Constituents of the Stem of *Angelica gigas* with Rat Lens Aldose Reductase Inhibitory Activity

Hyun Young Park[1], Soon Bae Kwon[2], Nam Kee Heo[2], Wan Joo Chun[3], Myong Jo Kim[4], and Yong Soo Kwon[1]*

[1]*College of Pharmacy, Kangwon National University, Chuncheon 200-701, Republic of Korea*
[2]*Speciality Crops Experimental Stations, GARES, Chunchon 200-150, Republic of Korea*
[3]*College of Medicine, Kangwon National University, Chuncheon 200-701, Republic of Korea*
[4]*Oriental Bio-Herb Research Institute, Kangwon National University, Chuncheon 200-701, Republic of Korea*

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Eleven compounds were isolated from the stem of *Angelica gigas*. On the basis of spectral data, these compounds were identified as isoimperatorin (1), 7-methoxy-5-prenyloxycoumarin (2), imperatorin (3), decursin (4), bergapten (5), psoralen (6), xanthotoxin (7), *p*-hydroxyphenethyl *trans*-ferulate (8), visamminol (9), scopoletin (10), and 3'-hydroxyxanthyletin (11). All isolates were evaluated *in vitro* for their inhibitory activities on the rat lens aldose reducatase. Tested compounds (1-11) exhibited inhibitory activities against rat lens aldose reductase with IC_{50} values ranging from 2.59 to 191.91 μM.

Key words: *Angelica gigas*, ^{13}C-nuclear magnetic resonance, chromone, coumarins, diabetic complications, 3'-hydroxyxantyltein, inhibitory activity, rat lens aldose reductase, stem

Acceleration of the polyol pathway [Yabe-Nishimura, 1998], nonenzymatic glycation [Friedman, 1999], activation of protein kinase C [Koya and King, 1998] and oxidative stress [Baynes and Thorpe, 1999] are considered as the prime mechanisms underlying complications such as cataracts, retinopathy, and nephropathy of diabetes mellitus.

Aldose reductase (AR), the key enzyme in the polyol pathway, has been reported to play an important role in the pathogenesis of diabetic complications [Santiago, 1993; Feldman *et al*., 1997]. AR inhibitors are considered as preventive agents used for diabetic complications [Boel *et al*., 1995]. Thus, many studies have reported various natural compounds from plant sources, which act as AR inhibitors [Shimizu *et al*., 1984; Ueda *et al*., 2004; Manzanaro *et al*., 2006; Jung *et al*., 2008; Endo *et al*., 2009].

Although *A. gigas* has been reported to be a good source of coumarins, which exhibit AR inhibitory activity [Ryu and Yook, 1967; Ryu *et al*., 1990; Okada *et al*., 1995; Kang *et al*., 2001; Lee *et al*., 2002; Kang and Kim, 2007], the chemical composition and biological activities of *A. gigas* have not been fully elucidated [Kim *et al*., 2009]. Therefore, the present study examined the constituents of the stem of *A. gigas* and their biological activity, with focus on isolation, structure elucidation, and AR inhibitory activity.

*Corresponding author
Phone: +82-33-250-6921; Fax: +82-33-255-7865
E-mail: yskwon@kangwon.ac.kr

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Materials and Methods

General procedures. Melting point was determined on a Fisher Johns melting point apparatus (Philadelphia, PA). UV/Vis spectra were measured on a V-530 spectrophotometer (JASCO, Tokyo, Japan). Optical rotation was measured on a DIP 1000 Digital Polarimeter (JASCO). Infrared (IR) spectrum was measured on a Fourier transform infrared (FTIR)–4200 (JASCO). MS (Mass spectroscopy) spectrum was measured on a Autospec M363 (Micromass, Manchester, England). Nuclear magnetic resonance (NMR) spectrum was recorded on DPX 400 and AVANCE 600 (Bruker, Rheinstetten, Germany). The chemical shifts were represented as parts per million (ppm) referenced to the residual solvent signal. Column chromatography was carried out using Kieselgel 60, 400-230 mesh (Merck, Darmstadt, Germany) and YMC gel octadecyl silyl-silica gel (ODS)-A, 150 mm (YMC, Kyoto, Japan). Thin-layer chromatography (TLC) was performed on glass backed Kieselgel 60 F_{254} and RP F_{254s}

plates (Merck).

Plant material. *A. gigas* was collected from Tae-Baek (September, 2009), Korea. A voucher specimen (KNUH-S-0903) was deposited in the herbarium of the College of Pharmacy, Kangwon National University, Korea.

Extraction and isolation. The dried stem of *A. gigas* (1.8 kg) was refluxed with hot methanol (MeOH) for 3 h, three times. The MeOH extract (71 g) was suspended in water and then successively partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH. Each soluble fraction was evaporated *in vacuo* to yield the residues of *n*-hexane (7 g), $CHCl_3$ (50 g), and *n*-BuOH (10 g) extracts. Among these extracts, $CHCl_3$ extracts exhibited 85% inhibition on AR (10 μg/mL). The $CHCl_3$ soluble fraction (50 g) was column chromatographed on a silica gel (1 kg, 10×50 cm) using isocratic elution with benzene:ethyl acetate (EtOAc) (4:1 to 1:2, gradient), to afford ten fractions (Fr. 1-Fr. 10). Fr. 2 was re-chromatographed on silica gel (100 g, 2.5×50 cm) by elution with hexane:EtOAc (9:1) to afford nine sub-fractions (Fr. 1-1–Fr. 1-9). Fr. 2-4 was re-chromatographed on silica gel (30 g, 3×20 cm) by elution with benzene: EtOAc (39:1) to give compound **1** (20 mg). Fr. 2-5 was re-chromatographed on a silica gel (30 g, 3×20 cm) by elution with benzene:EtOAc (39:1) to give compound **2** (4 mg). Fr. 2-8 was re-chromatographed on silica gel (30 g, 3×20 cm) by elution with benzene:EtOAc (39:1) to afford four sub-fractions (Fr. 2-8-1–2-8-4). Fr. 2-8-3 was re-chromatographed on an ODS column (30 g, 3×20 cm) and silica gel column (30 g, 3×20 cm) by elution with MeOH:H_2O (70:30) and benzene: EtOAc (39:1) to give compound **3** (3 mg). Fr. 3 was re-chromatographed on ODS column (300 g, 10×50 cm) by elution with MeOH:H_2O (80:20) to afford three sub-fractions (Fr. 3-1–3-3). Compound **4** (5 g) was isolated through ODS column (100 g, 2.5×50 cm) of Fr. 3-2 with an acetonitril:H_2O (70:30). Fr. 3-1 was re-chromatographed on ODS column (30 g, 3×20 cm) by elution with MeOH:H_2O (50:50) to afford seven sub-fractions (Fr. 3-1-1–3-1-7) from which compound **5** (230 mg) was obtained. Compounds **6** (10 mg) and **7** (40 mg) were isolated through additional silica gel column chromatography (30 g, 3×20 cm) of Fr. 3-1-4 with benzene: EtOAc (49:1). Fr. 4 was re-chromatographed on silica gel (100 g, 2.5×50 cm) by elution with *n*-hexane:EtOAc (2:1) to afford five sub-fractions (Fr. 4-1–Fr. 4-5). Fr. 4-4 was re-chromatographed silica gel column (80 g, 2.5×50 cm) and ODS column (30 g, 3×20 cm) by elution with *n*-hexane:EtOAc (2:1) and benzene: EtOAc (9:1) and MeOH:H_2O (70:30) to give compound **8** (250 mg). Fr. 6 was re-chromatographed on a silica gel (100 g, 2.5×50 cm) by elution with chloroform ($CHCl_3$): MeOH (49:1) and divided into four sub-fractions (Fr. 6-1–Fr. 6-4). Fr. 6-2 was re-chromatographed on an ODS column (60 g, 2.5×50 cm) by elution with acetonitril: MeOH (50:50) to afford eight sub-fractions (Fr. 6-2-1–6-2-8). Fr. 6-2-4 was re-chromatographed on a silica gel (30 g, 3×20 cm) by elution with $CHCl_3$:MeOH (39:1) to give compound **9** (25 mg). Fr. 7 was re-chromatographed on a silica gel (100 g, 2.5×50 cm) by elution with benzene: EtOAc (4:1) to afford three sub-fractions (Fr. 7-1–7-3). Fr. 7-2 was re-chromatographed on an ODS column (30 g, 3×20 cm) by elution with MeOH:H_2O (50:50) to give compounds **10** (7 mg), and **11** (45 mg).

Compound **11:** UV (MeOH) λ_{max} 206, 248, 295, 333; ^{1}H-NMR (600 MHz, acetone-d_6) and ^{13}C-NMR (125 MHz, acetone-d_6), see Table 1 for spectral data; EI-MS *m/z*: 244 $[M]^+$

Assay for AR inhibition activity. Rat Lens were removed from the eyes of 8 weeks old Sprague-Dawley rats (Dae Han Bio Link Co., Umsung, Korea) each weighing 130-150 g and homogenized in 100 mM sodium phosphate buffer (pH 6.2). The homogenate was centrifuged at 10,000 rpm at 4°C for 20 min to obtained supernatant as the crude rat lens aldose reductase (RLAR). Reaction mixture contained 0.15 mM nicotine amide dinucleotide phosphate reduced form (NADPH), 10 mM DL-glyceraldehyde, 100 μL RLAR, and 10 μL test sample solution or dimethylsulfoxide (DMSO) to a total volume of 1 mL of 100 mM sodium phosphate buffer (pH 6.2). The reaction mixtures were pre-incubated at 25°C for 3 min, and the reaction was started by addition of the enzyme. Decrease in absorbance was measured at 340 nm using a JASCO V-530 spectrophotometer. The inhibitory activity (%) was estimated as follows: [1−(ΔA sample/min−ΔA blank/min)/(ΔA control/min−ΔA blank/min)]×100. ΔA sample/min showed a decrease in absorbance for 1 min with a sample, ΔA blank/min with DMSO and water instead of sample and substrate, and ΔA control/min with DMSO instead of sample and substrate.

Result and Discussion

The structures of **1-10** were identified as isoimperatorin (**1**) [Wei and Ito, 2006], 7-methoxy-5-prenyloxycoumarin (**2**) [Kang *et al.*, 2001], imperatorin (**3**) [Wei and Ito, 2006], decursin (**4**) [Lee *et al.*, 2002], bergapten (**5**) [Bergendorff *et al.*, 1997], psoralen (**6**) [Masuda *et al.*, 1998], xanthotoxin (**7**) [Sasaki *et al.*, 1982], Hydroxyphenethyl *trans*-ferulate (**8**) [Nakata *et al.*, 1982], visamminol (**9**) [Baba *et al.*, 1981], and scopoletin (**10**) [Gözler *et al.*, 1984] by comparison of their spectral data with those of literature values (Fig. 1). Isoimperatorin (**1**) and imperatorin (**3**) have been reported to exhibit β-secretase inhibition activity [Marumoto and Miyazwa, 2010], antiproliferative effects [Kim *et al.*, 2007], and

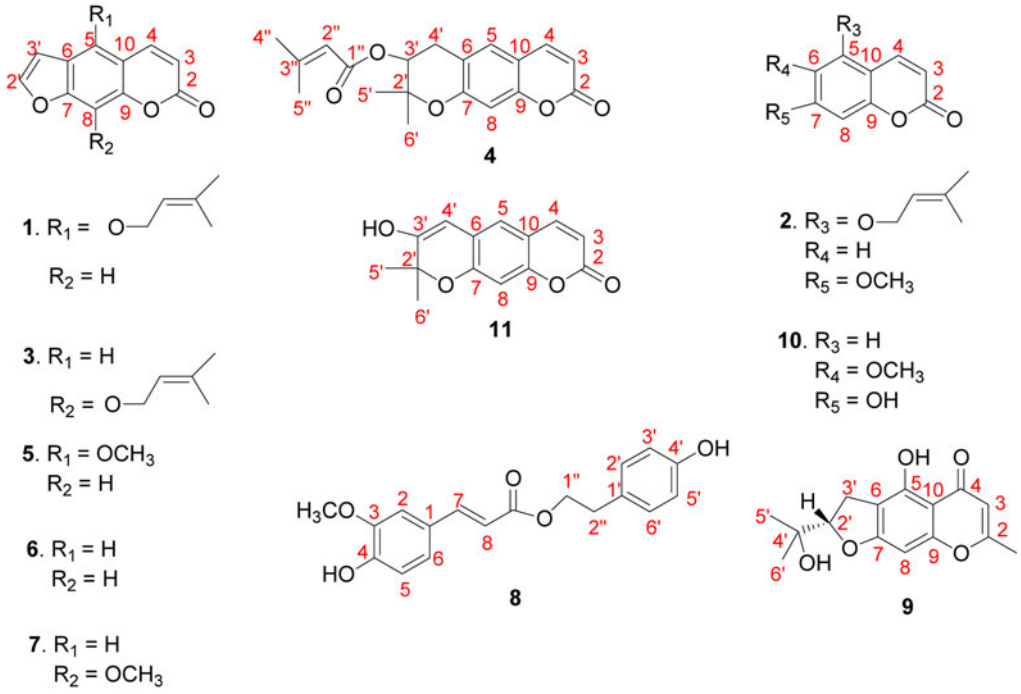

Fig. 1. Structures of 1-11 isolated from the stem of *A. gigas*.

acetylcholinesterase inhibition activity [Kim *et al*, 2002]. 7-Methoxy-5-prenyloxycoumarin (**2**) was reported to possess neuroprotective effect [Epifano *et al*, 2008] and an acetylcholineseterase inhibition effect [Kang *et al*., 2001], and decursin (**4**) was shown to exhibit acetylcholineseterase inhibitory effects [Kang *et al*., 2001] and anticancer properties [Kim *et al*., 2010]. Bergapten (**5**), psoralen (**6**), and xanthotoxin (**7**) have been reported to possess topoisomerase I inhibitory activity [Diwan and Malpathak, 2009]. *p*-Hydroxyphenethyl *trans*-ferulate (**8**) was shown to exhibit cancer preventive activity [Xiao and Parkin, 2006] and serotonergic activity [Deng *et al*., 2006]. Visaminol (**9**) has been reported to possess antiplatelet aggregation activity [Chen *et al*., 1996]. Scopoletin (**10**) was demonstrated to exhibit adipocyte differentiation inhibitory activity [Shin *et al*., 2010], anti-

Table 1. ^{1}H- and ^{13}C-NMR data for 11 (in acetone-d_6)

Position	δ_H (J=Hz)		δ_C	
	11	3'-hydroxyxanthyletin[a]	**11**	3'-hydroxyxanthyletin[a]
2			160.05	‡
3	6.36 (d, 9.6)	6.38 (d, 9.6)	114.07	114.8
4	8.06 (d, 9.6)	7.79 (d, 9.6)	144.40	144.3
5	7.84 (s)	7.61 (s)	119.96	100.2
6			126.02	‡
7			156.18	‡
8	7.46 (s)	7.45 (s)	98.86	100.0
9			151.88	‡
10			115.39	‡
2'			68.19	71.0
3'			166.93	164.0
4'	6.78 (s)	6.64 (s)	99.85	119.7
2×Me-2'	1.63 (s)	1.70 (s)	28.35	28.9

The assignments were based on HMBC, HSQC, and COSY experiments.
‡Not observed
[a]Data from Satyajit *et al*., 1995.

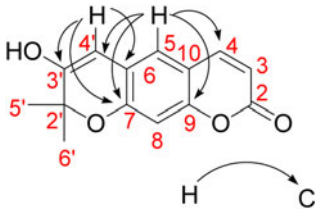

Fig. 2. Important HMBC correlations of 11.

Table 2. Inhibitory effects of 1-11 of *A. gigas* on aldose reductase

Compound	Inhibitory effect (IC_{50} values)[a]	
	μg/mL	μM
1	1.40±0.06	5.19
2	8.42±2.28	32.38
3	-	-
4	-	-
5	5.41±2.12	25.05
6	9.60±1.30	51.62
7	22.28±10.24	103.15
8	60.26±13.21	191.91
9	7.36±2.54	26.66
10	0.50±0.007	2.59
11	1.03±0.21	4.23
Quercetin	1.25±0.001	4.14
TMG	1.76±0.16	9.45

[a]The concentrations of compounds to achieve 50% inhibition (IC_{50}) of rat lens aldose reductase. IC_{50} values were calculated from the dose inhibition curve. Inhibitory effect was expressed as mean±SD (μg/mL) and mean (μM) of triplicate experiments. Quercetin and 3,3,-Tetramethyleneglutaric acid (TMG) were used as positive controls.
'-' indicates that compound had no effect.

convulsant activity [Mishra *et al*., 2010], antidepressant activity [Capra *et al*., 2010], and anti-arthritic activity [Pan *et al*., 2010].

Satyajit *et al*. [1995] isolated and identified compound **11** as 3'-hydroxyxanthyletin from *Boronia algida*. However, they were unable to obtained full carbon NMR (^{13}C-NMR) and heteronuclear multiple bond connectivity (HMBC) spectra due to the small quantity of sample. In the present study, our group able to generate enough sample for structure elucidation of compound **11**. The proton nuclear magnetic resonance (^{1}H-NMR) and mass spectrometry (MS) spectra of **11** were very similar to those of literature values, whereas ^{13}C-NMR spectrum showed slightly different chemical shift (Table 1). To assign the signals of carbon, spectrum analysis was performed on heteronuclear single quantum coherence (HSQC) and HMBC (Fig. 2), which revealed a chemical shift of C-5 and C-4' responsible for the conclusive data for structure elucidation of **11** was misidentified. These data allowed the assignment of all positions of 3'-hydroxyxanthyletin. All isolates obtained in the present study were evaluated for their potential to inhibit the AR (Table 2). Among them, isoimperatorin (**1**), scopoletin (**10**), and 3'-hydroxyxanthyletin (**11**) showed good inhibitory activity with IC_{50} values of 5.1, 2.59, and 4.23 μM, respectively. 7-Methoxy-5-prenyloxycoumarin (**2**), bergapten (**5**), psoralen (**6**), and visamminol (**9**) also showed activity in the assay with IC_{50} values of 32.38, 25.03, 51.62, and 26.66 μM, respectively. *p*-Hydroxy-phenethyl *trans* ferulate (**8**) and xanthotoxin (**7**) showed weak activity with IC_{50} values of 191.92 and 103.15 μM, respectively. Interestingly, visaminol (**9**), a chromone derivative, showed relatively good inhibitory activity in the present study. Imperatorin (**3**) and decursin (**4**) did not show any significant activity. These results show that, although the number of compounds tested is not sufficient, coumarins appear to possess AR inhibitory activity. In particular, in terms of the structure-activity relationship, it is believed that simple coumarins should possess a hydroxyl group at either the position 6 or 7, furano-cumarins at the position 5, and pyranocumarins at the position 3. These results suggested that the extracts from the stem of *A. gigas* would be potential candidates as therapeutic or preventive agents for diabetic complications.

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