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Inhibitory Effects of Naturally Occurring Flavonoids on Rat Lens Aldose Reductase

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Abstract – Aldose reductase (AR), the key enzyme of the polyol pathway, is known to play important roles in the diabetic complications. The inhibitors of AR, therefore, would be potential agents for the prevention of diabetic complications. In order to clarify the structural requirements of flavonoids for aldose reductase inhibitory activity, thirty flavonoids were examined. Among the thirty flavonoids, flavonols such as quercetin (**5**), reyneutrin (**7**), quercitrin (**9**), isoquercitrin (**11**), and avicularin (**14**) were found to exhibit much stronger AR inhibition. Lonicerin (**10**), amentoflavone (**27**) and sophoraflavanone B (**30**) were also showed strong inhibitory activity. Especially, quercitrin and reyneutrin exhibited the most inhibitory potency on rat lens (RL) AR. The results suggested that flavonol having the 7-hydroxyl and/or catechol moiety at the B ring exhibit strong activity. In additon, flavonols having 3-*O*-monosaccharide also showed stronger inhibition than free flavonols at the 3-position. These results suggested that quercitrin and reyneutrin are attributed to be the promising compounds for the prevention and/or treatment of diabetic complications.

Keywords – Aldose reductase, Quercitrin, Reyneutrin, Diabetic complications

Introduction

Aldose reductase (AR), the key enzyme in the polyol pathway, has been demonstrated to play important roles not only in the cataract formation in the lens (Van Heyningen, 1959), but also in the pathogenesis of diabetic complications such as neuropathy (Ward, 1973), nephropathy (Beyer-Mears *et al.*, 1984) and retinopathy (Engerman and Kern, 1984). Evidence suggests that compounds that inhibit AR could be effective for the prevention of diabetic complications. A number of structurally diverse naturally occurring and synthetic AR inhibitors have been studied *in vivo* to clarify their effectiveness for prevention of diabetic complications in clinical trials (Handelsman and Turtle, 1981) as well as experimental animals (Beyer-Mears and Cruz, 1985). Since the mid Seventies, several studies on the inhibition of aldose reductase by flavonoids have been reported, and Matsuda *et al.* reported structure-activity relationship of flavonoids and related compounds for aldose reductase inhibitory activity (Matsuda *et al.*, 2002).

In a series of investigations to evaluate potential AR inhibitors from the Korean plants, we have shown that some hot water and methanol extracts from natural products exhibited a significant inhibition of bovine lens (BL) or RLAR *in vitro* (Shin *et al.*, 1993; Jung *et al.*, 2003), and a number of flavonoid compounds were isolated and characterized as AR inhibitors from plants (Shin *et al.*, 1994; 1995; Jung *et al.*, 2002).

In a previous paper, we synthesized flavonoid derivatives and their inhibitory effects on RLAR, platelet aggreggation and antioxidant effects have been tested. Some chalcone derivatives were found to possess AR inhibition and antioxidant activities *in vitro* as well as inhibition in the accumulation of sorbitol *in vivo*, in which 3,4,2',4'-tetrahydroxychalcone (butein) was the most promising compound for the prevention or treatment of diabetic complications (Lim *et al.*, 2000; 2001).

In the present study, naturally occurring flavonoids were examined to clarify the further structural requirements of flavonoids for aldose reductase inhibitory activity

Experimental

Flavonoids – Tested flavonoids were provided by Prof. Dr. Sam Sik Kang (Natural Products Research Institute, Seoul National University).

Preparation of RLAR – Crude RLAR was prepared

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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). The 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.

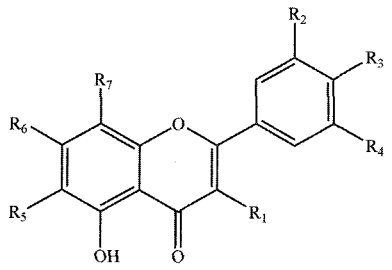
Measurement of RLAR activity – RLAR activities were assayed spectrophotometrically by measuring the decrease in absorption of NADPH at 340 nm over a 4 min period with DL-glyceraldehyde as a substrate (Sato and Kador, 1990). Each 1.0 ml cuvette contained equal units of enzyme, 0.10 M sodium phosphate buffer (pH 6.2), 0.3 mM NADPH with or without 10 mM substrate and inhibitor. The concentration of inhibitors giving 50% inhibition of enzyme activity (IC_{50}) was calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity.

Results and Discussion

The chemical structures of the naturally occurring flavonoid derivatives tested are listed in Table 1. Their inhibitory effects of 18 flavonols, 5 flavones ands 7 other flavonoids on RLAR using DL-glyceraldehyde as a substrate are shown in Table 2.

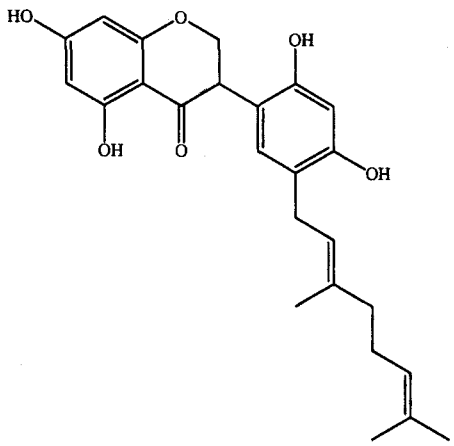
Flavonols such as quercetin (**5**), reyneutrin (**7**), quercitrin (**9**), isoquercitrin (**11**), and avicularin (**14**) were found to exhibit much stronger AR inhibition (more than 85% inhibition at 5 μg/ml) than other flavonoids. Lonicerin (**10**),

Table 1. Chemical structure of tested flavonoids on RLAR

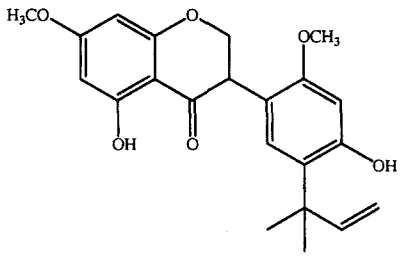


	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Anhydroicaritin-3-*O*-Rha (**1**)	*O*-Rha	H	OCH_3	H	H	OH	Pre
Kaempferol-3-*O*-Glc-(1 → 2)-Rha (**2**)	*O*-Glc-(1 → 2)-Rha	H	OH	H	H	OH	H
Icariin (**3**)	*O*-Rha	H	OCH_3	H	H	*O*-Glc	Pre
Quercetin (**5**)	OH	OH	OH	H	H	OH	H
Populnin (**6**)	OH	H	OH	H	H	*O*-Glc	H
Reyneutrin (**7**)	*O*-Xyl	OH	OH	H	H	OH	H
Linarin (**8**)	H	H	OCH_3	H	H	*O*-Rut	H
Quercitrin (**9**)	*O*-Rha	OH	OH	H	H	OH	H
Lonicerin (**10**)	H	OH	OH	H	H	*O*-Neo	H
Isoquercitrin (**11**)	*O*-Glc	OH	OH	H	H	OH	H
Quercetin 3,3'-dimethyl ether (**12**)	OCH_3	OCH_3	OH	H	H	OH	H
6,5'-Diprenylquercetin (**13**)	OH	OH	OH	Pre	Pre	OH	H
Avicularin (**14**)	*O*-Ara(*f*)	OH	OH	H	H	OH	H
Laricitrin-3-*O*-Rut (**15**)	*O*-Rut	OCH_3	OH	OH	H	OH	H
Isorhamnetin-3-*O*-Rut (**16**)	*O*-Rut	OCH_3	OH	H	H	OH	H
Diosmetin-7-*O*-glucoside (**17**)	H	OH	OCH_3	H	H	*O*-Glc	H
Kaempferol-3-*O*-Rut (**20**)	*O*-Rut	H	OH	H	H	OH	H
3,3'-Dimethylquercetin 4'-*O*-Glc (**21**)	OCH_3	OCH_3	*O*-Glc	H	H	OH	H
Kaempferol-3-*O*-(6"-*O*-*p*-coumaroyl-Glc-(1 → 2)-Rha (**22**)	*O*-(6"-*O*-*p*-coumaroyl-Glc-(1 → 2)-Rha	H	OH	H	H	OH	H
Jaceosidin (**23**)	H	OCH_3	OH	H	OCH_3	OH	H
Isorhamnetin (**25**)	OH	OCH_3	OH	H	H	OH	H
Anhydroicaritin (**26**)	OH	H	OCH_3	H	H	OH	Pre
Diosmin (**29**)	H	OH	OCH_3	H	H	*O*-Rut	H

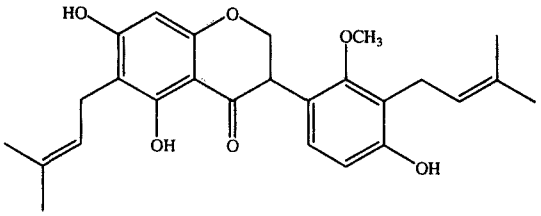
Glc: β-D-glucopyranosyl; Rha: α-L-rhamnopyranosyl; Xyl: β-D-Xylophyrhnooyl; Ara: α-L-arabinopyranosyl; Ara(*f*): α-L-arabinofuranosyl; Rut: rutinosyl; Neo: neohesperodosyl; Pre: prenyl



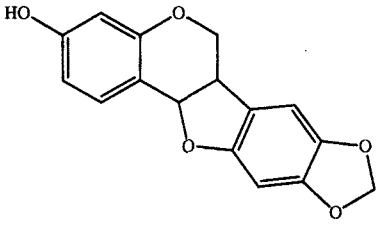
Kenusanone A (**4**)



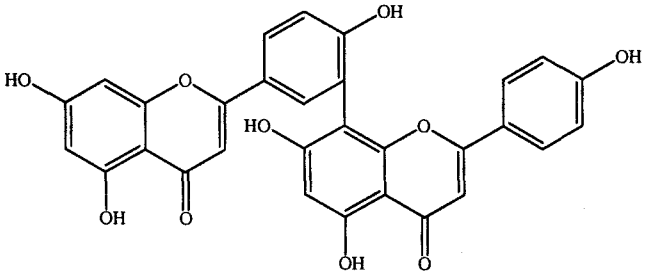
Echinoisosophoranone (**18**)



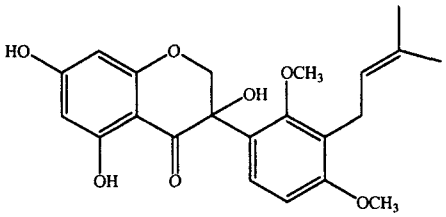
Isosophoranone (**19**)



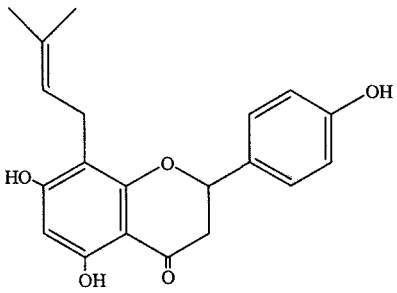
Maackiain (**24**)



Amentoflavone (**27**)



Echinoisoflavanone (**28**)



Sophoraflavanone B (**30**)

amentoflavone (**27**) and sophoraflavanone B (**30**) also showed strong inhibitory activities.

To evaluate the AR inhibitory activities between active flavonoids more precisely, their inhibitory potencies and IC_{50} values were estimated and indicated in Table 2.

Although slightly less potent than epalrestat (ONO Co.), known as one of typical AR inhibitors (IC_{50} value, 0.082 $\times 10^{-6}$ M), the inhibitory potencies of quercitrin and reyneutrin

Table 2. Inhibitory effect of flavonoids on RLAR

Flavonoids	Inhibition %[b]	IC_{50} values (μg/ml)	Micromolar IC_{50} values of active flavonoids (μM)
TMG [a]	92.3	–	1.99
Epalrestat	99.9	–	0.082
1	68.1	2.25	–
2	36.4	> 5	–
3	19.4	> 5	–
4	28.7	> 5	–
5	87.4	0.49	1.62
6	78.0	1.57	–
7	91.9	0.12	0.27
8	20.4	> 5	–
9	99.6	0.072	0.15
10	90.1	0.31	0.52
11	83.1	0.80	1.72
12	82.6	1.34	–
13	68.0	2.15	–
14	91.8	0.60	1.38
15	55.0	> 5	–
16	72.6	2.07	–
17	35.1	> 5	–
18	8.0	> 5	–
19	15.2	> 5	–
20	70.7	2.76	–
21	52.1	> 5	–
22	67.5	3.05	–
23	42.8	> 5	–
24	29.1	> 5	–
25	69.0	2.95	–
26	17.1	> 5	–
27	93.4	1.34	–
28	39.7	> 5	–
29	66.1	2.43	–
30	94.9	0.24	0.71

[a]Tetramethyleneglutaric acid.
[b]Each sample concentration was 5 μg/ml.

as expressed by IC_{50} values, were 0.15×10^{-6} M and 0.27×10^{-6} M, respectively.

Varma and Kinoshita (1976) have indicated some possible relationships of structure to the inhibiting potencies of flavones. The hydroxylation in the 4'-position has beneficial effects, and the abolition of the double bond between C-2 and C-3 leads to a decrease of inhibition. In support of these findings, the 4'-hydroxyl group (R_3 position of flavonoid in Table 1) of tested compounds was important for showing the inhibition of AR activity than the methoxy group at the same poisiton. The double bond of C-2,3 was also important in enhancing the activity.

Our previous study demonstrated that some synthetic chalcone derivatives were found to possess AR inhibition *in vitro* as well as inhibition in the accumulation of sorbitol *in vivo*. We postulated the possible relationships of structure to the inhibitory activities of flavonoids: 1) 4'-hydroxyl at the B ring show stronger activity; 2) chalcones having catechol moiety at the B ring (the 3,4-ortho-dihydroxyl moiety) show stronger activity; 3) chalcone shows a stronger activity than dihydrochalcone, in which 3,4,2',4'-tetrahydroxychalcone was the most potent inhibitor for aldose reductase (Lim *et al.*, 2000). In the present study, our results indicated that ring-closure form (quercetin-like structure) of 3,4,2',4'-tetrahydroxychalcone exhibit stronger activities than other flavonoids. Flavonols having 3-*O*-monosaccharide (quercitrin) also showed stronger inhibition than free flavonols at the 3-position (quercetin).

In conclusion, from *in vitro* data, quercitrin and reyneutrin were found to show potent inhibitory activities, therefore, these flavonoids could be offered as leading compounds for further study as a new drug for diabetic complications.

Acknowledgements

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