Studies on Lens-Aldose-Reductase Inhibitor in Medicinal Plants. II. Active Constituents of *Monochasma savatierii* FRANCH. *et* MAXIM.

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The 70% acetone extract of *Monochasma savatierii* FRANCH. *et* MAXIM. showed very strong inhibition of rabbit lens aldose reductase (AR). From the active fraction, five iridoid glucosides along with the two phenolic glycosides, acteoside and dehydroacteoside, have been isolated. Among them, acteoside showed the highest activity, being about 2.5 times more potent than baicalein, a known natural inhibitor of AR ($IC_{50} = 9.8 \times 10^{-7}$ M). Demethylmussaenoside and 7-*O*-acetyl-8-epi-loganic acid, which are iridoid glucosides, had weak inhibitory activity.

Keywords *Monochasma savatierii*; lens aldose reductase; acteoside; iridoid glucoside

A large quantity of sorbitol causes various complications of chronic diabetes, such as cataracts. In connection with our systematic isolation and structure studies on biologically active constituents from crude drugs and medicinal plants, we have already reported the result of preliminary screening for lens aldose reductase (AR)-inhibitory activity.[1)]

The 70% acetone extract of *Monochasma savatierii* was found to have AR-inhibitory activity. The components of *M. savatierii* have already been reported.[2)]

In the present paper, we describe the components of *M. savatierii* with AR-inhibitory activity.

Materials and Methods

Aerial parts of *M. savatierii* were collected at Hondo-shi, Kumamoto. Voucher specimens are on deposit at the Experimental Station of Medicinal Plants, Hiroshima University School of Medicine.

Bioassay The enzyme assay was performed at 25 °C in 0.1 M sodium phosphate buffer (pH 6.2) containing 0.3 M ammonium sulfate, 10 mM DL-glyceraldehyde, 0.15 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH) and an appropriate amount of enzyme (2.5 μg) in a total volume of 3.0 ml. AR I_b, prepared from rabbit lens by the method described in the previous paper, was used in this study.[3)] The effects on the enzyme activity were determined by the reported method.[3)] Most samples were dissolved in methanol (0.17%) or dimethylsulfoxide (0.15%), which were found to have no effect on the enzyme activity.

Results and Discussion

The MeOH-soluble fraction of the 70% acetone extract was separated by using a combination of Sephadex LH-20 and MCIgel CHP-20P column chromatography to afford 11 fractions. Fraction (fr.) 9, which was the most active, was applied to a column of μBondpack C_{18} and then a column of Sephadex LH-20 to give acteoside (**4**), which was an active compound. Fractions 10 and 6, which were fairly active, were applied to the same columns to give demethylmussaenoside (**5**) from fr. 10 and 7-*O*-acetyl-8-epi-loganic acid (**6**)[4)] and dehydroacteoside (**7**) from fr. 6. In addition, three iridoid glucosides, catalpol (**1**), bartisioside (**2**) and aucubin (**3**), were isolated from other fractions.

The isolated compounds **1**—**7** were tested for inhibitory activity (Table I) on AR. Among them, **4** showed very strong inhibitory activity on AR with an IC_{50} of 3.9×10^{-7} M. Compound **6** showed a slightly more potent inhibitory effect than **5**. Compounds **6** and **5** exhibited inhibition of AR with IC_{50} values of 5.6×10^{-5} M and 6.14×10^{-5} M, respectively. The AR-inhibitory effects of both iridoid compounds were much less than that of **1** or baicalein.[5)] However, both effects were more potent than those reported for brazilin and haematoxylin.[6)] These compounds have not previously been reported to have inhibitory activity of AR. Compounds **1**, **2** and **3** had no inhibitory activity. Compound **4** seems to be the main AR inhibitor in the 70% acetone extract of *M. savatierii*.

In order to determine the type of inhibition, the kinetics of inhibition of AR by **4** were plotted according to Lineweaver and Burk and **4** was found to be an uncompetitive inhibitor (Fig. 1). We are now investigating the AR-inhibitory effect of related phenolic and iridoid compounds.

TABLE I. Inhibition of Lens Aldose Reductase by Compounds **1**—**7** from *M. savatierii*

Compound	IC_{50} (M)
1	—
2	—
3	—
4	3.90×10^{-7}
5	6.14×10^{-5}
6	5.60×10^{-5}
7	Not tested
Baicalein	9.80×10^{-7}

Baicalein was tested as a reference in this study.

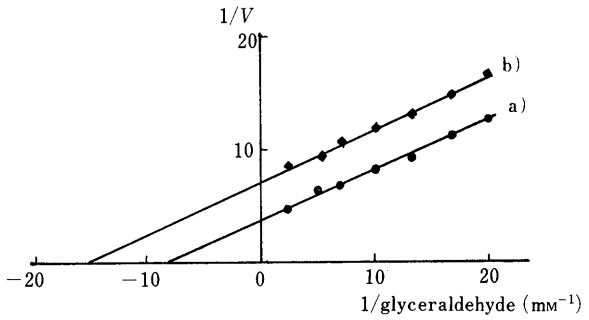

Fig. 1. Uncompetitive Inhibition of Aldose Reductase by **4**
a) None. b) The concentration of **4** was 3.0×10^{-7} M.

References and Notes

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2) S. Yahara, T. Nohara, H. Kohda, K. Shimomura and M. Satake, *Yakugaku Zasshi*, **106**, 725 (1986).
3) T. Tanimoto, H. Fukuda and J. Kawamura, *Chem. Pharm. Bull.*, **31**, 2395 (1983).
4) Though this compound was previously reported to be 7-*O*-acetyl loganic acid,[2] it was found here to be 7-*O*-acetyl-8-epi-loganic acid.
5) M. Shimizu, T. Ito, S. Terashima, T. Hayashi, M. Arisawa, N. Morita, S. Kurokawa, K. Ito and Y. Hashimoto, *Phytochemistry*, **23**, 1885 (1984). Baicalein was also tested as a reference in this study ($IC_{50} = 9.8 \times 10^{-7}$ M).
6) C. K. Moon, Y. P. Yun, J. H. Lee, H. Wagner and Y. S. Shin, *Planta Medica*, **1985**, 66.