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Antioxidant Activities of Decursinol Angelate and Decursin from *Angelica gigas* Roots

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Abstract – The anti-oxidant activities of decursinol angelate (**1**) and decursin (**2**) isolated from *Angelica gigas* were investigated. These two coumarins exhibited decrease in serum transaminase activities elevated by hepatic damage induced by CCl_4-intoxication in rats. They also showed increase in anti-oxidant enzymes such as hepatic cytosolic superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-px) in CCl_4-intoxicated rats. These results suggest that decursinol angelate (**1**) and decursin (**2**) from *A. gigas* possess not only the anti-oxidant, but also the hepatoprotective activities in rats.
Keywords – *Angelica gigas*, Umbelliferae, anti-oxidant enzymes, decursinol angelate, decursin, s-transaminase CCl_4-intoxication

Introduction

Reactive oxygen species (ROS) are generated as byproducts of biological reactions or from exogenous factors (Cerutti, 1991). The involvement of ROS in the pathogenesis of a large number of diseases is well documented (Cross, 1987). It is suggested that free radical damage to cells leads to the pathological changes associated with aging (Beckman and Ames, 1998). Free radicals may also be a contributory factor in a progressive decline in the function of the immune system (Pike and Chandra, 1995). The cooperative defense systems that protect the body from free radical damage include the anti-oxidant nutrients and enzymes. The anti-oxidant enzymes include superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-px), and indirectly glutathione reductase. Their roles as protective enzymes are well known and have been investigated extensively both *in vivo* and *in vitro* model systems. The consequences of oxidative stress are serious, and in many cases are manifested by increased activities of enzymes involved in oxygen detoxification. Identification of new anti-oxidants remains a highly active research area because anti-oxidants may reduce the risk of various chronic diseases caused by free radicals.

The roots of *A. gigas* were used under the Korean name Zam Dang Gui. *A. gigas* has been used as a traditional medicine not only for treatment of anemia but also as a sedative, an anodyne or a tonic agent (Yook, 1990). *A. gigas* has been studied extensively and are shown to contain a variety of substances including coumarins (Chi, 1969; Jung *et al.*, 1991; Konoshima *et al.*, 1968; Pachaly *et al.*, 1996; Ryu *et al.*, 1990), essential oils (Chi and Kim, 1988), and polyacetylenes (Choi *et al.*, 2000). Decursin exhibited significant prolongation of hexobarbital-induced hypnosis as well as significant inhibition of hepatic microsomal drug metabolizing enzyme activities (Shin *et al.*, 1996). Decursin and decursinol antagonized against the voluntary activity in mice (Kim *et al.*, 1980). Decursinol represented the highest inhibitory activity toward acetyl cholinesterase (Kang *et al.*, 2001).

This paper deals with the isolation and characterization of anti-oxidant as well as hepatoprotective principles from *A. gigas*.

Experimental

Instruments and reagents – IR spectra were recorded with a Jasco FT/IR-300E instrument on KBr disc. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AVANCE 400 NMR spectrometer in $CDCl_3$ using TMS as internal standard. MS spectra were measured with a Jeol JMS-AX505WA mass spectrometer. Sodium azide, ethylenediamine tetraacetic acid (EDTA), β-nicotinamide adenine dinucleotide

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phosphate, reduced form (NADPH), cumene hydroperoxide, glutathione reductase, carbon tetrachloride (CCl_4), xanthine, potassium cyanide (KCN), sodium dodecylsulfate, cytochrome C, and pyridine were purchased from Sigma Chem. Co. (St. Louis, MO). All other chemicals and reagents were analytical grade.

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 *et al.*, 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 the 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).

A portion of the 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** (789 mg, 37:3) and **2** (5 g, 37:3).

Decursinol angelate (**1**); white crystals from MeOH. 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''); EI-MS *m/z* (rel. int., %): 328 (5.1) $[M]^+$, 228 (32.7), 213 (100), 147 (1.8), 83 (21.8), 55 (21.5).

Decursin (**2**); white crystals from MeOH. 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); EI-MS *m/z* (rel. int., %): 328 (4.6) $[M]^+$, 228 (33.8), 213 (100), 147 (1.8), 83 (38.3), 55 (11.5).

Anti-oxidant assay *in vivo* – Male Sprague-Dawley rats weighing 200-250 g were used in all experiments. Animals were maintained on 12 hr light/dark cycle at approximately 22°C and allowed food and water *ad libitum.* All treatments were conducted between 9:00 and 10:00 oclock to minimize variations in animal response due to circadian rhythm. Rats were injected i.p. with a mixture of CCl_4 in olive oil (1:1) at a dose of 0.6 ml/kg to induce hepatotoxicity. Control animals were given the vehicle alone. Rats were pretreated with silymarin, decursin, and decursinol angelate given p.o. at a dose of 30 mg/kg/day for seven consecutive days prior to the administration of CCl_4. Animals were sacrified 24 hr after CCl_4 dosing and blood was collected by decapitation for the determination of serum transaminase.

Hepatic tissues were carefully excised and homogenized in cold 1.15% KCl-10 mM phosphate buffer with EDTA (pH 7.4) and centrifuged at 10,000 rpm for 10 min. The supernatant was further centrifuged at 40,000 rpm for 60 min to obtain cytosolic extract for the measurement of liver cytosolic SOD, catalase, and GSH-px activities. The protein content was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Hepatocellular damage was estimated by measuring sGOT and sGPT activities (Reitman and Frankel, 1957). Blood was collected from the abdominal aorta of each rat. The blood was centrifuged at 3,000 rpm at 4°C for 10 min to separate the serum. sGOT and sGPT activities were expressed as Karmen unit.

SOD was assayed by the method of McCord and Fridovich (1969). The reaction mixture containing 0.5 mM xanthine as substrate (300 μl), 0.05 mM KCN (100 μl), 1% sodium deoxycholate (100 μl), xanthine oxidase (20 μl), cytosolic extract (20 μl) and 0.1 mM cytochrome C (300 μl) was placed in a 1 cm cuvette and the rate of increase in absorbance at 550 nm was recorded for 5 min. SOD activity was expressed as unit/mg protein. Catalase was assayed by the method of Rigo and Rotilio (1977). The cytosolic extract of liver (40 μl) diluted 10 times was added with 0.13 mM phosphate buffer (pH 7.0, 500 μl), distilled water (660 μl) and 15 mM H_2O_2 (1800 μl), and thoroughly mixed. The rate of changes in the absorbance

at 240 nm for 5 min was recorded. Catalase activity was expressed as unit/mg protein. GSH-px was assayed by the method of Burk *et al.* (1978). The reaction mixture containing 0.3 mM phosphate buffer with 4.0 mM EDTA (pH 7.2, 1000 μl), 26.56 mM sodium azide (500 μl), 294.37 mM GSH (60 μl), 8.4 mM NADPH (110 μl), 1 mM cumene hydroperoxide (320 μl), glutathione reductase (5 μl), and cytosolic solution (30 μl) was placed in 1 cm cuvette and the rate of changes in absorbance was recorded at 340 nm for 5 min. GSH-px activity was expressed as unit/mg protein.

Statistical analysis – Multiple comparison test was applied for detecting the significance of difference between different groups.

Results and Discussion

A chromatographic separation of the Et_2O fraction from *A. gigas* led to the isolation of compounds **1** and **2**. According to extensive NMR experiments and published data, the chemical structures of isolated compounds were elucidated as decursinol angelate (**1**) (Ryu *et al.*, 1990) and decursin (**2**) (Konoshima *et al.*, 1968).

Decursinol angelate (**1**) and decursin (**2**) obtained from *A. gigas* were tested for their free radical scavenging effects, and the effects on hepatocellular damage in CCl_4-intoxicated rats were evaluated. Carbon tetrachloride (CCl_4) is widely used to induce lipid peroxidation and toxicity. CCl_4 is metabolized by cytochrome P450 2E1 (CYP2E1) to the trichloromethyl radical ($CCl_3\cdot$), which is assumed to initiate free radical-mediated lipid peroxidation leading to the accumulation of lipid-derived oxidation products that cause liver injury (Recknagel *et al.*, 1989). A number of investigators have previously demonstrated that antioxidants prevent CCl_4 toxicity, particularly hepatotoxicity, by inhibiting

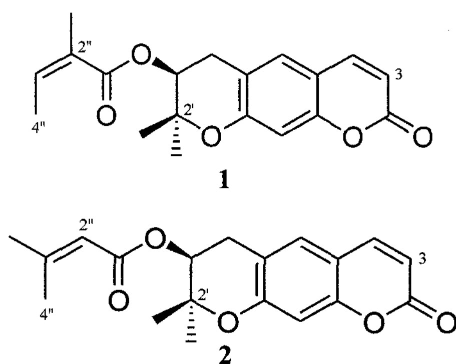

Table 1. Effects of decursinol angelate (**1**) and decursin (**2**) from *A. gigas* on the sGOT and sGPT activities

Treatments	sGOT (Karmen unit)	sGPT (Karmen unit)
Control	205.83± 8.78	117.38±16.75
Silymarin	167.04±10.25*	55.50± 6.60*
1	176.23± 3.61*	68.42± 3.84*
2	175.16± 7.15*	66.30± 1.96*

Rats were pretreated with silymarin, compounds **1** and **2** given at a dose of 30 mg/kg for 7 days, prior to the administration of CCl_4. Rats were sacrified 24 hr after CCl_4. Heparinized blood sample was collected. Hepatocellular damage was estimated by measuring sGOT and sGPT activities. Significantly different from the control; *p<0.05.

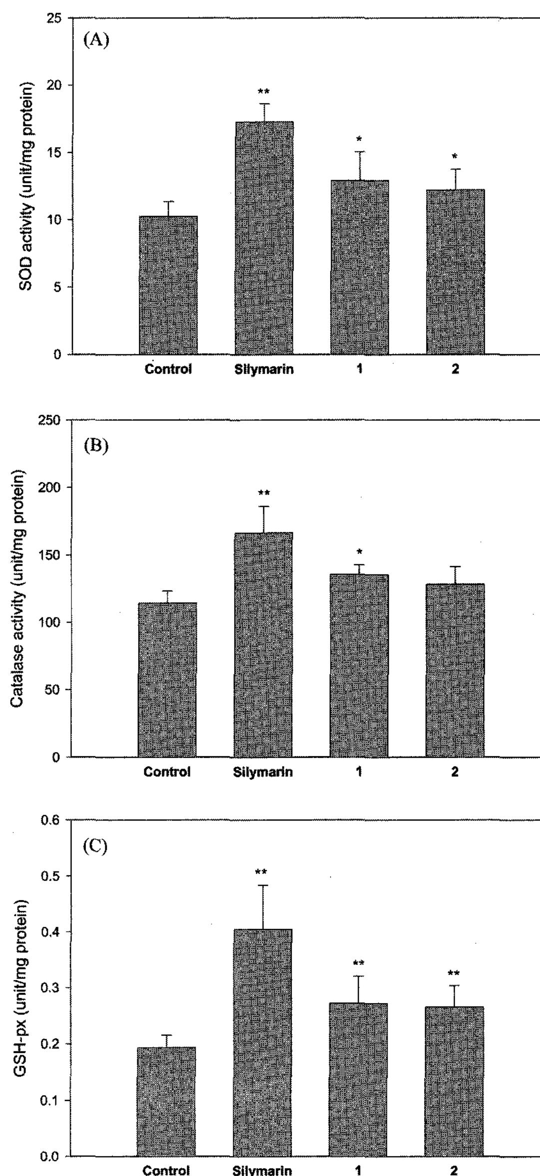

Fig. 1. Effects of decursinol angelate (**1**) and decursin (**2**) from *A. gigas* on the liver cytosolic SOD (**A**), catalase (**B**), and GSH-px (**C**) activities.
Rats were pretreated with silymarin, compounds **1** and **2** given at a dose of 30 mg/kg for 7 days, prior to the administration of CCl_4. Hepatic tissues were carefully excised, homogenized, and centrifuged to obtain cytosolic fractions for the measurement of liver cytosolic SOD, catalase, and GSH-px activities. The test method is the same as that described in anti-oxidant assay *in vivo*. Significantly different from the control; *p<0.05, **p<0.01.

lipid peroxidation, and increasing antioxidant enzyme activity (Kumaravelu *et al.*, 1995). The effects of decursinol angelate (**1**) and decursin (**2**) from *A. gigas* on serum transaminase in CCl_4-intoxicated rats were estimated and the results are shown in Table 1. In the CCl_4-intoxicated control group, the sGOT and sGPT activities were increased. In contrast, the group treated with decursinol angelate (**1**) and decursin (**2**) decreased these elevated transaminase activities. Decursinol angelate (**1**) and decursin (**2**) inhibited the sGOT & sGPT activities by 14.38 & 41.71%, and 14.90 & 43.52%, respectively. Silymarin inhibited the sGOT and sGPT activities by 18.85 and 52.72%, respectively.

Decursinol angelate (**1**) and decursin (**2**) cause an elevation of the free radical scavenging enzyme activities such as SOD, catalase and GSH-px. As shown in Fig. 1, silymarin caused the elevation of catalase and SOD activity. Increase in the catalase activity with respect to CCl_4 treatment indicates that decursinol angelate (**1**) and decursin (**2**) can play an important role in scavenging hydrogen peroxide. Restoration of SOD activity indicates that decursinol angelate (**1**) and decursin (**2**) can help in cellular defense mechanisms by preventing cell membrane oxidation. Similarly, an increase in glutathione peroxidase activity indicates that decursinol angelate (**1**) and decursin (**2**) also helps in the restoration of vital molecules such as NAD, cytochrome, and glutathione.

In conclusion, the present study demonstrated that decursinol angelate (**1**) and decursin (**2**) from *A. gigas* possess not only the anti-oxidant, but also the hepatoprotective activities in rats.

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