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Coumarins and a Polyacetylene from the Roots of *Angelica purpuraefolia*

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Abstract – Four coumarins (**1-4**) and one polyacetylene (**5**) were isolated from the roots of *Angelica purpuraefolia* Chung (Umbelliferae) through repeated column chromatography. Four coumarins, isoscopoletin (**1**), oxypeucedanin hydrate (**2**), arnottinin (**3**) and isokhellactone (**4**), and a polyacetylene, (+)-9(*Z*),17-octadecadiene-12,14-diyne-1,11,16-triol (**5**), were identified by spectroscopic analysis including two dimensional NMR and mass. These compounds were examined for their anti-complement activity against the classical pathway of the complement system. However, compounds **1-5** were inactive in this assay system.
Keywords – *Angelica purpuraefolia*, Umbelliferae, coumarin, polyacetylene, anti-complement activity

Introduction

Angelica species (Umbelliferae) are endemic to Northeast Asia and are important medicinal plants that have attracted considerable attention on account of their biological and chemical diversity. Studies of the chemical components of *Angelica* have led to the identification of many compounds including coumarins, chromones, phenylpropanoids, sesquiterpenes, and polyacetylenes (An *et al.*, 2005). Some of them have been shown to have 5α-reductase type I activity (Seo *et al.*, 2002), anti-proliferative activity (Fujioka *et al.*, 1999), anti-HIV-1 activity (Zhou *et al.*, 2000), anti-acetylcholinesterase activity (Kim *et al.*, 2002), and activity in inhibiting prostaglandin E2 (Ban *et al.*, 2003) and TNF-α production (Cho *et al.*, 1998). *Angelica purpuraefolia* Chung is one of substitute sources of the Chinese herbal drug "Gangwhal" (Lee, 1996). The roots of this plant have been used to treat the common cold, headache, neuralgia, and arthralgia (Woo *et al.*, 1982). This study is part of an ongoing study on the bio-active compounds from natural sources.

The activation of the complement system plays a dual role in the disease process. First, complement activation has many protective functions in immunity, both as a first line defense mechanism against invading pathogens and as a potentiator of acquired immunity. However, complement activation is a major cause of tissue damage in many pathological conditions. Three different pathways via which the complement system can be activated have been identified. These are the classical pathway, the alternative pathway, and the MBL/MASP (mannan binding lectin/MBL-associated serine protease) pathway (Ember & Hugli, 1997). The proteolytic cascade allows for significant amplification since each proteinase molecule activated at one step can generate many multiple copies of the activated enzyme later in the cascade. These in turn cleave non-enzymatic components such as C3, C4, and C5 (i.e., C3b, C4b, and C5b), which are involved in the biological effector functions, such as opsonization, phagocytosis, and immunomodulation. However, the small molecules, C3a, C4a, and C5a, which are known as an anaphylatoxins, induce the release of various mediators from mast cells and lymphocytes, such as the chemotaxis of leukocytes, and the degranulation of phagocytic cells, mast cells, and basophils, which cause a variety of diseases (i.e., rheumatoid arthritis, osteoarthritis, atopic dermatitis, lung fluid inflammation, and atherosclerotic lesion) as well as hyperacute xenograft rejection (Oh *et al*,. 1999). Therefore, the ability of certain compound to modulate the complement activity will be useful treating inflammatory diseases. As a part of an ongoing study aimed at identifying novel anti-complement active compounds from medicinal plants, this paper reports the isolation and the anti-complement effect of coumarins and polyacetylene of the isolated from the roots of *A. purpuraefolia*.

Experimental

Optical rotations were measured with a JASCO DIP-370 automatic digital polarimeter in $CHCl_3$ or MeOH. The NMR spectra were recorded on a Varian NMR

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Table 1. The ^{1}H-NMR (400 MHz) spectroscopic data of compounds **1-4**

proton	**1**[a]	**2**[a]	**3**[b]	**4**[b]
3	6.27 d (9.6)	6.29 d (9.6)	6.16 d (9.6)	6.23 d (9.6)
4	7.59 d (9.6)	8.17 d (9.6)	7.82 d (9.6)	7.90 d (9.6)
5	6.92 s		7.30 d (8.4)	7.60 s
6			6.80 d (8.4)	6.80 s
7				
8	6.85 s	7.17 s		
OCH_3	3.96 s			
1'			3.56 d (2H, 7.6)	
2'		7.61 d (2.8)	5.53 td (7.6, 1.6)	
3'		6.99 d (2.8)		4.37 d (4.4)
4'			3.89 m	5.35 d (4.4)
5'			1.87 s	
CH_3				1.29 s, 1.28 s
1"		4.54 dd (10.0, 3.0)		
		4.44 dd (10.0, 7.8)		
2"		3.92 dd (7.8, 3.0)		
CH_3		1.36 s, 1.31 s		

[a]$CDCl_3$ [b]MeOH-d_4. δ values in ppm and coupling constant (in parentheses) in Hz.

System 400 MHz, with chemical shifts being represented in ppm and tetramethylsilane used as an internal standard. ESIMS was measured on a HP5989 DIP mass spectrometer. Medium pressure liquid chromatography (MPLC) separations were performed over LiChroprep RP C-18 (Merck, size B). High performance liquid chromatography (HPLC) separations were performed over YMC-Pack ODS-A (250 × 20 mm I.D.). The spots were detected under UV radiation and by spraying with 10% H_2SO_4, followed by heating.

Plant material – The roots of *A. purpuraefolia* were collected during September 2003 at Chiri-san, Korea, and dried at room temperature. A voucher specimen (PBS-2358.1) was deposited at Plant Extract Bank., Korea Research Institute of Bioscience and Biotechnology, Deajeon, Korea.

Isolation procedure – The dried and powdered roots of *A. purpuraefolia* (10 kg) were extracted with MeOH (3 × 10 L) for 7 days at 25 °C. The combined extracts were concentrated under reduced pressure. The residue (652 g) was diluted with water (1 L), and then partitioned successively with $CHCl_3$ (3 × 1 L) and EtOAc (3 × 1 L) to afford the $CHCl_3$- (340 g) and EtOAc-soluble fractions (10 g), respectively. The $CHCl_3$-soluble fraction was chromatographed on a silica gel column. The column eluted using a stepwise gradient of hexane and EtOAc to give nine fractions (Fr. 1 – Fr. 9; 4.6 g, 19.3 g, 6.2 g, 93.2 g, 137.1 g, 11.3 g, 11.0 g, 5.1 g, 9.0 g). Of nine fractions, Fr. 7 was loaded onto a silica gel column, which was eluted with hexane : EtOAc (50 : 1 → 1 : 1, v/v) to give six sub-fractions (sub-fr. 1 – sub-fr. 6; 0.044 g, 0.218 g, 4.73 g, 1.87 g, 1.06 g, 0.22 g). Repeat column chromatography of sub-fr. 3 on silica gel (hexane : EtOAc) and MPLC (RP C-18, 45% acetonitrile) afforded **5** (94 mg). Fr. 8 was chromatographed on a silica gel column with hexane : EtOAc (3 : 1 → 1 : 1) and $CHCl_3$: MeOH (9 : 1) to yield five sub-fractions (sub-fr. 1 – sub-fr. 5; 0.159 g, 6.564 g, 2.051 g, 2.225 g, 3.713 g). Column chromatography of sub-fr. 2 over silica gel (hexane : EtOAc, 3 : 2), RP C-18 MPLC (40% acetonitrile), and HPLC (ODS, 20% → 44% acetonitrile) resulted in the isolation of **1** (5.0 mg), **2** (7.7 mg), **3** (2.7 mg), and **4** (4.0 mg).

Table 2. The ^{13}C-NMR (100 MHz) spectroscopic data of compounds **1-4**

carbon	**1**[a]	**2**[a]	**3**[b]	**4**[b]
2	161.4	161.1	163.8	165.2
3	111.5	114.3	113.3	112.7
4	149.7	139.0	146.5	146.2
5	107.5	152.5	123.9	129.4
6	143.2	113.1	111.9	126.3
7	150.3	158.1	160.6	163.4
8	103.2	94.9	116.4	99.8
4a	113.5	107.3	123.6	114.5
8a	144.0	148.5	154.8	158.0
OCH_3	56.4			
1'			14.0	
2'		145.3	113.6	71.8
3'		104.7	136.7	98.9
4'			68.8	72.5
5'			22.3	
CH_3				25.5, 25.3
1"		71.7		
2"		76.5		
3"		74.5		
CH_3		26.7, 25.1		

[a]$CDCl_3$ [b]MeOH-d_4.

Isoscopoletin (1) – yellow needle; mp 184-186 °C; ESI-MS *m/z*: 193 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2.

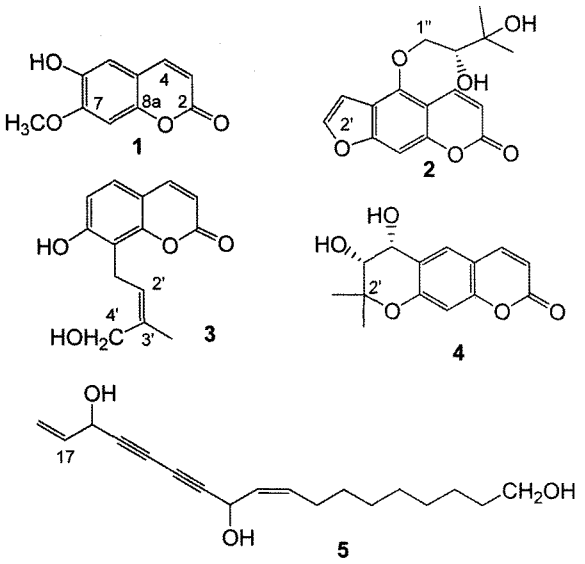

Fig. 1. Structures of compounds **1-5** from *A. purpurasefolia.*

Oxypeucedanin hydrate (2) – colorless needle; mp 132-134 °C; $[\alpha]_D$: +21° (c = 0.2, MeOH); ESI-MS *m/z*: 305 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2.

Arnottinin (3) – white amorphous powder; mp 192-194 °C; ESI-MS *m/z*: 247 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2.

Isokhellactone (4) – white amorphous powder; mp 226 - 227 °C; $[\alpha]_D$: +45.2° (c = 0.2, $CHCl_3$); ESI-MS *m/z*: 263 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2.

(+)-9(*Z*),17-Octadecadiene-12,14-diyne-1,11,16-triol (5) – colorless oil; $[\alpha]_D$: +185° (c = 0.2, $CHCl_3$); ESI-MS *m/z*: 291 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6) δ: 5.93 (1H, ddd, J = 17.0, 10.4, 5.5 Hz, H-17), 5.58 (1H, dd, J = 10.8, 7.5 Hz, H-9), 5.50 (1H, dd, J = 10.8, 8.2 Hz, H-10), 5.42 (1H, dt, J = 17.0, 1.2 Hz, H-18a), 5.21 (1H, dt, J = 10.4, 1.2 Hz, H-18b), 5.17 (1H, d, J = 8.2 Hz, H-11), 4.89 (1H, d, J = 5.5 Hz, H-16), 3.56 (2H, t, J = 6.6 Hz, H-1), 2.14 (2H, m, H-8), 1.54 (2H, m, H-7); ^{13}C-NMR (DMSO-d_6) δ: 137.1 (C-17), 133.1 (C-10), 128.9 (C-9), 115.6 (C-18), 80.1 (C-15), 78.8 (C-12), 69.1 (C-14), 67.8 (C-13), 62.8 (C-16), 62.0 (C-1), 57.9 (C-11), 32.7, 29.5 (×2), 29.4, 29.2, 27.5, 25.9.

Determination of anti-complement activity through the classical pathway (CP) – Anti-complement activity was determined by modified method of Mayer as described previously (Min *et al.*, 2003). For the classical pathway assay, a diluted solution of normal human serum (80 μl) collected from a healthy volunteer (Man) was mixed with gelatin veronal buffer (80 μl) with or without sample. Each sample was dissolved in DMSO, and used as a negative control. The mixture was pre-incubated at 37 °C for 30 min, and sensitized erythrocytes (sheep red blood cells, 40 μl) were added. After incubation under the same conditions, the mixture was centrifuged (4 °C, 1500 rpm) and the optical density of the supernatant (100 μl) measured at 405 nm. Tiliroside was employed as a positive control (Jung *et al.*, 1998).

Results and Discussion

Repeated column chromatography of the $CHCl_3$-soluble fraction of the MeOH extraction from the roots of *A. purpuraefolia* led to the isolation of four coumarins (**1-4**) and one polyacetylene (**5**).

Compound **1** was obtained as light yellow needles. The presence of 6,7-dioxygenated coumarin was indicated from the typical signals as δ 7.59 and 6.27 (each 1H, d, J = 9.6 Hz) for H-4 and H-3, and two singlet aromatic protons at δ 6.92 (H-5) and 6.85 (H-8) in the ^{1}H-NMR spectrum (Table 1), compared with that of scopoletin 7-*O*-β-D-sophoroside isolated from *Viburnum tinus* (Mohamed *et al.*, 2005). This observation was further supported by the carbon signals for a carbonyl carbon (δ 161.4), two olefinic carbons (δ 149.7 and 111.5), three oxygenated aromatic carbons (δ 150.3, 144.0, 143.2), and two aromatic carbons (δ 107.5, 103.2) in the ^{13}C-NMR spectrum (Table 2). In addition, the ^{1}H-NMR spectrum showed a methoxy group at δ 3.96, which correlated with the aromatic proton at δ 6.85 (H-8) in the NOESY spectrum, and led to the identification of the position of the methoxy group at C-7. Based on these results and on values previously reported in the literature (Cardona *et al.*, 1992), compound **1** was identified as isoscopoletin.

Compound **2** was obtained as colorless needles with a positive optical rotation ($[\alpha]_D$ +21°). The ^{1}H-NMR spectrum exhibited signals for two olefinic protons at δ 8.17, 6.29 (each 1H, d, J = 9.6 Hz) and an aromatic proton at δ 7.17 (s), which were assignable to 5,7-dihydroxy-6-substituted coumarin in the ^{1}H-NMR spectrum, compared with that of isoimperatorin isolated from *Angelica dahurica* (Baek *et al.*, 2000). This moiety was further supported by the ^{13}C-NMR signals for a carbonyl carbon (δ 161.1), two olefinic carbons (δ 139.0 and 114.3), three oxygenated aromatic carbons (δ 158.1, 152.5, 148.5), and three aromatic carbons (δ 113.1, 107.3, 94.9). The ^{1}H-NMR spectrum also showed the presence of 2,3-dihydroxy-3-methylbutyl group at δ 4.54 (1H, dd, J = 10.0, 3.0 Hz), 4.44 (1H, dd, J = 10.0, 7.8 Hz), 3.92 (1H, dd, J = 7.8, 3.0 Hz), 1.36 (3H, s), and 1.31 (3H, s). Furthermore, a proton doublet signal at δ 7.61 (d, J = 2.8 Hz) and its vicinal partner proton at δ 6.99 (d, J = 2.8 Hz) in the ^{1}H-NMR spectrum indicated the presence of a furan-ring in compound **2**. Thus, compound

2 was elucidated as oxypeucedanin hydrate by comparison of literature (Baek *et al.*, 2000).

Compound **3** was isolated as a white amorphous powder. The ^{1}H-NMR spectrum of compound **3** exhibited signals due to hydroxylmethyl protons at δ 3.89 (2H, m), one allylic methyl at δ 1.87 (s), adjacent methylene and olefinic methine protons at δ 3.56 (2H, d, J = 7.6 Hz) and 5.53 (1H, td, J = 7.6, 1.6 Hz), as well as signals arising from two olefinic protons at δ 7.82 and 6.16 (each 1H, d, J = 9.6 Hz), and two aromatic protons at δ 7.30 and 6.80 (each 1H, d, J = 8.4 Hz). These data indicated that compound **3** is a 7,8-disubstituted coumarin. This finding was further supported by signals for six non-protonated carbons at δ 163.8 (C-2), 160.6 (C-7), 154.8 (C-8a), 136.7 (C-3'), 123.6 (C-4a) and 116.4 (C-8), and eight protonated carbons at δ 146.5 (C-4), 123.9 (C-5), 113.6 (C-2'), 113.3 (C-3), 111.9 (C-6), 68.8 (C-4'), 22.3 (C-5') and 14.0 (C-1') in the ^{13}C-NMR spectrum. Thus, compound **3** was elucidated as arnottinin to have *Z*-configuration by comparison of literature (Ishii and Ishikawa, 1975).

Compound **4** was also obtained as a white amorphous powder. It exhibited two olefinic protons δ 7.90 and 6.23 (each 1H, d, J = 9.6 Hz), a gem-dimethyl group at δ 1.29 and 1.28 (each 3H, s), and two oxygenated methines at δ 5.35 and 4.37 (each 1H, d, J = 4.4 Hz), which were assignable to a pyranocoumarin in the ^{1}H-NMR spectrum, compared with that of khellactone (Matsuda *et al.*, 2000). However, the presence of 7-hydroxy-6-substituted pyranocoumarin in compound **4** was indicated from the signals for two aromatic protons at δ 7.60 and 6.80 (each 1H, s) in the ^{1}H-NMR spectrum. This moiety was further supported by the ^{13}C-NMR signals for two methyl carbons at δ 25.5 and 25.3, an oxygenated quaternary carbon at δ 71.8 (C-2'), and two oxygenated methines at δ 98.9 (C-3') and 72.5 (C-4'). Based on these results, compound **4** was identified as isokhellactone.

Compound **5** was isolated as colorless oil with a positive optical rotation, $[\alpha]_D$ +185°. The ^{13}C-NMR spectrum showed resonances of four nonprotonated acetylenic carbons at δ 80.1, 78.8, 69.1 and 67.8 (by HMQC), four olefinic carbons at δ 137.1, 133.1, 128.9 and 115.6, three oxygen-bearing sp^3 carbons at δ 62.8, 62.0 and 57.9, and seven methylenes. The ^{1}H-NMR spectrum showed signals for three olefinic protons at δ 5.93 (ddd, J = 17.0, 10.4, 5.5 Hz), 5.42 (dt, J = 17.0, 1.2) and 5.21 (dt, J = 10.4, 1.2 Hz), which were correlated to a methine at δ 4.89 (d, J = 5.5 Hz) in the ^{1}H-^{1}H COSY spectrum. The COSY spectrum also showed another hydroxy methine at δ 5.17 (d, J = 8.2 Hz) coupled to olefinic protons at δ 5.58 (dd, J = 10.8, 7.5 Hz) and 5.50 (dd, J = 10.8, 8.2 Hz). Furthermore, a terminal hydroxymethyl protons at δ 3.56 (2H, t, J = 6.6 Hz) was coupled to a methylene protons. Based on these results and on values previously reported in the literature, compound **5** was identified as (+)-9(*Z*),17-octadecadiene-12,14-diyne-1,11,16-triol (Papajewski *et al.*, 1998).

Table 3. Inhibitory activity of the compounds **1-4** against complement system of classical pathway

compound	IC_{50} values (μM)[a]
isoscopoletin (**1**)	> 200
oxypeucedanin hydrate (**2**)	> 200
arnottinin (**3**)	> 200
isokhellactone (**4**)	> 200
(+)-9(*Z*),17-octadecadiene-12,14-diyne-1,11,16-triol (**5**)	> 200
tiliroside[b]	102

[a]The value represent the mean ± S.D. of three experiments.
[b]Used as positive control.

The compounds (**1-5**) isolated from *A. purpuraefolia* were bioassayed for their classical pathway complement inhibitory activity in vitro using protocol previously reported (Min *et al.*, 2003). However, all compounds tested were completely inactive against complement-induced hemolysis through the classical pathway (Table 3).

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