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Anti-Tumor Activities of Decursinol Angelate and Decursin from *Angelica gigas*

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The *in vivo* anti-tumor activities of decursinol angelate (**1**) and decursin (**2**) isolated from the roots of *Angelica gigas* were investigated. These two compounds, when administered consecutively for 9 days at 50 and 100 mg/kg i.p. in mice, caused a significant increase in the life span and a significant decrease in the tumor weight and volume of mice inoculated with Sarcoma-180 tumor cells. These results suggest that decursinol angelate (**1**) and decursin (**2**) from *A. gigas* have anti-tumor activities.

Key words: *Angelica gigas*, Umbelliferae, Anti-tumor activity, Decursinol angelate, Decursin

INTRODUCTION

The roots of *A. gigas* Nakai (Umbelliferae) were used, under the Korean name Zam Dang Gui. *A. gigas* has been used as traditional medicine not only for treatment anemia but also as a sedative, and an anodyne or tonic agent (Yook, 1990; Han, 1992).

A. gigas has been studied extensively and 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 a significant prolongation of hexobarbital-induced hypnosis, as well as significant inhibition of hepatic microsomal drug metabolizing enzyme activities (Shin *et al.*, 1996). Both decursin and decursinol antagonized against the voluntary activity in mice (Kim *et al.*, 1980), and the latter represented the highest inhibitory activity toward acetyl cholinesterase (Kang *et al.*, 2001).

In previous papers, Ahn *et al.* reported the *in vitro* cytotoxic activity of decursinol angelate and decursin from *A. gigas* (Ahn *et al.*, 1996; Ahn *et al.*, 1997), but there have been no reports on their *in vivo* anti-tumor activities.

This paper has shown the *in vivo* anti-tumor activities of decursinol angelate (**1**) and decursin (**2**) from the roots of *A. gigas*.

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MATERIALS AND METHODS

Plant materials

The roots of *Angelica gigas* Nakai (Umbelliferae) 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 was deposited at the Herbarium of Natural Products Research Institute (NPRI), Seoul National University, Korea.

Isolation of compounds 1 and 2

The extraction and fractionation of *A. gigas* roots have been reported as earlier. 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 decursinol angelate (**1**) and decursin (**2**) (Lee *et al.*, 2002).

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); EI-MS *m/z* (rel. int., %): 328 (5.1) $[M]^+$, 228 (32.7), 213 (100), 147 (1.8), 83 (21.8), 55 (21.5); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.59 (1 H, d, *J* = 9.5 Hz, H-4), 7.17 (1 H, s, H-5), 6.79 (1 H, s, H-8), 6.23 (1 H, d, *J* = 9.5 Hz, H-3), 6.11 (1 H, q, *J* = 7.2 Hz, H-3"),

5.14 (1 H, t, J = 4.9 Hz, H-3'), 3.24 (1 H, dd, J = 17.0, 4.9 Hz, H-4'$_a$), 2.90 (1 H, dd, J = 17.0, 4.9 Hz, H-4'$_b$), 1.89 (3 H, d, J = 7.2 Hz, H-4"), 1.85 (3 H, s, 2"-CH_3), 1.41 (3 H, s, gem-CH_3), 1.39 (3 H, 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 (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); EI-MS *m/z* (rel. int., %): 328 (4.6) [M]$^+$, 228 (33.8), 213 (100), 147 (1.8), 83 (38.3), 55 (11.5); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.58 (1 H, d, J = 9.5 Hz, H-4), 7.15 (1 H, s, H-5), 6.77 (1 H, s, H-8), 6.20 (1 H, d, J = 9.5 Hz, H-3), 5.65 (1 H, s, H-2"), 5.07 (1 H, t, J = 4.8 Hz, H-3'), 3.18 (1 H, dd, J = 17.1, 4.7 Hz, H-4'$_a$), 2.90 (1 H, dd, J = 17.1, 4.7 Hz, H-4'$_b$), 2.13 (3 H, s, 3"- CH_3), 1.86 (3 H, s, H-4"), 1.37 (3 H, s, gem-CH_3), 1.35 (3 H, 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).

Animals

Four-week-old and specific pathogen-free male ICR mice weighing 23±2 g were supplied from the Daehan Animal Center. The maintenance and animal experiments were carried out in the SPF barrier zone of Seoul National University Hospital. They were fed on laboratory chows and water *ad lib.*, and were housed at 23±0.5°C and 10% humidity in a 12 h light-dark cycle.

Cell line

The Sarcoma-180 tumor cell line was supplied from the Korea Cell Line Bank, Seoul National University, and maintained by intraperitoneally inoculation of 1×10^6 cells in ICR mice. The viable cells were counted by a hemocytometer using the trypan blue exclusion method.

Anti-tumor activities

The effects of decursinol angelate (**1**) and decursin (**2**) on the tumor growth and host survival, in the Sarcoma-180 tumor cell line, of the mice were estimated by evaluating the tumor volume, and weight, and the percentage increase in the life span of the tumor (ILS) hosts. On day 0, groups of nine mice were inoculated i.p. with 1×10^6 Sarcoma-180 cells/mouse. The treatments with decursinol angelate (**1**) and decursin (**2**) (50 and 100 mg/kg, i.p.) were started 12 h after the tumor cell inoculation, and continued for nine consecutive days. The control group was treated with carboxy methylene chloride only. The anti-tumor activities of the two compounds were estimated by measuring the ILS, which were expressed as the median survival time (MST). For solid tumor development, mice were injected with 0.1 mL of a Sarcoma-180 suspension into the right hind limbs. Six days after tumor transplantation, the mice were randomized into six groups and injected i.p. with each of the two compounds (50 and 100 mg/kg, i.p.) and 5-fluorouracil (25 mg/kg) once a day for 9 days. Eight days later, the animals were killed by cervical dislocation, the solid tumors removed, and weighed for their wet weight. The tumor volume was measured using digital calipers.

Statistical analysis

All the data were expressed as the mean±S.E.M. A statistical analysis was performed using an unpaired student's *t*-test; Value at $p<0.05$ was considered as statistically significant.

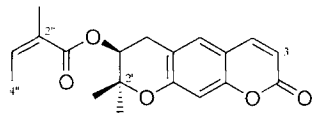
RESULTS AND DISCUSSION

The effects of decursinol angelate (**1**) and decursin (**2**) from *A. gigas* on the survival time of the Sarcoma-180 ascitic tumor in the mice were tested, and the results are summarized in Table I. The median survival time (MST) for the Sarcoma-180 in the control group was 22.4 days, while the MSTs were dose dependently increased by the treatments with the two compounds, at doses of 50 and 100 mg/kg, for nine consecutive days. The MSTs for the two doses were 32.3 and 34.2, and 29.0 and 39.6 days

Table I. Effects of compounds **1** and **2** on the survival time of the Sarcoma-180 ascitic tumors in mice

Treatments	Dose (mg/kg)	Median survival time (Days)	Increase in life span (%)
Control	–	22.4 ± 2.2	–
5-Fluorouracil	25	55.2 ± 5.5**	146.8
Decursinol angelate (**1**)	50	32.3 ± 1.6**	44.2
	100	34.2 ± 1.6**	52.7
Decursin (**2**)	50	29.0 ± 2.0*	29.5
	100	39.6 ± 2.9**	76.8

Mice were inoculated i.p. with 0.1 mL of ascitic tumors (1×10^7 cells/mL) on day 0. 5-Fluororacil was used as a positive control. Mice were administered i.p. with compounds **1** and **2**, at 50 and 100 mg/kg, for 9 consecutive days, starting 12 h after implantation of the tumor cells. The survival times were measured. Significantly different from the control; *$p < 0.05$, **$p < 0.01$



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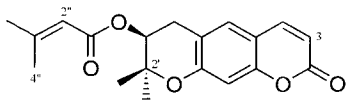

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Fig. 1. Structures of compounds **1** and **2**

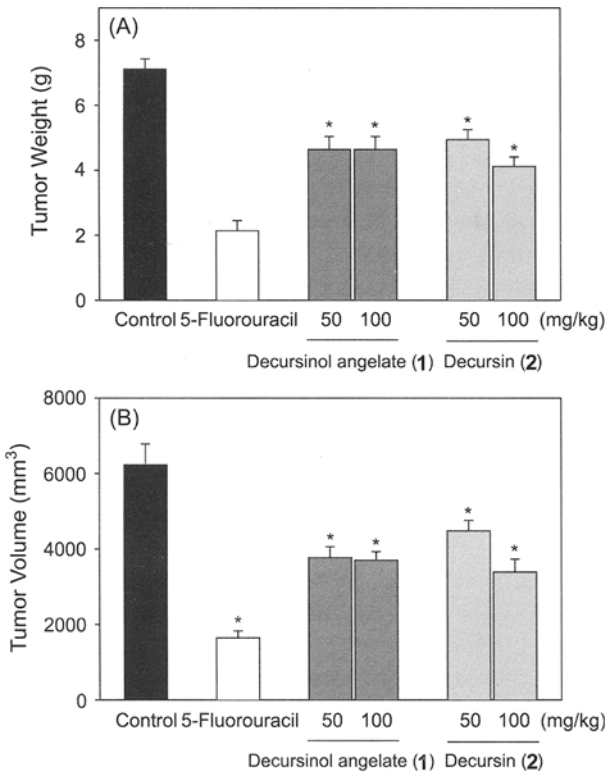

Fig. 2. Effects of compounds **1** and **2** on the tumor weights (**A**) and volumes (**B**) of the Sarcoma-180 tumors in mice. Solid-type Sarcoma-180 was prepared by subcutaneous transplantation of 1×10^6 cells into the right groins of mice on day 0. Six days after tumor cell inoculation, the treatments with saline, compounds **1** and **2** (50 and 100 mg/kg) and 5-fluorouracil (25 mg/kg) were commenced. Eight days later, the solid tumors were removed and their wet weights and volumes measured. The data are expressed as the mean±S.E.M. of nine mice. Significantly different from the control; *p<0.001.

for decursinol angelate (**1**) and decursin (**2**), respectively. The animal group treated with a dose of 25 mg/kg 5-fluorouracil, as a positive drug, showed a much stronger enhancement of the MST.

The results of the effect of decursinol angelate (**1**) and decursin (**2**) on the solid tumors induced by Sarcoma-180 tumor cells in the mice as measured by the tumor volumes and weights are shown in Fig. 2. As shown in Fig. 2, the average tumor volume in the control group was 6229.5± 552.4 mm^3. The level of the tumor volume in the animals treated with 5-fluorouracil injection decreased by 73.7% (1641±187.4 mm^3) compared with the controls. Decursinol angelate (**1**) and decursin (**2**), at the 50 mg/kg dose caused a 39.5% (3768.8±292.5 mm^3) and 28.1% (4479.1 ±279.3 mm^3) inhibition of the tumor volumes, and at the 100 mg/kg dose by 40.6% (3700.3±231.0 mm^3) and 45.6% (3388.8±341.9 mm^3), respectively. Decursinol angelate (**1**) and decursin (**2**), when injected i.p. at 50 and 100 mg/kg, caused decreases in the tumor weights by 35.2% (4.6± 0.4 g) and 31.0% (4.9±0.3 g), and 35.2% (4.6±0.4 g) and 42.3% (4.1±0.3 g), respectively, while 5-fluorouracil, at 25 mg/kg, inhibited the tumor weight by 70.4% (2.1±0.3 g) compared with the controls (7.1±0.3 g).

From this work, it was clear that decursinol angelate (**1**) and decursin (**2**) from *A. gigas* are anti-tumor agents. In previous papers, decursinol angelate (**1**) and decursin (**2**) from *A. gigas* were shown to have *in vitro* cytotoxic activities. Decursin (**2**) has displayed toxic activity against various human cancer cell lines and activated protein kinase C (PKC) *in vitro*, which indicates that the cytotoxic activity of decursin (**2**) may be related to the activation of PKC (Ahn *et al.*, 1996). Decursinol angelate (**1**), a structural isomer of decursin (**2**), has shown similar *in vitro* cytotoxicity and PKC activating activity to decursin (**2**) (Ahn *et al.*, 1997).

Our work has shown the *in vivo* anti-tumor activities of decursinol angelate (**1**) and decursin (**2**) from *A. gigas*. Decursin (**2**) was more active than decursinol angelate (**1**). The comparison of the anti-tumor activities of decursinol angelate (**1**) and decursin (**2**) suggested that the senecioylic acid moiety of decursin (**2**) was more important in exhibiting the anti-tumor effects than the angeloylic acid moiety of decursinol angelate (**1**).

Accordingly, decursin (**2**) is the main anti-tumor agent from *A. gigas*. Of the other naturally occurring coumarins, aurapten from *Poncirus trifoliata* has exhibited cytotoxic activity against L1210 cells (Kang *et al.*, 1985).

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