) using *n*-hexane-CHCl₃-EtOH (25:20:1) as eluting solvent.

Imperatorin (3): colorless prisms (CHCl₃-EtOH), mp 101-102°C; ¹H NMR: Table I; ¹³C NMR: Table II.

Knidilin (4): yellowish prisms (*n*-hexane-EtOAc), mp 114-115°C; ¹H-NMR: Table I; ¹³C-NMR: Table II.

ADE-11 was applied on silica gel column eluting with *n*-hexane-EtOAc-acetone (4:6:1) to give compound **5** (oxypeucedanin hydrate, 517 mg).

Oxypeucedanin hydrate (5): pale yellowish prisms (CHCl₃-EtOH), mp 134-135°C; ¹H NMR: Table I; ¹³C NMR: Table II.

Extraction of *Angelica dahurica* in acetone

The plant material (50 g) was extracted by shaking with acetone (500 mL) at room temperature for 5 h. The same treatment for the extract as previous procedures yielded

EtOAc extract (1.2 g). The extract was developed on the silica gel TLC (*n*-hexane-EtOAc=3:1) and compared with oxypeucedanin hydrate-3^{''}-butyl ether (R_f value=0.46)

RESULTS AND DISCUSSION

The MeOH extract of the plant was fractionated with

Table II. ¹³C-NMR data of furanocoumarins from the root of *Angelica dahurica* Bentham et Hooker (100 MHz)*

No. of C	1	2	3	4	5
2	160.7	161.2	160.4	160.4	163.4
3	117.8	112.6	114.3	112.5	112.9
4	139.1	139.4	139.6	139.3	141.6
4a	106.6	107.2	116.3	107.3	108.0
5	148.5	152.5	113.1	150.7	153.4
	113.3	113.9	125.8	114.3	114.9
7	157.6	158.1	148.4	144.6	159.4
8	93.3	94.2	131.4	126.6	94.8
8a	152.1	148.8	143.6	144.2	150.3
2'	144.5	144.9	146.5	145.0	146.4
3'	104.7	104.9	106.6	105.0	106.1
1 ^{''}	69.1	74.2	70.0	70.2	75.5
2 ^{''}	118.8	76.7	119.7	119.7	78.0
3 ^{''}	139.0	75.6	139.6	139.5	72.9
4 ^{''}	25.4	21.3	25.7	25.7	27.0
5 ^{''}	17.9	21.1	18.0	17.9	26.3
<i>n</i> -butyloxy		60.9		60.6	
1 ^{'''} , 2 ^{'''}		32.4			
3 ^{'''} , 4 ^{'''}		19.4			
		13.8			

***1, 2, 3, 4** in CDCl₃; **5** in CD₃OD

Table I. ¹H-NMR data of furanocoumarins from the root of *Angelica dahurica* Bentham et Hooker (400 MHz)*

No. of H	1	2	3	4	5
3	**6.04, d, 9.8	6.16, d, 9.8	6.34, d, 9.5	6.25, d, 9.8	6.16, dd, 9.8, 0.8
4	7.91, dd, 9.8, 0.5	8.14, d, 9.8	7.78, d, 9.5	8.10, d, 9.8	8.23, d, 9.8
5			7.36, s		
8	6.84, br s	7.03, br s			6.92, br s
2'	7.42, d, 2.4	7.50, d, 2.2	7.67, d, 2.2	7.62, d, 2.2	7.61, dd, 2.3, 0.7
3'	6.78, dd, 2.4, 1.0	6.93, dd, 2.2, 0.7	6.81, d, 2.2	7.00, d, 2.2	7.06, d, 2.3
1 ^{''}	4.74, d, 7.1	4.53, dd, 10.0, 3.0 4.31, dd, 10.0, 7.8	4.99, d, 7.1	4.83, d, 7.3	4.69, dd, 10.0, 2.2 4.39, dd, 10.0, 8.8
2 ^{''}	5.36, t, 7.1	3.85, dd, 7.8, 3.0	5.60, t, 7.1	5.60, t, 7.3	3.91, dd, 8.8, 2.2
4 ^{''}	1.57, s	1.17, s	1.73, s	1.69, s	1.36, s
5 ^{''}	1.65, s	1.19, s	1.73, s	1.73, s	1.37
1 ^{'''}		3.33, t, 6.3		4.18, s	
2 ^{'''}		1.45, m			
3 ^{'''}		1.29, m			
4 ^{'''}		0.83, t, 7.3			

***1, 2, 3, 4** in CDCl₃; **5** in CD₃OD

** , coupling pattern, *J* in Hz

EtOAc and H₂O. Five furanocoumarins were isolated by repeated silica gel column chromatographies of EtOAc fraction.

The compound **1**, **3**, **4**, and **5** were identified as isoimperatorin, imperatorin, knidilin, and oxypeucedanin hydrate by comparison of spectral and physical data with those of literatures (Gu *et al.*, 1990; Harkar *et al.*, 1984; Hata *et al.*, 1981; Kozawa *et al.*, 1981; Sasaki *et al.*, 1982).

The compound **2**, obtained as pale brownish prisms in CHCl₃-EtOH, showed the absorbance band at 3465, 3015, 1725, 1620 and 1520 cm⁻¹ in the IR spectrum (CHCl₃) indicating the presence of hydroxyl (3465 cm⁻¹), α-pyrone (1725, 1520 cm⁻¹) and an aromatic ring system (1620 cm⁻¹), respectively. The UV spectrum exhibited typical bands of 5-substituted linear furanocoumarin ring at 252, 269, and 315 nm. The ¹H and ¹³C NMR data of compound **2** were very similar to those of oxypeucedanin hydrate (**5**). In the ¹H NMR spectrum of compound **2** (400 MHz, CDCl₃), two proton doublet signals (1H, *J*=9.8 Hz) at δ 6.16 and 8.14 were assignable to the protons of pyrone ring. A proton doublet signal (1H, *J*=2.2 Hz) at δ 7.50 and another proton doublet-doublet signal (1H, *J*=2.2, 0.7 Hz) at δ 6.93 were attributable to the protons of furan ring. The signal for H-4 was observed rather lower field (δ 8.14) to indicate the absence of proton at C-5 (Harkar *et al.*, 1984). And the signal for H-3' was observed as a doublet-doublet (*J*=2.2, 0.7 Hz) to mean the occurrence of long-range coupling with H-8 because long-range coupling between H-3' and H-5 in the linear furanocoumarin skeleton was not usually observed (Sasaki *et al.*, 1982). Therefore, a side chain

was suggested to be substituted at C-5 position. The presence of 2-hydroxy-3-methyl-3-oxy-butyl unit was confirmed by the proton signals observed at δ 4.53 and δ 4.31 (H-1''), δ 3.85 (H-2''), δ 1.17 and δ 1.19 (H-4'', 5''). Additionally, a oxygenated methylene (δ 3.33, t), two methylenes (δ 1.45, δ 1.29, both m) and a methyl (δ 0.83, t) proton signals indicated the presence of *n*-butyloxy unit, which was supposed to be linked to 3''-hydroxy from the fact that C-4'' and C-5'' of compound **2** were upfield-shifted by 5.76 and 5.15 ppm, respectively, and C-3'' was downfield-shifted by 2.66 ppm, compared to those of oxypeucedaninhydrate (**5**) in the ¹³C-NMR spectrum. Furthermore, it was confirmed by long-range correlation of H-1'' with C-3'' in the HMBC spectrum of compound **2** and the comparison of NMR-data with 3''-methoxyl oxypeucedanin hydrate (Lee and Woo, 1982) or 3''-ethoxyl oxypeucedanin hydrate (Harkar *et al.*, 1984). In the ¹³C-NMR spectrum of **2** (100 MHz, CDCl₃), four carbon signals due to *n*-butyloxy group were observed at δ 60.92 (t), 32.39 (t), 19.37 (t) and 13.82 (q). Finally, chemical structure of compound **2** was determined to be oxypeucedanin hydrate-3''-butyl ether.

From Umbelliferae plants, derivatives of oxypeucedanin hydrate such as 3''-methoxyl (Lee and Woo, 1982) or 3''-ethoxyl (Harkar *et al.*, 1984) have been isolated, but 3''-butoxyl derivative has not been reported. During extraction or isolation procedures, *n*-BuOH was not used, therefore, oxypeucedanin hydrate-3''-butyl ether (**2**) was not thought to be an artifact. To confirm it, the root of *Angelica dahurica* was extracted in acetone and partitioned between EtOAc and H₂O. The presence of compound **2** in EtOAc fraction was affirmed by direct comparison of silica gel TLC for the EtOAc layer and compound **2**.

Preliminary anticonvulsant test for the isolated five furanocoumarins exhibited significant activity. More intensive study is proceeding.

Some of linear furanocoumarins have been known to show ⁵*J* long-range coupling in the ¹H-NMR spectrum, between H-8 and H-3, H-4, H-2' or H-3' (Lee and Woo, 1982). The coupling constants are varied with just minute change of stereostructure of the linear furanocoumarin skeleton (Pavia *et al.*, 1996), which is affected by the kind and position of substitutional group. ⁵*J* Coupling between H-4 and H-8 was observed in isoimperatorin (**1**) which has a 3-methyl-2-butenyl substitution at C-5 (Gu *et al.*, 1990). And ⁵*J* coupling between H-3' and H-8 was observed in isoimperatorin (**1**) and oxypeucedanin hydrate-3''-butyl ether (**2**) which has a 2-hydroxy-3-methyl-3-butoxybutyl at C-5. And ⁶*J* coupling between H-3 and H-8, and ⁵*J* coupling via oxygen atom between H-2' and H-8 were observed only in oxypeucedaninhydrate (**5**), which has 2,3-dihydroxy-3-methylbutyl at C-5 (Harkar *et al.*, 1984). In addition, long-range coupling between H-5 and other protons was not observed in imperatorin (**3**) (Sasaki *et al.*, 1982; Harkar *et al.*, 1984; Lee & Woo, 1982). The pre-

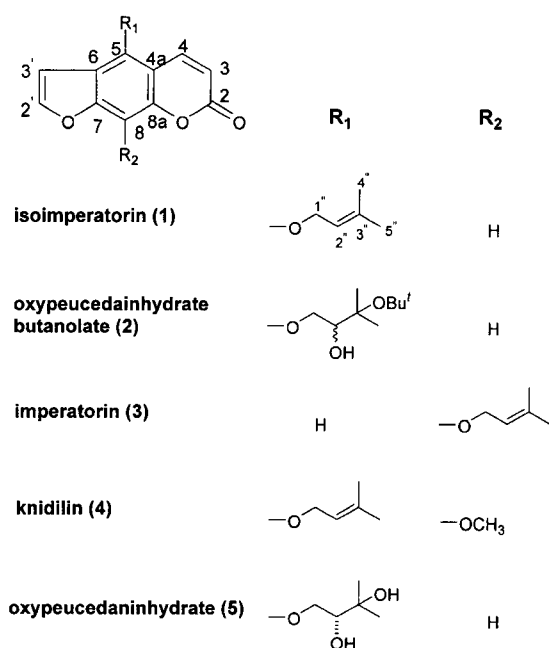


Fig. 1. Chemical structure of furanocoumarins isolated from the root of *Angelica dahurica* Bentham et Hooker

viously described results for long-range couplings will be useful for structural determination of linear furanocoumarins.

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