응용약물학회지, The Journal of Applied Pharmacology, **10**, 85-88(2002)

Coumarins from *Angelica gigas* Roots having Rat Lens Aldose Reductase Activity

Sanghyun Lee, Sang Hoon Jung, Yeon Sil Lee and Kuk Hyun Shin*

Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

(Received May 15, 2002 ; accepted June 22, 2002)

Abstract – Systematic fractionation of *Angelica gigas* roots led to the isolation of linear coumarins such as decursinol angelate (**1**), decursin (**2**) and nodakenin (**3**). These compounds were tested for their effects on rat lens aldose reductase. Nodakenin (**3**) was demonstrated to exhibit significant inhibition of rat lens aldose reductase activity with IC_{50} value of 7.33 μM.

Keywords □ *Angelica gigas*, Umbelliferae, rat lens aldose reductase activity, coumarin, nodakenin

The enzyme, aldose reductase, together with sorbitol dehydrogenase, which catalyzes the reduction of aldose to polyol, has been demonstrated to play a central role in the cataract formation in galactosemia and diabetes (Kinoshita and Nishimura, 1988). Aldose reductase inhibitors are considered to be effective in preventing cataract onset and various diabetic complications.

A. gigas roots belonging to Umbelliferae have been used as traditional medicine not only for treatment anemia but also as a sedative, an anodyne or a tonic agent (Yook, 1990).

A. gigas had been studied extensively and were shown to exhibit a variety of activities. Decursin and decursinol angelate displayed toxic activity against various human cancer cell lines (Ahn *et al.*, 1996; Ahn *et al.*, 1997). Decursin and decursinol antagonized against the voluntary activity in mice (Kim *et al.*, 1980). Decursinol was demonstrated to exhibit a potent inhibitory activity toward acetyl cholinesterase (Kang *et al.*, 2001).

In the course of a series of studies for the purpose of evaluating naturally occurring aldose reductase inhibitors, we found that the hot water extract of this plant showed inhibition of bovine lens aldose reductase activity at 10^{-1} mg/ml (Shin *et al.*, 1993).

Based on this result, we attempted to isolate and characterize active principles from this plant. As results, three major linear coumarins such as decursinol angelate (**1**), decursin (**2**) and nodakenin (**3**) were isolated as active principles. Among them, nodakenin (**3**) was demonstrated to be a most promising compound for the inhibition of aldose reductase.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Reagents

^1H- and ^{13}C-NMR spectra were recorded with Bruker AVANCE 400 NMR spectrometer. MS spectra were measured with Jeol JMS-AX505WA mass spectrometer. β-Nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), phenylmethylsulfonylfluoride (PMSF), DL-glyceraldehyde, 2-mercaptoethanol and tetramethyleneglutaric acid (TMG) were purchased from Sigma Chem. Co. (St. Louise). Other reagents of first grade were commercially available.

Plant materials

The roots of *Angelica gigas* Nakai were purchased from Kyungdong market, Seoul, Korea in Mar. 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, 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. 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 frac-

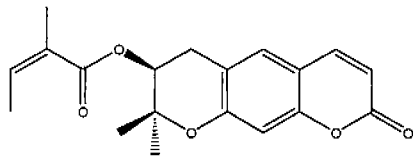
tionated successively with equal volumes of Et_2O and *n*-BuOH, leaving residual H_2O soluble fraction. Each fraction was evaporated *in vacuo* to yield the residues of Et_2O soluble fraction (518 g) and *n*-BuOH soluble fraction (445 g).

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 decursinol angelate (**1**) (789 mg, 37:3) and decursin (**2**) (5 g, 37:3). The portion of *n*-BuOH fraction (34 g) was chromatographed on silica gel eluting with a gradient of $CHCl_3$-MeOH to afford nodakenin (**3**) (2.9 g, 37:3) (Lee *et al.*, 2002).

Decursinol angelate (**1**); EI-MS (rel. int. %): *m/z* 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 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 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**); EI-MS (rel. int. %): *m/z* 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 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 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).

Nodakenin (**3**); EI-MS (rel. int. %): *m/z* 408 (23.1) $[M]^+$, 229 (66.5), 213 (37.4), 187 (100); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.91 (1H, d, J = 9.5 Hz, H-4),



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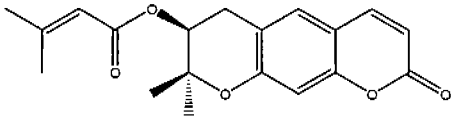

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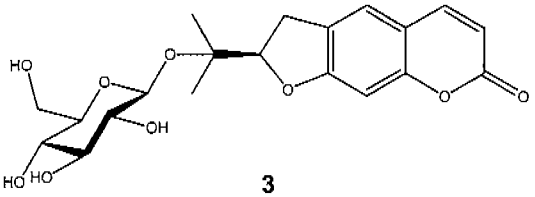

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7.46 (1H, s, H-5), 6.78 (1H, s, H-8), 6.20 (1H, d, J = 9.5 Hz, H-3), 4.85 (1H, m, H-2'), 4.4. (1H, d, J = 7.7 Hz, glycosyl H-1"), 3.19 (2H, m, H-3'), 1.25 (3H, s, CH_3), 1.22 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 163.5 (C-2), 161.0 (C-7), 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').

Measurement of rat lens aldose reductase activity *in vitro*

Crude rat lens aldose reductase was prepared as follows. Rat lenses were removed from Sprague-Dawley rats weighing 250~280 g and frozen until use. The supernatant fraction of the rat lens homogenate was prepared according to Hayman and Kinoshita (1965), and then partially purified according to Inagaki *et al.* (1982). Partially purified enzyme with a specific activity of 6.5 mU/mg was routinely used to test enzyme inhibition. The partially purified material was separated into 1.0 ml aliquots and stored at −40°C.

The effects of the test materials on rat lens aldose reductase were estimated as described previously (Brubaker *et al.*, 1986). In brief, rat lens were homogenized and centrifuged at 12,000 g and the supernatant was used as an enzyme source. Aldose reductase activities were measured using 10 mM DL-glyceraldehyde as substrate, by determining the decrease in absorbance

(340 nm) of NADPH (16 mM) for 5 min in the presence or absence of the test compounds. Appropriate blanks contained all reagents except the substrate. The percent inhibition of each compound was calculated by comparing the reaction rate of the solution containing both substrate and only inhibitor with that of the control solution containing only the substrate. IC_{50} values, the concentration of the inhibitor that caused 50% inhibition, were calculated from regression equations.

RESULTS AND DISCUSSION

Systematic fractionation of the MeOH extract from the roots of *A. gigas* led to the isolation of two linear coumarins from the Et_2O fraction and a coumarin glucoside from the *n*-BuOH fraction, and their chemical structures were elucidated as decursinol angelate (**1**), decursin (**2**) and nodakenin (**3**) by spectral analysis and the comparison of the published data (Ryu *et al.*, 1990; Konoshima *et al.*, 1968; Pachaly *et al.*, 1996).

Table I. Effects of major coumarins (**1**, **2** and **3**) from the roots of *A. gigas* on rat lens aldose reductase

Samples	Inhibition (%)[a)]
TMG*	82.1
Decursinol angelate (**1**)	34.9
Decursin (**2**)	26.2
Nodakenin (**3**)	72.1

Each sample concentration was 10 μM.
[a)]Inhibition rate was calculated as percentage with respect to the control value.
*TMG: tetramethylene glutaric acid, a reference compound as one of typical aldose reductase inhibitors.

Table II. Inhibitory potency of nodakenin (**3**) from the roots of *A. gigas* on rat lens aldose reductase

Samples	Concentrations (μM)	Inhibition (%) [a)]	IC_{50} (μM) [b)]
TMG*	10	82.1	0.63
	1	53.7	
	0.1	29.8	
Nodakenin (**3**)	10	72.1	7.33
	5	25.6	
	1	11.1	

[a)] Inhibition rate was calculated as percentage with respect to the control value.
[b)] IC_{50} values were calculated from the least-squares regression equations in the plot of the logarithm of at three graded concentrations vs % inhibition.
*TMG: tetramethylene glutaric acid, a reference compound as one of typical aldose reductase inhibitors.

Three major compounds, decursinol angelate (**1**), decursin (**2**) and nodakenin (**3**) were subjected to test for rat lens aldose reductase activity at 10 mM of the test compounds and the results were shown in Table I. As shown in Table I, nodakenin (**3**), a coumarin glucoside, was found to exhibit a very strong aldose reductase inhibitory activity (72.1% inhibition) which was a little weaker than TMG. But other coumarins such as decursinol angelate (**1**) and decursin (**2**) were exhibited a far weaker inhibitory activity, their inhibitory activities at the same dose level, being 34.9 and 26.2%, respectively.

To evaluate the aldose reductase inhibitory potency of nodakenin, more precisely effects on aldose reductase were estimated at three graded concentrations, and its IC_{50} value was calculated and indicated in Table II.

Although slightly less potent than tetramethylene glutaric acid (TMG) known as one of typical aldose reductase inhibitors (IC_{50} value, 0.63 μM), the inhibitory potency of nodakenin (**3**) as expressed by IC_{50} value was 7.33 μM.

In diabetes, the high extracellular levels of glucose disturb the cellular osmoregulation and sorbitol is formed intracellularly due to the intracellular polyol pathway, which is suspected to be one of the key processes in the development of diabetic complications and associated cellular dysfunctions.

Aldose reductase inhibitors thus have been shown to prevent or delay significantly diabetic complications, and synthetic aldose reductase inhibitors are currently available and many have been tested for their clinical use, albeit with limited success (Raskin and Rosenstock, 1987); Synthetic compounds with diverse structures such as sorbinil (Beyer-Mears and Cruz, 1985), epalrestat (Terashima *et al.*, 1984), other hydantoin derivatives (Inagaki *et al.*, 1982), and flavonoids (Shimizu *et al.*, 1984), isoliquiritigenin (Aida *et al.*, 1990), luteolin (Shin *et al.*, 1995) and coumarins (Moon *et al.*, 1988; Shin *et al.*, 1994) from natural origin have been extensively studied and reported to inhibit aldose reductase.

The present study was carried out in a search for the new potential aldose reductase inhibitors useful for the treatment of diabetic complications from the roots of *A. gigas*, and we found that nodakenin (**3**) was the most active principle utilizable as a lead compound, for

the inhibition of aldose reductase and is attributed to be a promising compound for the prevention and/or treatment of diabetic complications.

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