Arch Pharm Res Vol 26, No 6, 449-452, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Antibacterial Coumarins from *Angelica gigas* Roots

Sanghyun Lee[1], Dong-Sun Shin[1], Ju Sun Kim[1], Ki-Bong Oh[1,2], and Sam Sik Kang[1]

[1]Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea and [2]Graduate School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

(Received April 2, 2003)

Systematic fractionation of *Angelica gigas* roots led to the isolation of linear furano(pyrano)coumarins such as bergapten (**1**), decursinol angelate (**2**), decursin (**3**), nodakenetin (**4**) and nodakenin (**5**). The antibacterial activities of those compounds against pathogenic bacteria were investigated. Among the compounds tested, decursinol angelate (**2**) and decursin (**3**) exhibited significant antibacterial activity against *Bacillus subtilis* with the minimum inhibitory concentrations (MICs) of 50 and 12.5 μg/mL, respectively.

Key words: *Angelica gigas*, Umbelliferae, Antibacterial activity, Coumarin, MIC.

INTRODUCTION

It is well known that intensive use of an antibiotic is often followed by the appearance of resistance strains. In view of this propensity of microorganisms to drug resistance, the search for new antimicrobial agents continues unabated. In this connection, medicinal plants are promising resources The use of medicinal plants as a screening pool for novel antibiotics has several advantages related to safety, availability, and minimizing the risk of side-effect and addiction (Hahm *et al.*, 2001; Lee and Kim, 1999).

Angelica gigas belongs to the family Umbelliferae. It is abundantly distributed throughout the northern Asia. *A. gigas* has been used as traditional medicine not only for treatment anemia but also as a sedative, an anodyne or a tonic agent (Yook, 1990). It has been studied extensively and shown to exhibit a variety of activities by coumarins. Decursin and decursinol angelate displayed cytotoxic activity against various human cancer cell lines (Ahn *et al.*, 1996; Ahn *et al.*, 1997) and antagonized against the voluntary activity in mice (Kim *et al.*, 1980). In addition, decursinol represented the inhibitory activity toward acetyl cholinesterase (Kang *et al.*, 2001). Decursin exhibited significant prolongation of hexobarbital-induced hypnosis as well as significant inhibition of hepatic microsomal drug metabolizing enzyme activities (Shin *et al.*, 1996).

Correspondence to: Sam Sik Kang, Natural Products Research Institute, Seoul National University, 28, Yeongun-dong, Jongno-gu, Seoul 110-460, Korea
Tel: 82-2-740-8925, Fax: 82-2-743-3323
E-mail: sskang@snu.ac.kr

In connection with our systematic isolation and structure studies on biologically active compounds from crude drugs and medicinal plants, we noticed that the ether fraction of *A. gigas* roots possessed antibacterial activity. In present paper, we report the molecular characteristics of the active principle of *A. gigas* roots, and describe the results of antibacterial activity of purified compounds.

MATERIALS AND METHODS

Instruments and reagents

^{1}H- and ^{13}C-NMR spectra were recorded with Bruker AVANCE 400 NMR spectrometer in $CDCl_3$ or DMSO-d_6 using TMS as internal standard. IR spectra were recorded with Jasco FT/IR-300E instrument on KBr disc. EI-MS spectra were measured with Jeol JMS-AX505WA mass spectrometer. Bacterial strains were purchased from Korea Research Institute of Bioscience and Biotechnology. Other reagents were commercially available first grade material.

Plant materials

The roots of *Angelica gigas* Nakai were purchased from Kyung Dong Market, Seoul, Korea in March 2001 and verified by Prof. Emeritus H. J. Chi, Seoul National University, Korea. A voucher specimen of this plant was deposited at the Herbarium of Natural Products Research Institute (NPRI), Seoul National University, Korea.

Extraction and isolation

The air-dried powdered roots of *A. gigas* (5 Kg) were extracted three times with MeOH under reflux by method

described as earlier (Lee *et al.*, 2002). The resultant extracts were combined and concentrated under reduced pressure to afford 1125 g of the residue. The MeOH extract was suspended in water, and then fractionated successively with equal volumes of Et_2O and *n*-BuOH, leaving residual H_2O soluble fraction. Each fraction was evaporated *in vacuo* to yield the residues of Et_2O soluble fraction (518 g) and *n*-BuOH soluble fraction (445 g).

The portion of Et_2O fraction (34 g) was chromatographed on a silica gel column (7×60 cm) eluting with a gradient of *n*-hexane-EtOAc to afford compounds **1** (5.8 mg, 38 : 2), **2** (789 mg, 37 : 3), **3** (5 g, 37 : 3) and **4** (4.2 mg, 30 : 10). The portion of *n*-BuOH fraction (34 g) was chromatographed on silica gel eluting with a gradient of $CHCl_3$-MeOH to afford compound **5** (2.9 g, 37 : 3).

Bergapten (1)

EI-MS *m/z* (rel. int., %): 216 $[M]^+$ (100), 201 (32.0), 188 (20.0), 173 (84.9), 145 (38.0), 129 (4.8), 89 (19.2), 75 (7.8); IR ν_{max} (KBr) cm^{-1}: 1732 (α-pyrone ring), 1634, 1560, 1479 (aromatic C=C), 1218, 1121 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 8.18 (1H, d, *J* = 9.8 Hz, H-4), 7.62 (1H, d, *J* = 2.4 Hz, H-2'), 7.17 (1H, s, H-8), 7.05 (1H, d, *J* = 2.4 Hz, H-3'), 6.30 (1H, d, *J* = 9.8 Hz, H-3), 4.30 (3H, s, 5-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C (ppm): 161.3 (C-2), 158.4 (C-7), 152.7 (C-9), 149.5 (C-5), 144.8 (C-2'), 139.2 (C-4), 112.7 (C-6), 112.6 (C-3), 106.4 (C-10), 105.0 (C-3'), 93.9 (C-8), 60.1 (5-OCH_3).

Decursinol angelate (2)

EI-MS *m/z* (rel. int., %): 328 (5.1) $[M]^+$, 228 (32.7), 213 (100), 147 (1.8). 83 (21.8), 55 (21.5); IR ν_{max} (KBr) cm^{-1}: 1732 (α-pyrone ring), 1626, 1561, 1494 (aromatic C=C), 1229, 1134 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.59 (1H, d, *J* = 9.5 Hz, H-4), 7.17 (1H, s, H-5), 6.79 (1H, s, H-8), 6.23 (1H, d, *J* = 9.5 Hz, H-3), 6.11 (1H, q, *J* = 7.2 Hz, H-3"), 5.14 (1H, t, *J* = 4.9 Hz, H-3'), 3.24 (1H, dd, *J* = 17.0, 4.9 Hz, H-4'$_a$), 2.90 (1H, dd, *J* = 17.0, 4.9 Hz, H-4'$_b$), 1.89 (3H, d, *J* = 7.2 Hz, H-4"), 1.85 (3H, s, 2"-CH_3), 1.41 (3H, s, gem-CH_3), 1.39 (3H, s, gem-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C (ppm): 167.0 (C-1"), 161.2 (C-2), 156.4 (C-7), 154.2 (C-9), 143.1 (C-4), 139.4 (C-3"), 128.6 (C-5), 127.3 (C-2"), 115.8 (C-6), 113.2 (C-3), 112.8 (C-10), 104.6 (C-8), 76.6 (C-2'), 70.0 (C-3'), 27.8 (C-4'), 25.0 (gem-CH_3), 23.2 (gem-CH_3), 20.5 (2"-CH_3), 15.7 (C-4").

Decursin (3)

EI-MS *m/z* (rel. int., %): 328 (4.6) $[M]^+$, 228 (33.8), 213 (100), 147 (1.8). 83 (38.3), 55 (11.5); IR ν_{max} (KBr) cm^{-1}: 1726 (α-pyrone ring), 1626, 1563, 1494 (aromatic C=C), 1226, 1135 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.58 (1H, d, *J* = 9.5 Hz, H-4), 7.15 (1H, s, H-5), 6.77 (1H, s, H-8), 6.20 (1H, d, *J* = 9.5 Hz, H-3), 5.65 (1H, s, H-2"), 5.07 (1H, t, *J* = 4.8 Hz, H-3'), 3.18 (1H, dd, *J* = 17.1, 4.7 Hz, H-4'$_a$), 2.90 (1H, dd, *J* = 17.1, 4.7 Hz, H-4'$_b$), 2.13 (3H, s, 3"- CH_3), 1.86 (3H, s, H-4"), 1.37 (3H, s, gem-CH_3), 1.35 (3H, s, gem-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C (ppm): 165.7 (C-1"), 161.2 (C-2), 158.4 (C-3"), 156.4 (C-7), 154.1 (C-9), 143.1 (C-4), 128.6 (C-5), 115.9 (C-6), 115.5 (C-2"), 113.1 (C-3), 112.7 (C-10), 104.6 (C-8), 76.7 (C-2'), 69.0 (C-3'), 27.8 (C-4'), 27.4 (C-4"), 24.9 (gem-CH_3), 23.1 (gem-CH_3), 20.3 (3"-CH_3).

Nodakenetin (4)

EI-MS *m/z* (rel. int., %): 246 (70.2) $[M]^+$, 228 (4.4), 213 (23.3), 187 (100), 175 (14.2), 160 (22.4), 147 (3.3), 131 (11.1), 115 (2.0), 102 (3.1), 81 (3.7), 69 (5.9), 59 (20.6); IR ν_{max} (KBr) cm^{-1}: 3479 (OH), 1699 (α-pyrone ring), 1630, 1569, 1486 (aromatic C=C), 1268, 1132 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.61 (1H, d, *J* = 9.5 Hz, H-4), 7.24 (1H, s, H-5), 6.77 (1H, s, H-8), 6.24 (1H, d, *J* = 9.5 Hz, H-3), 4.76 (1H, t, *J* = 8.7 Hz, H-2'), 3.24 (2H, m, H-3'), 1.40 (3H, s, CH_3), 1.26 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C (ppm): 163.1 (C-2), 161.0 (C-7), 155.7 (C-10), 143.6 (C-4), 125.0 (C-6), 123.3 (C-5), 112.8 (C-9), 112.3 (C-3), 97.9 (C-8), 91.0 (C-2'), 71.6 (C-4'), 29.4 (C-3'), 26.1 (C-6'), 24.2 (C-5').

Nodakenin (5)

EI-MS *m/z* (rel. int., %): 408 (23.1) $[M]^+$, 229 (66.5), 213 (37.4), 187 (100); IR ν_{max} (KBr) cm^{-1}: 3352 (OH), 1717 (α-pyrone ring), 1627, 1568, 1487 (aromatic C=C), 1265,

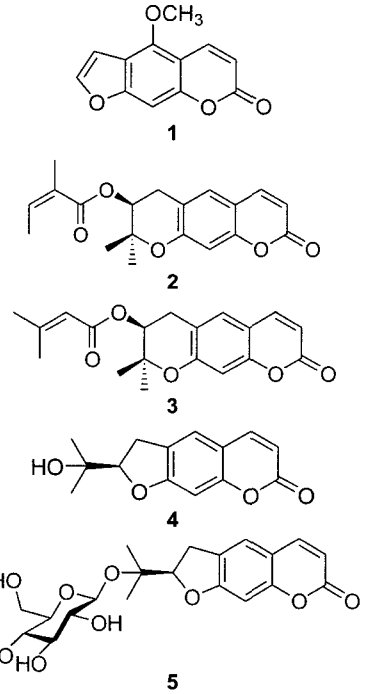

Fig. 1. Compound **1-5**

1170 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 7.91 (1H, d, J = 9.5 Hz, H-4), 7.46 (1H, s, H-5), 6.78 (1H, s, H-8), 6.20 (1H, d, J = 9.5 Hz, H-3), 4.85 (1H, m, H-2'), 4.40 (1H, d, J = 7.7 Hz, glycosyl H-1"), 3.19 (2H, m, H-3'), 1.25 (3H, s, CH_3), 1.22 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C (ppm): 163.5 (C-2), 161.0 (C-7), 155.4 (C-10), 145.2 (C-4), 126.1 (C-6), 124.4 (C-5), 112.6 (C-9), 111.6 (C-3), 97.6 (C-1"), 97.2 (C-8), 90.5 (C-2'), 77.5 (C-5"), 77.4 (C-4'), 76.9 (C-3"), 73.9 (C-2"), 70.4 (C-4"), 61.2 (C-6"), 29.2 (C-3'), 23.4 (C-6'), 21.1 (C-5').

Antibacterial activity test

Escherichia coli ATCC 35218, *Proteus vulgaris* ATCC 3851 *Salmonella typhimurium* ATCC 14028, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermis* ATCC 12228 and *Staphylococcus aureus* ATCC 65389 were used for the antibacterial activity test. The effect of isolated compounds on the bacterial growth was determined by the 2-fold microtiter broth dilution method (Wu and Hancocks, 1999). Briefly, bacteria were grown overnight into m-plate count broth (Difco), harvested and then washed twice with sterile distilled water. Stock solutions of test compounds were prepared in 100% dimethyl sulfoxide (DMSO) and stored at -20°C. Each stock solution was diluted with broth medium to prepare serial 2-fold dilutions in the range of 200 to 0.62 μg/mL before use. One hundred μL of the broth containing about 10^5 CFU/mL of test bacteria was added to each well of a 96-well microtiter plate and the minimum inhibitory concentration (MIC) was determined after overnight incubation at 37°C. The MIC was taken as the concentration at which no growth was observable.

RESULTS AND DISCUSSION

In the course of isolation, each fraction obtained at various steps was assayed for its growth inhibitory activity against various bacteria. As shown in Table I, among those fractions (ether, *n*-butanol and water), the ether fraction showed the highest antibacterial activity; therefore, this fraction was further purified by the silica gel column chromatography. Further systematic fractionation of the ether and *n*-butanol fractions led to the isolation of five linear furano(pyrano)coumarins. According to extensive NMR experiments and published data, the chemical structures of isolated compounds were elucidated as bergapten (**1**) (Chung, 1970), decursinol angelate (**2**) (Ryu *et al.*, 1990), decursin (**3**) (Konoshima *et al.*, 1968), nodakenetin (**4**) (Chi, 1969) and nodakenin (**5**) (Pachaly *et al.*, 1996).

Table I. Antibacterial activities of various fractions from *A. gigas* roots

	Organisms	MIC (μg/mL)		
		Et_2O fr.	*n*-BuOH fr.	H_2O fr.
Gram (+)	*Bacillus subtilis*	25	>200	>200
	Staphylococcus epidermis	>200	>200	>200
	Staphylococcus aureus	>200	>200	>200
Gram (−)	*Escherichia coli*	>200	>200	>200
	Proteus vulgaris	50	>200	>200
	Salmonella typhimurium	50	>200	>200

Table II. Antibacterial activities of coumarins from *A. gigas* roots

	Organisms	MIC (μg/mL)					
		1	**2**	**3**	**4**	**5**	**Tc***
Gram (+)	*Bacillus subtilis*	>200	50	12.5	>200	>200	<0.10
	Staphylococcus epidermis	>200	>200	>200	>200	>200	0.39
	Staphylococcus aureus	>200	>200	>200	>200	>200	<0.10
Gram (−)	*Escherichia coli*	>200	>200	>200	>200	>200	0.78
	Proteus vulgaris	>200	>200	>200	>200	>200	<0.10
	Salmonella typhimurium	>200	>200	>200	>200	>200	<0.10

Tc* is tetracycline as a positive control.

The isolated five coumarins were subjected to test their antibacterial activities against various bacteria and the results are summarized in Table II. Pyranocoumarins such as decursinol angelate (**2**) and decursin (**3**) revealed potent antibacterial activities against *Bacillus subtilis*, whereas furanocoumarins such as bergapten (**1**), nodakenetin (**4**) and nodakenin (**5**) did not exhibit any inhibitory effect upon bacterial cell growth at the highest concentration tested (200 μg/mL). The MICs of decursinol angelate (**2**) and decursin (**3**) against *B. subtilis* were calculated to be about 50 and 12.5 μg/mL, respectively.

Interestingly, when compared the inhibitory potency of decursinol angelate (**2**) and decursin (**3**), which are the structural isomers with the same molecular weight, decursin (**3**) was found to be more active than decursinol angelate (**2**). Decursin (**3**) contained senecioylic acid moiety, while decursinol angelate (**2**) contained angeloylic acid moiety. Furthermore, coumarins with six-membered ring (**2** and **3**) exhibited inhibitory active, whereas coumarins with five-membered ring (**1**, **4** and **5**) did not. These results suggest that the six-membered ring and senecioylic acid type side chain closely related to the enhanced antibacterial activities of coumarins against *B. subtilis*. Although a precise mechanism has not yet been clarified, these phenomena might be considered to occur due to the differences in the binding affinities of both compounds on the active sites of the enzymes or receptors from the differences in the position of the side chain moiety.

Coumarins are phenolic substances made of fused benzene and α-pyrone rings. Their fame has come mainly from their antithrombotic (Thastrup *et al.*, 1985), anti-inflammatory (Piller, 1975), vasodilatory (Namba *et al.*,

1988), and antiviral activities (Berkada, 1978). Other several coumarins have antimicrobial properties. Hydroxycinnamic acids, related to coumarins, seem to be inhibitory to gram-positive bacteria (Fernandez *et al.*, 1996). Also, phytoalexins, which are hydroxylated derivatives, are produced carrots in response to fungal infection and can be presumed to have antifungal activity (Hoult and Paya, 1996). All in all, data about specific antibiotic properties of coumarins are scarce, although many reports give reason to believe that some utility may reside in these phytochemicals (Hamburger and Hostettmann, 1991). Further research is warranted.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Korea Health 21st R & D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ2-PG6-01NA01-002). The authors gratefully acknowledge Dr. H. J. Chi for the identification of this plant material.

REFERENCES

Ahn, K-S., Sim, W-S., and Kim, I-H., Decursin: a cytotoxic agent and protein kinase C activator from the root of *Angelica gigas*. *Planta Med.*, 62, 7-9 (1996).

Ahn, K-S., Sim, W-S., Lee, I-K., Seu, Y-B., and Kim, I-H., Decursinol angelate: a cytotoxic and protein kinase C activating agent from the root of *Angelica gigas*. *Planta Med.*, 63, 360-361 (1997).

Berkada, B., Preliminary report on warfarin for the treatment of herpes simplex. *J. Irish. Coll. Phys. Surg.*, 22 (suppl.), 56 (1978).

Chi, H.-J., Studies on the components of Umbelliferous plants in Korea (IV). *Yakhak Hoeji*, 13, 47-50 (1969).

Chung, B. S., Studies on the components of *Evodia daniellii* Hemsley in Korea. *Kor. J. Pharmacogn.*, 1, 119-124 (1970).

Fernandez, M. A., Garcia, M. D., and Saenz, M. T., Antibacterial activity of the phenolic acids fraction of *Scrophularia frutescens* and *Scrophularia sambucifolia*. *J. Ethnopharmacol.*, 53, 11-14 (1996).

Hahm, D.-H., Yeom, M.-J. Lee, E. H. Shim, I. Lee, H.-J., and Kim, H.-Y., Effect of Scutellariae radix as a novel antibacterial herb on the ppk (polyphosphate kinase) mutant of *Salmonella typhimurium*. *J. Microbiol. Biotechnol.*, 11, 1061-1065 (2001).

Hamburger, H. and Hostettmann, K., The link between phyto-chemistry and medicine. *Phytochemistry*, 30, 3864-3874 (1991).

Hoult, J. R. S. and Paya, M., Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. *Gen. Pharmacol.*, 27, 713-722 (1996).

Kang, S. Y., Lee, K. Y. Sung, S. H. Park, M. J., and Kim, Y. C., Coumarins isolated from *Angelica gigas* inhibit acetyl cholinesterase: structure-activity relationships. *J. Nat. Prod.*, 64, 683-685 (2001).

Kim, H-S., Park, J-S., Park, H-J., and Chi, H-J., A study of the effects of the root components of *Angelica gigas* Nakai on voluntary activity in mice. *Kor. J. Pharmacogn.*, 11, 11-14 (1980).

Konoshima, M., Chi, H.-J., and Hata, K., Coumarins from the root of *Angelica gigas* Nakai. *Chem. Pharm. Bull.*, 16, 1139-1140 (1968).

Lee, S. H. and Kim, C. J., Antibacterial activity of antimycotic miconazole against methicillin-resistant *Staphylococcus aureus*. *J. Microbiol. Biotechnol.*, 9, 572-575 (1999).

Lee, S., Kang, S. S., and Shin, K. H., Coumarins and a pyrimidine from *Angelica gigas* roots. *Nat. Prod. Sci.*, 8, 58-61 (2002).

Namba, T., Morita, O., Huang, S-L., Goshima, K., Hattori, M., and Kakiuchi, N., Studies on cardio-active crude drugs. I. Effect of coumarins on cultured myocardial cell. *Planta Med.*, 54, 277-282 (1988).

Pachaly, P., Treitner, A., and Sin, K. S., Neue Cumaringlykoside aus *Angelica gigas*. *Pharmazie*, 51, 57-61 (1996).

Piller, N. B., A comparison of the effectiveness of some anti-inflammatory drugs on thermal oedema. *Br. J. Exp. Pathol.*, 56, 554-559 (1975).

Ryu, K. S., Hong, N. D., Kim, N. J., and Kong, Y. Y., Studies on the coumarin constituents of the root of *Angelica gigas* Nakai Isolation of decursinol angelate and assay of decursinol angelate and decursin. *Kor. J. Pharmacogn.*, 21, 64-68 (1990).

Shin, K. H., Han, J. M., and Lee, I. R., Effect of the constituents of *Angelicae gigantis* radix on hepatic drug metabolizing enzymes. *Kor. J. Pharmacogn.*, 27, 323-327 (1996).

Thastrup, O., Knudsen, J. B., Lemmich, J., and Winther, K., Inhibition of human platelet aggregation by dihydropyrano- and dihydrofurano-coumarins, a new class of camp phosphodiesterase inhibitors. *Biochem. Pharmacol.*, 34, 2137-2140 (1985).

Wu, M. and Hancocks, R. E. W., Interaction of the cyclic antimicrobial cationic peptide bactenecin with the outer and cytoplasmic membrane. *J. Biol. Chem.*, 274, 29-35 (1999).

Yook, C. S., *Coloured medicinal plants of Korea*, Academy Book Co., Seoul, p. 390 (1990).