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# Hyperin, An Aldose Reductase Inhibitor from *Acanthopanax senticosus* Leaves

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**Abstract** – Hyperin from the leaves of *Acanthopanax senticosus* was tested for its effect on rat lens aldose reductase, and demonstrated to exhibit a significant inhibition of rat lens aldose reductase activity with $IC_{50}$ value of 2.63 μM.
**Keywords** – *Acanthopanax senticosus*, Araliaceae, rat lens aldose reductase activity, hyperin

## Introduction

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

*A. senticosus* is genus of the family Araliaceae and distributed in Korea, Japan and China. It has traditionally been used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes (Perry, 1980; Yook, 1990).

Investigations on the compounds have revealed the presence of phenolic compounds such as isofraxidin, eleutherosides B and E from the stem barks (Nishibe *et al.*, 1990), eleutheroside $E_2$ and isomaltol 3-*O*-α-D-glucopyranoside from the roots (Li *et al.*, 2001), *etc.*

*A. senticosus* had been studied extensively and were shown to exhibit a variety of activities such as anti-bacterial, anti-cancer, anti-inflammatory, anti-gout, anti-hepatitis, anti-hyperglycemic, anti-leishmanicidic, anti-oxidant, anti-pyretic, anti-xanthine oxidase, choleretic, hemostatic, immuno-stimulatory, hypo-cholesterolemic and radio-protectant effects (Davydov and Krikorian, 2000).

In the course of a series of studies for the purpose of evaluating naturally occurring aldose reductase inhibitors, we attempted to isolate and characterize active principles from *Acanthopanax senticosus*. Hyperin isolated from this plant leaves was demonstrated to be a most promising compound for the inhibition of aldose reductase.

This paper describes the aldose reductase inhibitory activity of hyperin isolated from the leaves of *A. senticosus*.

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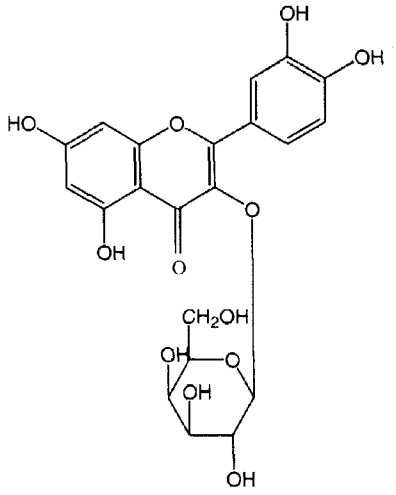
## Experimental

**Instruments and reagents** – Melting point was determined with Mitamura Riken apparatus and uncorrected. MS spectra were measured with Jeol JMS-AX505WA mass spectrometer. IR spectrum was recorded with Jasco FT/IR-300E instrument on KBr disc. $^1$H- and $^{13}$C-NMR spectra were recorded with Bruker AVANCE 400 NMR 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 leaves of *Acanthopanax senticosus* Harms were collected at Kong Ju, Korea in Oct. 2000, and verified by Prof. S. H. Cho, Kong Ju University of Education, 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 leaves of *A. senticosus* were extracted three times with MeOH under reflux. The MeOH extract was suspended in water, and then fractionated successively with equal volumes of $CH_2Cl_2$, EtOAc and *n*-BuOH, leaving residual water-soluble fraction. The EtOAc fraction was chromatographed on silica gel eluting with a gradient of $CHCl_3$-MeOH to afford hyperin.

**Hyperin**; mp: 253-254°; FABMS *m/z*: 465 $[M + H]^+$; EIMS (rei. int. %) *m/z*: 302 (100) $[M\text{-Gal}]^+$, 273 (7.3), 245 (4.2), 207 (11.1), 153 (6.1), 137 (7.4), 128 (7.1); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3316, 2900, 1655, 1607, 1060; $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta_H$ (ppm): 12.64 (1H, s, 5-O*H*), 7.67 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 7.53 (1H, d, *J* = 2.0 Hz, H-2'), 6.82 (1H, d, *J* = 8.5 Hz, H-5'), 6.41 (1H, d, *J* = 1.9 Hz, H-8), 6.21



(1H, d, $J$ = 1.9 Hz, H-6), 5.38 (1H, d, $J$ = 7.8 Hz, galactosyl H-1"); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta_c$ (ppm): 177.9 (*C*=O), 164.5 (C-7), 161.6 (C-5), 156.6 (C-2, C-9), 148.9 (C-4'), 145.2 (C-3'), 133.9 (C-3), 122.4 (C-6'), 121.5 (C-1'), 116.3 (C-5'), 115.6 (C-2'), 104.3 (C-10), 102.2 (C-1"), 99.1 (C-6), 93.9 (C-8), 76.2 (C-5"), 73.6 (C-3"), 71.6 (C-2"), 68.3 (C-4"), 60.5 (C-6").

**Preparation of rat lens aldose reductase** – 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 enzyme was separated into 1.0 ml aliquots and stored at –40°C.

**Measurement of rat lens aldose reductase activity *in vitro*** – 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 (Brubaker *et al.*, 1986). 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

Hyperin from *A. senticosus* leaves was subjected to test for rat lens aldose reductase activity, and the result was shown in Table 1. As shown in Table 1, hyperin was found to exhibit a very strong aldose reductase inhibitory activity (91.6% inhibition at 10 μM) that was a little weaker than TMG.

To evaluate the aldose reductase inhibitory potency of hyperin, more precisely effects on aldose reductase were estimated at three graded concentrations, and its $IC_{50}$ value was calculated and indicated in Table 2. 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 hyperin as expressed by $IC_{50}$ value was 2.63 μM.

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 Rosentstock, 1987); Synthetic compounds with diverse structures such as sorbinil (Beyer-Mears and Cruz, 1985), epalrestat (Terashima *et al.*, 1984), 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; Lee *et al.*, 2002) 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

**Table 1.** Effect of hyperin from *A. senticosus* leaves on rat lens aldose reductase

| Sample | Inhibition (%) [a)] |
|---|---|
| TMG* | 82.1 |
| Hyperin | 91.6 |

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

**Table 2.** Inhibitory potency of hyperin from *A. senticosus* leaves on rat lens aldose reductase

| Sample | Concentrations (μM) | Inhibition (%)[a)] | $IC_{50}$ (μM)[b)] |
|---|---|---|---|
| TMG* | 10 | 82.1 | 0.63 |
| | 1 | 53.7 | |
| | 0.1 | 29.8 | |
| Hyperin | 10 | 91.6 | 2.63 |
| | 1 | 47.1 | |
| | 0.5 | 31.8 | |

[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 references compound as one of typical aldose reductase inhibitors.

treatment of diabetic complications from the leaves of *A. senticosus*, and we found that hyperin was an 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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