Effect of *Polygonum hydropiper* Sulfated Flavonoids on Lens Aldose Reductase and Related Enzymes

Hiroyuki Haraguchi,* Isao Ohmi, Sachiko Sakai, and Ayumi Fukuda

Faculty of Engineering, Fukuyama University, Gakuen-cho, Fukuyama 729-02, Japan

Yoshie Toihara, Tadashi Fujimoto, Nubuyuki Okamura, and Akira Yagi

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Gakuen-cho, Fukuyama 729-02, Japan

Received September 18, 1995[⊗]

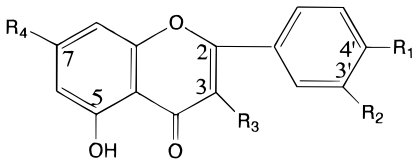
The sulfated flavonoids in *Polygonum hydropiper* showed potent inhibiton against lens aldose reductase. Among these flavonoids isorhamnetin 3,7-disulfate (**5**) was most potent. Kinetic analysis showed that **5** exhibited noncompetitive inhibition against both *dl*-glyceraldehyde and NADPH.

Aldose reductase (EC 1.1.1.21), a member of the NADPH-dependent aldo−keto reductase family, is a key enzyme in the polyol pathway.[1] This enzyme catalyzes the reduction of various aldehydes, and the reduction of glucose to sorbitol provides a common link in the onset of diabetic complications that result in tissue and functional changes in the cornea, lens, retina, iris, peripheral nerve, and kidney.[2,3] The intracellular accumulation of sorbitol leads to locally hyperosmotic conditions responsible for the loss of clarity in the lens.[4] Therefore, the inhibition of aldose reductase may be effective in preventing cataract formation in diabetes.

Flavonoids are commonly ingested from fruits and vegetables in the diet.[5] They have no nutritive value but are capable of exerting various pharmacological activities,[6] including antioxidative,[7−9] superoxide-scavenging,[10,11] and aldose reductase inhibitory activity.[12−14] However, one of the serious limitations to the use of flavonoids for any therapeutic activity has been their insolubility in water.[15] In order to overcome this solubility, some sulfated quercetin derivatives were synthesized, and assays were carried out for aldose reductase inhibition.[15]

In our earlier work, antioxidative and superoxide anion-scavenging flavonoids were isolated from the water-soluble fraction of *Polygonum hydropiper* L. (Polygonaceae),[16,17] a medicinal herb used as a spice in Japanese cuisine.[18] Lipid peroxidation is also one of the causes of cataracts.[19,20] This paper deals with the inhibitory activities of sulfated flavonoids isolated from this plant on lens aldose reductase and other related enzymes. We also compare their activities with the corresponding flavonoid aglycons and glycosides. The structures of flavonoids in *P. hydropiper* discussed in the present work are listed in Figure 1.

The degree of inhibition of porcine lens aldose reductase by sulfated, methylated, and glycosidal flavonoids in *P. hydropiper* is presented in Table 1. The nonsulfated aglycon, quercetin (**1**), and its 3-glucoside isoquercitrin (**8**) inhibited lens aldose reductase, as reported earlier.[12,13] Among the flavonoids in *P. hydropiper*, isorhamnetin 3,7-disulfate (**5**) (IC_{50} 1.8 μM) showed most potent inhibitory activity on lens aldose reductase. The removal of 7-sulfate from **5**, as in percicarin (**4**), decreased aldose reductase inhibition (IC_{50} 69.0 μM).



	R_1	R_2	R_3	R_4
1	OH	OH	OH	OH
2	OH	OH	OSO_3K	OH
3	OH	OCH_3	OH	OH
4	OH	OCH_3	OSO_3K	OH
5	OH	OCH_3	OSO_3K	OSO_3K
6	OH	OCH_3	OH	OCH_3
7	OH	OCH_3	OSO_3K	OCH_3
8	OH	OH	O-Glc	OH
9	OCH_3	OH	O-Glc	OSO_3K

Figure 1. Flavonoids isolated from *P. hydropiper*.

Table 1. Effect of Flavonoids Isolated from *P. hydropiper* on Lens Aldose Reductase, Kidney Aldehyde Reductase, and Liver NADH Oxidase

	IC_{50} (μM)[a]		
compd	aldose reductase	aldehyde reductase	NADH oxidase
1	50.1	>91.0	65.3
2	50.9	55.6	>71.0
3	>95.0	>95.0	88.6
4	69.0	>69.0	>69.0
5	1.8	49.5	>54.0
6	>91.0	>91.0	>91.0
7	30.1	43.5	>67.0
8	16.0	>64.0	>64.0
9	5.0	>50.0	>50.0

[a] Inhibitory activity was expressed as the mean of 50% inhibitory concentration of triplicate determinations, obtained by interpolation of concentration-inhibition curves.

The removal of both the 7- and 3-sulfates from **5**, as in isorhamnetin (**3**), further decreased the activity (IC_{50} > 95.0 μM). On the other hand, the 3-sulfate of quercetin (**2**) showed aldose reductase inhibition to an extent similar to that of its aglycon (**1**). Methylation of C-3′ (**1** to **3**) caused a decrease in the inhibitory activity (IC_{50} 50.1 and > 95.0 μM), respectively. Further, an additional C-7 methylation of **3** resulted in a great loss of activity; rhamnazin (**6**) showed almost no effect on aldose reductase up to 91 μM. However, the addition of a C-3 sulfate to **6**, as in rhamnazin-3-sulfate (**7**), resulted in an inhibitory activity of IC_{50} 30.1 μM. As reported in the literature,[13] the monoglucosides are stronger inhibitors than the corresponding aglycons on

[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1996.

0163-3864/96/3259-0443$12.00/0

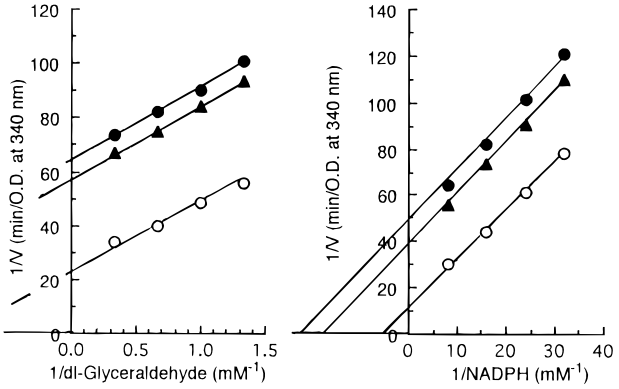


Figure 2. Inhibitory effect of **5** on porcine lens aldose reductase. Lineweaver-Burk plots in the absence (○) and in the presence (●, 1.8 μM; ▲, 0.54 μM) of **5** are shown.

aldose reductase: isoquercitrin (**8**) IC_{50} 16.0 μM, quercetin (**1**) IC_{50} 50.1 μM. 7-Sulfation and 3′-methylation of **8** forms tamarixetin 3-glucoside 7-sulfate (**9**) and causes an increase in inhibitory activity against lens aldose reductase.

Kinetic analysis for aldose reductase inhibition by a potent inhibitor (**5**) using Lineweaver−Burk plots is shown in Figure 2. When the concentration of the substrate *dl*-glyceraldehyde was changed, the slopes obtained from the uninhibited enzyme and two different concentrations of **5** were in parallel. The reciprocal plot curves of enzyme activities and concentration of cofactor NADPH also showed noncompetitive inhibition. These figures indicate that **5** inhibits lens aldose reductase noncompetitively with respect to both *dl*-glyceraldehyde and NADPH.

Many aldose reductase inhibitors also inhibit aldehyde reductase (EC 1.1.1.20).[21−24] It has been proposed that both aldose and aldehyde reductases may play a role in the development of chronic diabetic pathologies.[25] The sulfated flavonoids, which showed potent inhibition on lens aldose reductase, also inhibited kidney aldehyde reductase but to a lesser extent. The effects of flavonoids isolated from *P. hydropiper* are shown in Table 1. Glycosidal conjugation may contribute to selectivity but sulfation did not.

Aldose reductase is an NADPH-dependent oxidoreductase. The inhibition of NADH oxidase leads to the interference of normal glucose metabolism.[26] Some flavonoids have been reported to inhibit NADH oxidase in liver mitochondria.[27−29] As shown in Table 1, isorhamnetin (**3**) also affected NADH oxidase. On the other hand, all sulfated flavonoids (**2**,**4**,**5**,**7**,**9**) in *P. hydropiper* showed no effect on NADH oxidase.

The sulfated flavonoids in *P. hydropiper* showed potent inhibitory activity against lens aldose reductase, but a clear selectivity between aldose reductase and aldehyde reductase inhibition was not observed. As previously reported,[17] these compounds possessed potent antioxidative and superoxide-scavenging activity. Inasmuch as the young shoots of this plant are eaten and its extract used as an additive of "miso" and vinegar in Japan, present results on these water-soluble flavonoids provide considerable interests in both food science and preventive medicine. With a view to preventing cataract formation, the effects of these flavonoids on sorbitol accumulation in vivo are under investigation.

Experimental Section

Chemicals. All flavonoids (**1**−**9**) examined in this study were isolated from the leaves of *P. hydropiper*.[16,17] β-Mercaptoethanol and *dl*-glyceraldehyde were purchased from Sigma Chemical Co. (St. Louis, MO). NADPH and NADH were obtained from Oriental Yeast Co. (Tokyo, Japan).

Animal Tissues. Porcine eyeball and kidney and bovine liver were obtained from Nippon Ham Co. Ltd. (Osaka, Japan).

Preparation of Lens Aldose Reductase. Lenses were removed from porcine eyes and homogenized in 3 vol of 135 mM phosphate buffer (pH 7.0) containing 10 mM β-mercaptoethanol.[30] The homogenate was centrifuged at 10 000 × g for 15 min. The supernatant fluid was saturated with 75% $(NH_4)_2SO_4$. The precipitate obtained by centrifugation was dissolved in the same buffer and used as an enzyme preparation.[31]

Preparation of Kidney Aldehyde Reductase. The porcine kidneys were dissected into cortical and medullary regions, and the cortices were homogenized in 3 vol of 20 mM phosphate buffer (pH 7.5) containing 0.25 M sucrose, 0.5 mM EDTA, and 1 mM β-mercaptoethanol. The homogenate was centrifuged at 15 000 × g for 20 min. The supernatant fluid was saturated with 60% $(NH_4)_2SO_4$. The precipitate obtained by centrifugation was dissolved in 10 mM Tris buffer (pH 7.4) containing 0.5 mM EDTA and 1 mM β-mercaptoethanol and was subjected to a Sephadex G-25 column equilibrated with the same buffer. The enzyme eluate was chromatographed on a DEAE-cellulose column equilibrated with the same Tris buffer and developed with a 0−0.4 M linear gradient of NaCl. This partially purified aldehyde reductase was free of aldose reductase.[32]

Preparation of Liver Mitochondria. The bovine liver was homogenized in 10 vol of 0.25 M sucrose containing 0.1 mM EDTA (pH 7.4). The homogenate was centrifuged at 600 × g for 10 min. The supernatant fluid was decanted and centrifuged at 15 000 × g for 10 min.[33] The mitochondrial pellet was washed twice with the same solution and finally suspended in 3 mM Tris buffer (pH 7.4) containing 0.07 M sucrose, 0.21 M mannitol, and 0.1 mM EDTA.

Assay of Reductase Activity. Aldose reductase and aldehyde reductase were assayed spectrophotometrically on a Shimadzu MPS-2000 spectrophotometer equipped with a TCC temperature controller. The reaction mixture in a total volume of 3 mL contained 50 mM Na-phosphate buffer (pH 6.2), 0.125 mM NADPH, 400 mM Li_2SO_4, enzyme solution (equivalent to 4 mg protein), and 3 mM *dl*-glyceraldehyde as a substrate.[34] The reaction was initiated by the addition of NADPH. The reaction-rate was determined by tracing the decrease in the absorption of NADPH at 340 nm on a Shimadzu Graphic Printer PR-3.

Assay of NADH Oxidase. Mitochondrial NADH oxidase was assayed by measuring the decrease in absorbancy at 340 nm.[35] The reaction mixture in a total volume of 3 mL consisted of 50 mM phosphate buffer (pH 7.4), 0.1 mM NADH, and bovine liver mitochondrial protein (equivalent to 3 mg).

References and Notes

(1) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. *J. Med. Chem.* **1985**, *28*, 841.
(2) Kador, P. F. *Med. Res. Rev.* **1988**, *8*, 325.

(3) Kador, P. F.; Akagi, Y.; Terubayashi, H.; Wyman, M.; Kinoshita, J. H. *Arch. Ophthalmol.* **1988**, *106*, 1099.
(4) Chylack, L. T.; Kinoshita, J. H. *Invest. Ophthal.* **1969**, *8*, 401.
(5) Stavric, B.; Matula, T. I. In *Lipid-Soluble Antioxidant: Biochemistry and Clinical Applications*, Ong, A. S. H.; Packer, L., Ed.; Birkhäuser Verlag: Basel Switzerland, 1992; pp 274−294.
(6) Middleton, E., Jr.; Kandaswami, C. In *The Flavonoids: Advances in Research Since 1986*, Harborne, J. B., Ed.; Chapman & Hall: London, 1994; pp 619−652.
(7) Larson, R. A. *Phytochemistry* **1988**, *27*, 969.
(8) Ratty, A. K.; Das, N. P. *Biochem. Med. Metabol. Biol.* **1988**, *39*, 69.
(9) Cholbi, M. R.; Paya, M.; Alcaraz, M. J. *Experientia* **1991**, *47*, 195.
(10) Gryglewski, R. J.; Korbut, R.; Robak, J.; Swies, J. *Biochem. Pharmacol.* **1986**, *36*, 317.
(11) Robak, J.; Gryglewski, R. J. *Biochem. Pharmacol.* **1988**, *37*, 837.
(12) Varma, S. D.; Kinoshita, J. H. *Biochem. Pharmacol.* **1976**, *25*, 2505.
(13) Shimizu, M.; Ito, T.; Terashima, S.; Hayashi, T.; Arisawa, M.; Morita, N.; Kurokawa, S.; Ito, K.; Hashimoto, Y. *Phytochemistry* **1984**, *23*, 1885.
(14) Okuda, J.; Miwa, I.; Inagaki, K.; Horie, T.; Nakayama, M. *Chem. Pharm. Bull.* **1984**, *32*, 767.
(15) Varma, S. D. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships*, Cody, V.; Middleton, E.; Harborne, J. B., Eds. Alan R. Liss: New York, 1986; pp 343−358.
(16) Haraguchi, H.; Hashimoto, K.; Yagi, A. *J. Agric. Food Chem.* **1992**, *40*, 1349.
(17) Yagi, A.; Uemura, T.; Okamura, N.; Haraguchi, H.; Imoto, T.; Hashimoto, K. *Phytochemistry* **1994**, *35*, 885.
(18) Ohsuka, A. *Nippon Kagaku Zasshi* **1963**, *84*, 748.
(19) Zigman, S. In *Mechanism of Cataract Formation in the Human Lens*, Duncan, G., Ed.; Academic Press: New York, 1981; pp 117−150.
(20) Angusteyn, R. C. In *Mechanism of Cataract Formation in the Human Lens*, Duncan, G., Ed.; Academic Press: New York, 1981; pp 71−116.
(21) O'Brien, M. M.; Schofield, P. J.; Edwards, M. R. *J. Neurochem.* **1982**, *39*, 810.
(22) Srivasstava, S. K.; Petrash, J. M.; Sadana, J. J.; Ansari, N. H.; Partridge, C. A. *Curr. Eye Res.* **1982**, *2*, 407.
(23) DeJong, K. S.; Schofield, P. J.; Edwards, M. R. *Biochem. J.* **1987**, *242*, 143.
(24) Poulsom, R. *Biochem. Pharmacol.* **1986**, *35*, 2955.
(25) Mylari, B. L.; Beyer, T. A.; Siegel, T. W. *J. Med. Chem.* **1991**, *34*, 1011.
(26) Thierbach, G.; Kunze, B.; Reichenbach, H.; Höfle, G. *Biochim. Biophys. Acta* **1984**, *765*, 227.
(27) Bohmont, C.; Aaronson, L. M.; Mann, K.; Pardini, R. S. *J. Nat. Prod.* **1987**, *50*, 427.
(28) Hodnick, W. F.; Kung, F. S.; Roettger, W. J.; Bohmont, C. W.; Pardini, R. S. *Biochem. Pharmacol.* **1986**, *35*, 2345.
(29) Hodnick, W. F.; Roettger, W. J.; Kung, F. S.; Bohmont, C. W.; Pardini, R. S. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships*, Cody, Y., Middleton, E., Harborne, J. B., Eds.; Alan R. Liss: New York, 1986; pp 249−252.
(30) Aida, K.; Shindo, H.; Tawata, M.; Onaya, T. *Planta Med.* **1987**, *53*, 13.
(31) Hayman, S.; Kinoshita, J. H. *J. Biol. Chem.* **1965**, *240*, 877.
(32) Sato, S.; Kador, P. F. *Biochem. Pharmacol.* **1990**, *40*, 1033.
(33) Johnson, D.; Lardy, H. In *Methods in Enzymology*, Estabrook, R. W.; Pullman, M. E., Eds.; Academic Press: New York, 1967; Vol. 10, pp 94−96.
(34) Nishikawa, M.; Tsurumi, Y.; Namiki, T.; Toshida, K.; Okuhara, M. *J. Antibiot.* **1987**, *40*, 1394.
(35) Triphathi, R. K.; Gottlieb, D. *J. Bacteriol.* **1969**, *100*, 310.

NP9601622