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Cytotoxic Coumarins from the Roots of *Angelica gigas* NAKAI

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Abstract

Some known coumarins, decursin, nodakenetin, umbelliferone, 7-demethylsuberosin, columbianetin, decursinol angelate and decursinol, showing significant cytotoxic activities against P388 cell lines, were isolated from the roots of *Angelica gigas* (Umbelliferae). 7-Demethylsuberosin and columbianetin were obtained from *A. gigas* for the first time. Chemotaxonomic difference about coumarins components between the roots of *A. gigas* and those of *A. acutiloba* is also discussed.

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KEY WORDS : *Angelica gigas*; Umbelliferae; coumarin; 7-demethylsuberosin; columbianetin; P388 cells; cytotoxic activity; chemotaxonomy.

Angelica gigas Nakai(Umbelliferae) is called as "Cham Dang Gui", "Sung Geom Cho" and "To Dang Gui" in Korea and has been used for calm, ease from pain, anemia and women diseases. In our screening for antileukemic compounds from higher plants, a methanol extract prepared from the roots of *Angelica gigas* was found to exhibit an antileukemic activity against P388 cell lines. Here we described about the structures of coumarins isolated from *A. gigas*, showing antileukemic activities. Chemotaxonomic difference about coumarin constituents contained in between the roots of *A. gigas*, which have been used in Korea, and those of *A. acutiloba*, which have been used in Japan was also discussed. So far, the presence of some coumarins have been reported from the roots of *A. gigas* by Ryu *et al.*[1,2]

Methanol extract prepared from the roots of *A. gigas* was partitioned between *n*-hexane and water. The aqueous layer was further partitioned with methylene chloride and *n*-BuOH, successively. When the antileukemic activity against P-388 cell lines was carreid out, the cytotoxic activity was concentrated into both the *n*-hexane and methylene chloride extracts, and the chromatogaphic purification of them with the guidance of cytotoxicity led to the isolation of some coumarins.

As the active components, decursin[2], decursinol angelate[2] and 7-demethylsuberosin[3] Were isolated from the *n*-hexane soluble fraction, and umbelliferone[1], nodakenetin[1], decursinol[4] and columbianetin[3,5] were obtained from the methylene chloride soluble fraction. Each compound was identified by comparison of the melting point and spectroscopic data involving mass(MS), proton and carbon nuclear magnetic resonances(H-, and C-NMR) spectral data with those of the authentic samples. Coumarins 3 and 7 were isolated from *A. gigas* for the first time.

The decursin is a major compound of *Angelica gigas*, when it is compared with that of Japanese variety (*A. acutiloba*)[6]. Then its content in *A. gigas*

was 40% more higher than that in *A. acutiloba.*

Some pharmacological activities of decursin[1] was reported by Chi[7], however, we have presented the cytotoxic activity of coumarins 1-7 from *A. gigas.* As can be seen from Table 1, nodakenetin, decursional angelate and decursin exhibited cytotoxic activity against P-388 cell lines and the others also have moderately activities.

Table 1. Cytotoxic Activities of[1-7] against P-388 Cells.

Compounds	IC50(μg/ml)
decursin[1]	4.5
nodakenetin[5]	2.8
umbelliferone[4]	12.0
7-demethylsuberosin[3]	19.0
columbianetin[7]	50.0
decursinol angelate[2]	4.5
decursinol[6]	36.0

EXPERIMENTAL

All melting points were recoreded on a Yanagimoto MP-3 micromelting point apparatus, Infrared Spectra(IR) on a JASCO A-302, NMR on a Bruker AM 400 and mass spectra(MS) on a VG Autospec. Medium pressure liquid chromatography (MPLC) was carried out on a CIG column system (Kusano Scientific CO. Tokyo) packed with 10μm silica gel as the stationary phase.

Extraction and separation

The roots of *Angelica gigas*(10kg) were extracted three times with hot methanol and concentration to give a methanolic extract(2kg). This aqueous extract was partitioned with *n*-hexane, methylene chloride and *n*-butanol. The *n*-hexane soluble fraction(457g) was separated by chromatography on silica gel with hexane and ethyl acetate solvent system into eight fractions(Fr.1 to 8). Further chromatographic purification of the major fraction was carried out on silica gel MPLC(toluene-ethyl acetate solvent system) and led to the isolation of decursin(1, 103g), decursinol angelate(2, 103g) and 7-demethylsuberosin(3, 38.4g). The methylene chloride soluble fraction was also chromatographed on silica gel with a mixture of methylene chloride and ethyl acetate as the eluent. Further purification with the silica gel MPLC(toluene-ethyl acetate solvent system) and ODS MPLC(60% MeOH) afforded umbelliferone(4, 890mg) and columbianetin (7, 234mg). The nodakenetin(5, 772mg) and decursinol(6, 543mg) were purified by ODS HPLC (30% MeCN), followed by using ODS MPLC(60% MeOH).

Columbianetin(7)

Colorless needles, mp 162-164℃, [α]D+104.3 (c0.14, $CHCl_3$), MS*m/z*(%) : 246(12), 213(91), 167(82), 91(100), IRνmax(cm^{-1}) : 3684(-OH), 1734(C=O), 1619, 1521(C=C), H-NMR($CDCl_3$, δ, ppm) : 1.23(3H, s, $-CH_3$), 1.36(3H, s, CH_3), 3.29 (1H, dd, J=8.6, 16.2Hz, C_4-H), 3.35(1H, dd, J=9.4, 16.2Hz, C_4-H), 4.79(1H, dd, J=8.6, 9.4Hz, C_5-H), 7.62(1H, d, J=9.5Hz, C -H), C_{13}-NNR($CDCl_3$, δ, ppm) : 24.0(q), 26.0(q), 71.8(s), 91.4(d), 106.7 (d), 112.9(d), 113.2(s), 114.2(s), 128.8(d), 130.8 (s), 161.0(s), 163.7(s)

7-Demethylsuberosin(3)

Colorless needles, mp 130-132℃, MS*m/z*(%) : 230(54), 215(20), 175(100), 147(19), IRνmax(cm^{-1}) : 3201(-OH), 1693(C=O), ^{1}H-NMR($CDCl_3$, δ, ppm) : 1.73(1H, s, CH_3), 1.77(1H, s, CH_3), 3.26 (2H, d, J=7.2Hz, C_3-H), 5.32(1H, br.t, J=1.3, 7.2Hz, C_3-H), 6.22(1H, d, J=9.4Hz, C_3-H), 7.07(1H, s, C_5-H), 7.19(1H, s, C_8-H), 7.66(1H, d, J-9,4Hz, C_4-H), ^{13}C-NMR($CDCl_3$, δ, ppm) : 17.8(q), 25.8 (q), 28.2(t), 103.1(d), 111.9(d), 112.7(s), 121.2 (d), 126.2(s), 128.1(d), 134.6(s), 144.6(d), 154.1 (s), 158.8(s), 162.8(s).

Cytotoxic activity against P388 cells

MTT(3 - [4,5 - dimethylthiazol - 2 - yl] - 2,5 - diphenyltetrazolium bromide) colorimetric assay

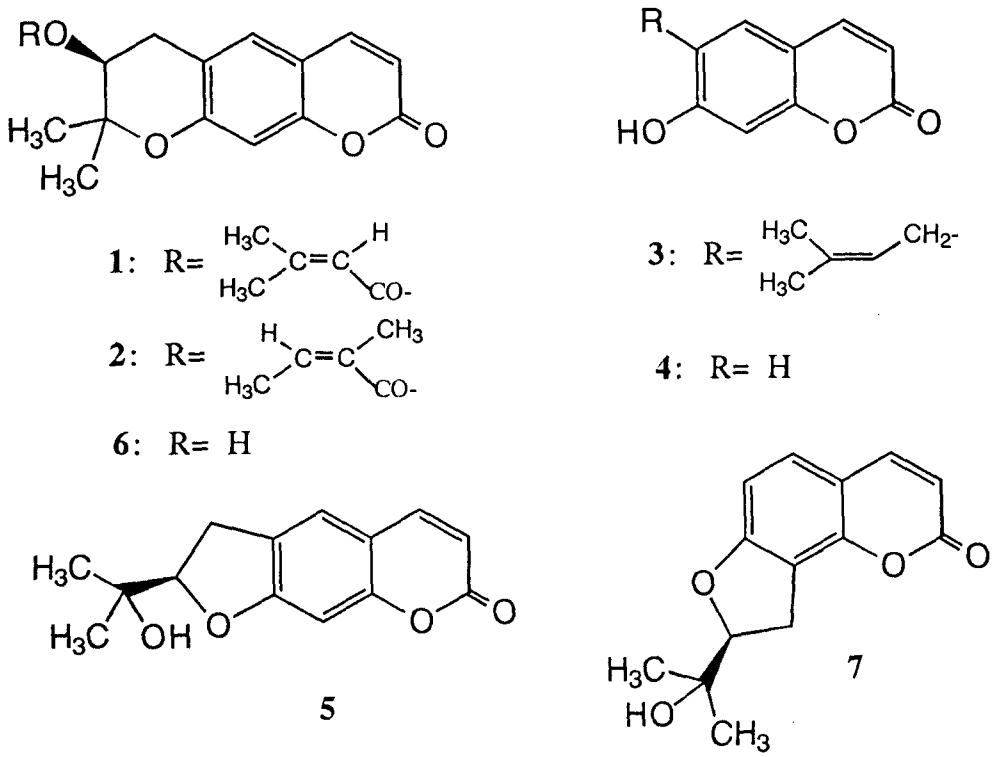

Fig. 1. Coumarins from the Roots of *Angelica gigas.*

and the plates were incubated for a further 4 hours, The resulting formazan was dissolved in 100 μl of 10% SDS(Sodium dodecyl sulfate) included 0.01N HCl. Each well was shaked with a pipet for 1 or 2 minute and read at 540nm. The IC(μg/ml) value was defind. It means the concentration of sample which achieved 50% reduction of sample-treat cell with respect to the control.

was performed in a 96-well plate. the assay is dependent on the reduction of MTT by the mitochondrial dehydrogenase of viable cell to a blue formozan product which can be measured spectrophotometrically. Mouse P388 leukemia cells (3×10^4 cells/mℓ) were inoculated in each well with 100mℓ of RPMI-1640 medium supplemented with 10% fetal calf serum and 10μg/mℓ of kanamycine, After overnight incubation(37℃, 5% CO_2), 100, 30, 10, 3, 1, 0, 0,3 and 0.1μg/mℓ of sample was added to the wells and the plates were incubated for 48hours. Then 20μl of MTT was added to each well

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