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INHIBITION OF LENS ALDOSE REDUCTASE BY FLAVONOIDS

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Key Word Index—Flavonoids; aldose reductase; inhibitors.

Abstract—The inhibitory activities of 73 flavonoids against rat aldose reductase were systematically investigated and cosmosiin, luteolin-7-glucuronide, lonicerin, 6-hydroxyluteolin, kaempferol-3-rhamnoside and avicularin were newly found to be highly active. The degree of inhibition appears to depend on the solvent system used. In general flavones are more active than flavonols and flavanones, glycosides are more active than aglycones, and the number of sugars present affects the activity.

INTRODUCTION

A high concentration of aldose in the human eye results in the accumulation of the corresponding polyol which appears to be responsible for the loss of clarity of the lens and this has been observed in experimental sugar cataracts [1]. Lens aldose reductase (AR) plays a central role in the reduction of aldose to polyol [2]. It has also been reported that cataract formation in diabetes and galactosemia is triggered by the accumulation in the lens of excessive sorbitol or dulcitol synthesized by the action of AR on glucose or galactose, respectively [3–5]. Recently, it has

Table 1. Inhibition of rat lens aldose reductase by flavones*

	Substituents							Inhibition (%)	
Compound	5	6	7	8	2′	3′	4′	10^{-5} M	10^{-6} M
Flavone type									
Flavone	—	—	—	—	—	—	—	8.3	0
Tectochrysin	OH	—	OMe	—	—	—	—	7.8	0
Cosmosiin	OH	—	O-R_1	—	—	—	OH	99.0	40.0
Rhoifolin	OH	—	O-R_2	—	—	—	OH	32.4	0
Linarin	OH	—	O-R_2	—	—	—	OMe	20.3	0
Linarin	OH	—	O-R'_2	—	—	—	OMe	18.2	0
Vitexin	OH	—	OH	C-R_1	—	—	OH	15.0	0
Baicalein	OH	OH	OH	—	—	—	—	61.9	0
Methylbaicalein	OMe	OMe	OMe	—	—	—	—	−4.2	0
Baicalin	OH	OH	O-R_1	—	—	—	—	41.7	0
Wogonin	OH	—	OH	OMe	—	—	—	13.1	0
Luteolin-7-R_1	OH	—	O-R_1	—	—	OH	OH	73.3	30.9
Luteolin-7-R_4	OH	—	O-R_4	—	—	OH	OH	88.2	68.0
Lonicerin	OH	—	O-R_2	—	—	OH	OH	91.5	55.6
Chrysoeriol	OH	—	OH	—	—	OMe	OH	31.3	0
Diosmetin	OH	—	OH	—	—	OH	OMe	16.7	0
Scutellarein	OH	OH	OH	—	—	—	OH	55.9	10.1
Methyl scutellarein	OMe	OMe	OMe	—	—	—	OMe	32.0	0
Sorbarin	OH	OH	O-R_3	—	—	—	OH	70.3	13.9
Pectolinarigenin	OH	OMe	OH	—	—	—	OMe	25.4	0
Pectolinarin	OH	OMe	O-R_2	—	—	—	OMe	41.5	0
Diacetyl cirsimaritin	OAc	OMe	OMe	—	—	—	OAc	34.7	0
6-Hydroxy luteolin	OH	OH	OH	—	—	OH	OH	86.7	53.3
Embinin	OH	C-R_2	OMe	—	—	—	OMe	12.7	0

*Assays were carried out as described in the Experimental Each compound was tested 3–5 times and the deviation from the figures listed was less than 5% R_1 = glucose, R_2 = rhamnose-glucose, R'_2 = R_2-4‴-OAc, R_3 = rhamnose, R_4 = glucuronic acid.

been suggested that the levels of AR and sorbitol dehydrogenase activities in whole lens are unaffected by diabetic conditions, but the distribution pattern in the lens of AR is different under normal and diabetic conditions [6].

Thus, inhibitors of AR would be expected to be effective in preventing cataract formation in diabetes. Some flavonoids are known to be highly potent inhibitors of lens AR [7–9]. In this paper, the inhibitory action of 73 flavonoids, 24 compounds of which were previously tested [7–9], was systematically tested on rat AR in order to determine structure–activity relationships.

RESULTS AND DISCUSSION

Inhibition of rat AR by 73 flavonoids was investigated. The flavonoids tested, apart from the compounds reported previously, and their inhibitory effects on rat AR (percentage of inhibition as compared to controls when the reaction was carried out without inhibitors) are presented in Tables 1 (flavones), 2 (flavonols) and 3 (isoflavones and other types). Cosmosiin (apigenin-7-glucoside), luteolin-7-glucuronide, lonicerin (luteolin-7-rutinoside), 6-hydroxyluteolin, kaempferol-3-rhamnoside and avicularin (quercetin-3-arabinoside) are newly shown

Table 2. Inhibition of rat lens aldose reductase by flavonols

	Substituents								Inhibition (%)	
Compound	3	5	6	7	2′	3′	4′	5′	10^{-5} M	10^{-6} M
Flavonol type										
Astragalin	O–R_1	OH	—	OH	—	—	OH	—	62 3	29.6
Kaempferol-3-R_3	O–R_3	OH	—	OH	—	—	OH	—	85 1	43.0
Kaempferol-3-R_5	O–R_5	OH	—	OH	—	—	OH	—	29.7	0
Juglanin	O–R_6	OH	—	OH	—	—	OH	—	64 8	6 3
Trifolin	O–R_7	OH	—	OH	—	—	OH	—	61 8	18 9
Populnin	OH	OH	—	O–R_1	—	—	OH	—	0 8	0
Kaempferol-7-R_3	OH	OH	—	O–R_3	—	—	OH	—	40.9	0
Rhamnetin	OH	OH	—	OMe	—	OH	OH	—	57.1	22 7
Isorhamnetin	OH	OH	—	OH	—	OMe	OH	—	8.9	0
Avicularin	O–R_6	OH	—	OH	—	OH	OH	—	84 0	32.4
Reynoutrin	O–R_8	OH	—	OH	—	OH	OH	—	71 0	33.8
Spiraeoside	OH	OH	—	OH	—	OH	O–R_1	—	51.2	8.9
Nelumboside	O–R_4–R_1	OH	—	OH	—	OH	OH	—	64 1	32.5
Quercimeritrin	OH	OH	—	O–R_1	—	OH	OH	—	44.4	19.6
Quercetagetin	OH	OH	OH	OH	—	OH	OH	—	71.4	29.1
Chrysosplenol B	OMe	OH	OMe	OMe	—	OMe	OH	—	27 6	0
Chrysosplenoside B	OMe	OH	OMe	OMe	—	OMe	O–R_1	—	0 6	0
Chrysosplenoside D	OMe	OH	OMe	OMe	—	OH	O–R_1	—	27.3	0
Oxyayanin A	OMe	OH	—	OMe	OH	—	OMe	OH	27.3	0
Chrysosplenoside A	OMe	OH	—	OMe	O–R_1	—	OMe	OH	14.7	0

Details of the experiments as in Table 1 R_5 = neohesperidose, R_6 = arabinose, R_7 = galactose, R_8 = xylose.

Table 3. Inhibition of rat lens aldose reductase by isoflavones and other types

	Substituents								Inhibition (%)	
Compound	5	6	7	8	2′	3′	4′	5′	10^{-5} M	10^{-6} M
Isoflavones										
Genistein	OH	—	OH	—	—	—	OH	—	50.3	0
Iridine	OH	OMe	O–R_1	—	—	OH	OMe	OMe	20.6	0
Sophoricoside	OH		OH		—	—	O–R_1	—	18.2	0
Flavanone										
Naringenin	OH	—	OH	—	—	—	OH	—	77.6	25.0
Biflavone										
Amentoflavone				apigenin (5′ — 8‴) apigenin					25.9	0

Details of the experiments as in Table 1.

to possess considerable activity (more than 80% at 10^{-5} M). In addition, quercitrin, isoquercitrin, orientin, hyperin, myricitrin and chrysosplenol D were also very active, which confirms earlier findings [7–9].

The results of our study allow us to draw the following conclusions. Some flavonoids show varying activities according to the solvent used (Table 4). In particular, apigenin dissolved in DMSO or propyleneglycol showed no activity, but high activity in other solvents [8]. Flavones are usually more active than the corresponding flavonols and flavanones (Fig. 1). Monoglycosides have a higher activity than the corresponding aglycones and the number of sugar substituents effects the activity (Table 5). Exceptionally, luteolin is more inhibitory than its glycosides.

Table 4. Flavonoids showing different activity according to the solvent (10^{-5} M)

	Solvent		
Compound	DMSO	Na_2CO_3–NaOH	Propylene glycol
Apigenin	20.3	84.2 (90)*	31.0
Cirsimaritin	26.2	23.0	31.0 (76)†
Cirsiliol	63.1	62.0	30 1 (89)†
Quercetin	54.8	84.3 (83)*	49.0

*According to Varma *et al.* dissolved by first suspending each sample in water and then adding a 2% solution of Na_2CO_3 in 0.1 N NaOH in small increments. When the solution was complete, the pH was adjusted to 7.0.

†According to Okuda *et al* [9].

EXPERIMENTAL

Material. Most flavonoids were obtained from plant sources by us. All flavonoids were dissolved in DMSO, which was found to have no effect on enzyme activity at below 0.1% concentration Shambhu *et al.* [8] dissolved flavonoids in alkali and then adjusted the pH to 7.0, but we found this affected the results with some flavonoids. Propylene glycol was also used, as in the experiments of Okuda *et al* [9], but it is not a very good solvent for these substances.

Preparation of crude rat lens aldose reductase (AR). Lens were removed from eyes of 10 male rats of the Wistar strain weighing 150–200 g and homogenized in 2 ml cold sodium phosphate buffer (pH 6.2) containing 1 mM mercaptoethanol and 1 mM NADP. The homogenate was centrifuged at 10000 *g* for 15 min at 4° and the supernatant was stored at 0° until needed.

Assay of inhibition. Assays were performed at room temperature in 0.1 M sodium phosphate buffer (pH 6.2), 0.104 mM NADPH, 10 mM DL-glyceraldehyde and 25 μl AR solution in a total volume of 1.5 ml. The reference blank to correct for nonspecific reduction of NADPH contained all the above compounds except the substrate. The reaction was initiated by the addition of AR and the rate of NADPH oxidation was followed by recording the decrease in absorbance at 340 nm. The effects of inhibitors on the enzyme activity were determined by

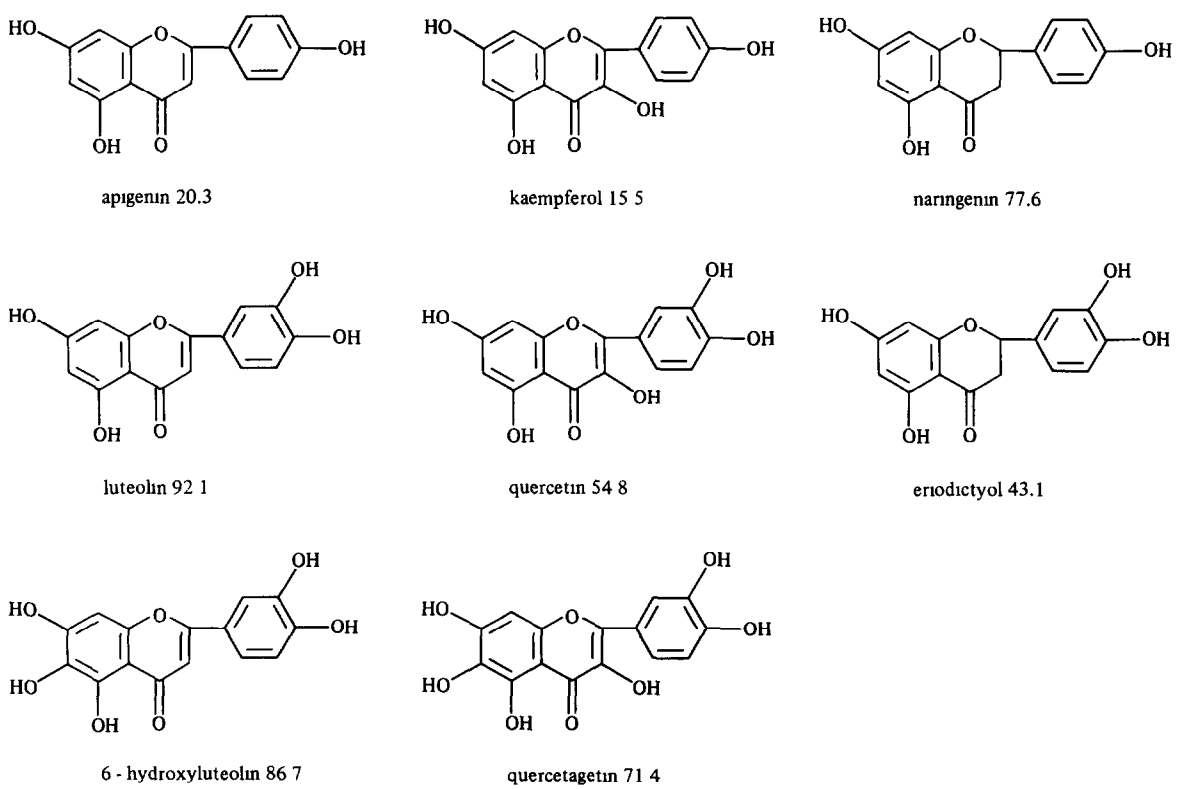

Fig. 1. Relative inhibitory activities of flavones, flavonols and flavanones.

Table 5. Effect of sugar substitution on inhibitory activity

Aglycone	Monosaccharide		Oligosaccharide
Apigenin 20.3	7-glucoside (cosmosiin) 99.0	8-*C*-glucoside (vitexin) 15.0	7-rutinoside (rhoifolin) 32.4 7-apiosylglucoside (apiin) 75.0
Luteolin 92.1	7-glucoside 73.3 7-glucuronide 88.2	8-*C*-glucoside (orientin) 95.0	7-rutinoside (lonicerin) 91.5
Kaempferol 15.5	3-glucoside (astragalin) 62.3 3-rhamnoside 85.1 3-galactoside (trifolin) 61.8	3-arabinoside (juglanin) 64.8 7-glucoside (populnin) 0.8 7-rhamnoside 40.9	3-neohesperidoside 29.7 7-rhamno-3-galactorhamnoside (robinin) 20.3
Quercetin 54.8	3-glucoside (isoquercitrin) 82.1 3-rhamnoside (quercitrin) 95.9 3-galactoside (hyperin) 92.8 3-arabinoside (avicularin) 84.0	3-xyloside (reynoutrin) 71.0 4′-glucoside (spiraeoside) 51.2 7-glucoside (quercimeritrin) 44.4	3-glucoglucuronide (nelumboside) 64.1 3-rutinoside (rutin) 75.4

including in the reaction mixture 15 μl of each compound being tested at different concentrations. The inhibitory activity was expressed as the rate of $A_{340\,nm}$ due to utilization of NADPH.

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