Structure–Activity Correlation of Flavonoids for Inhibition of Bovine Lens Aldose Reductase

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To clarify the structure–activity correlation of flavonoids for inhibition of aldose reductase, about fifty flavonoid compounds were screened. The presence of hydrophobic substituents on the A ring and hydrophilic substituents on the B ring of the flavonoid skeleton was suggested to improve the potency of inhibitory activity. The activities of extracts of *Scutellaria baicalensis*, *Andrographis paniculata* and *Gutierrezia microcephala* are also described.

Keywords aldose reductase inhibitor; flavonoid; structure–activity correlation; *Scutellaria baicalensis*; *Andrographis paniculata*; *Gutierrezia microcephala*

Since the mid-1970's, several papers on the inhibition of aldose reductase by flavonoid compounds have been published.[1–4] These papers generally discussed the structure–activity relationship only for compounds with hydroxyl and/or methoxyl group(s) at limited positions on the flavonoid nucleus. Thus, a broad, general view has not been obtained. To make matters worse, the nomenclature of flavonoid ring systems in those papers is often different from that currently used, raising the possibility of confusion. In the present study, flavonoid compounds not examined in the previous papers were tested for inhibitory activity against bovine lens aldose reductase and a broad structure–activity correlation was deduced.

Materials and Methods

Materials All flavonoid compounds in the present experiment except isoflavones and pterocarpans were obtained by synthesis. Some compounds related to naturally occurring flavonoids have been reported; **1**[5]; **7**, **8**, **9**, **11**, **23** and **24**[6]; **10**, **14**, **22**, **25**, **26**, **27** and **28**[7]; **29** and **30**[8]; **31** and **32**[9]; **33**, **34** and **35**[10]; **40** and **41**.[11] Isoflavones (**42** and **43**) and pterocarpans (**44** and **45**) isolated from the roots of *Euchresta japonica* according to Shirataki *et al.*[12] and **46** from *E. horsfieldii* were used.

Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Oriental Yeast Co., Ltd. (Osaka, Japan). DL-Glyceraldehyde and ammonium sulfate were from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Quercitrin was from Nakarai Chemicals Ltd. (Kyoto, Japan), and 3,3-tetramethyleneglutaric acid was from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Methods Purification of Aldose Reductase from Bovine Lenses: Bovine eyes were obtained from a local abattoir, and lenses were removed and frozen until needed. Aldose reductase was purified according to the method of Inagaki *et al.*[13] The enzyme was homogeneous on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis.

Assay of Aldose Reductase Activity: Aldose reductase assays were conducted according to the procedure of Inagaki *et al.*[14] except for the reaction temperature (37 °C instead of 30 °C). Assays were performed in 0.1 M sodium phosphate buffer (pH 6.2) containing 0.4 M ammonium sulfate, 10 mM DL-glyceraldehyde, 0.16 mM NADPH and the enzyme (10 mV/tube) in a total volume of 2.0 ml. The inhibitory effect of test compounds was determined by including 20 μl of inhibitor solution in the reaction mixture. The reaction was initiated by the addition of substrate, and the rate of NADPH oxidation was followed by recording the decrease in absorbance at 340 nm on Hitachi model U-2000 spectrophotometer.

Results and Discussion

Inhibitory activities of chalcones, flavones including flavanones and flavonols, and other phenolic compounds (isoflavones, pterocarpans, *etc.*) against bovine lens aldose reductase are shown in Tables I, II and III. The inhibitory activities for quercitrin and 3,3-tetramethyleneglutaric acid (TMGA) obtained in the present experiment seem to be a little lower than values reported previously.[2,3]

Generally the presence of hydroxyl groups, in particular, on the B ring moiety is essential for inhibitory activity against aldose reductase. In spite of the presence of two hydroxyl groups located at C-2 and C-2′ compound **1** showed no activity, but this may be because of hydrogen bonding between a carbonyl group and the hydroxyl group at C-2′. Introduction of another hydroxyl group into the β-position, as in **2**, results in keto-enol tautomerism as shown in Chart 2, and **2** showed a moderate inhibitory

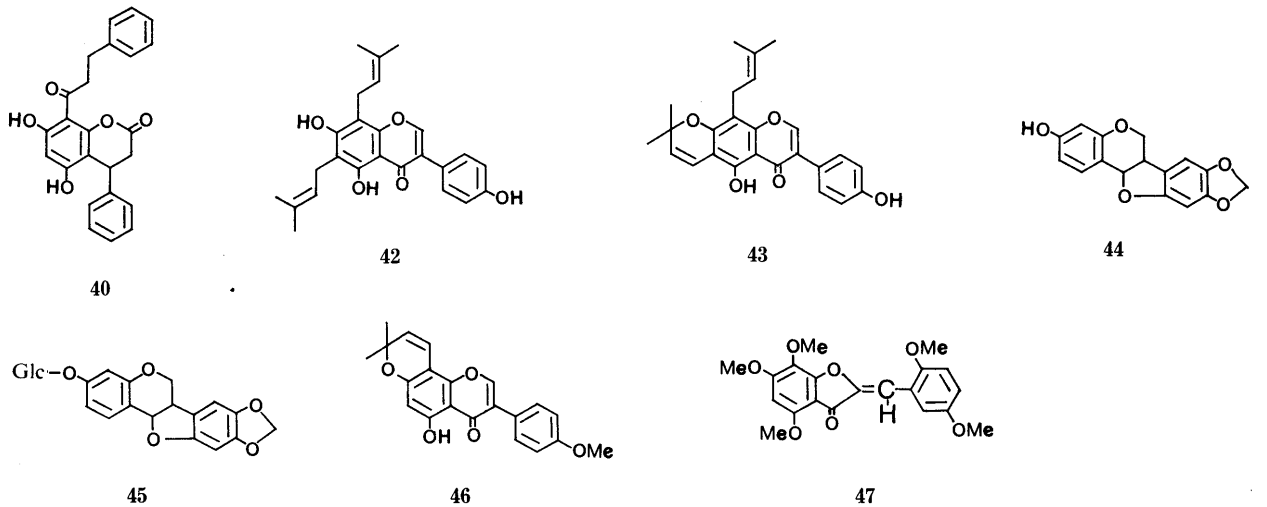
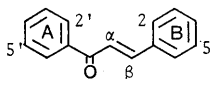


Chart 1

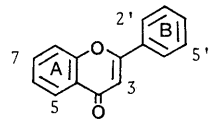
TABLE I. Inhibition of Bovine Lens Aldose Reductase by Chalcone Derivatives



	Percentage of inhibition	
	1×10^{-5} M	1×10^{-7} M
2,2′-Dihydroxy- (**1**)	0	0
2,2′,β-Trihydroxy- (**2**)	15	3
2′,4′,6′-Trihydroxy- (**3**)	5	0
2′,4′,6′-Trihydroxy-[a] (**4**)	3	0
2′-Hydroxy-4′,6′-dimethoxy- (**5**)	11	1
3,4-Dibenzyloxy-2′-hydroxy-4′-methoxy- (**6**)	0	0
4,5′-Dibenzyloxy-2′-hydroxy-3,4′,6′-trimethoxy- (**7**)	0	0
5′-Benzyloxy-2′-hydroxy-3,4,4′,6′-tetramethoxy- (**8**)	5	0
5′-Benzyloxy-2′-hydroxy-3,4-methylenedioxy-4′,6′-dimethoxy- (**9**)	0	0
2′-Hydroxy-2,4-diisopropyloxy-3′,4′,6′-trimethoxy- (**10**)	12	0
3,4-Methylenedioxy-2′,4′,5′,6′-tetramethoxy- (**11**)	0	0
2,5,2′-Trihydroxy-4′,6′-dimethoxy- (**12**)	4	0
2,5,2′-Trihydroxy-4′,6′-dimethoxy-[a] (**13**)	3	0
2′-Hydroxy-2,5,3′,4′,6′-pentamethoxy- (**14**)	28	10
2,4′-Dibenzyloxy-2′-hydroxy-3,3′,5′,6′-tetramethoxy- (**15**)	0	0
4-Benzyloxy-2′-hydroxy-3,5,4′,5′,6′-pentamethoxy- (**16**)	0	0
2′-Hydroxy-2,4,5,4′,5′,6′-hexamethoxy- (**17**)	37	2
2′-Hydroxy-2,4,6,4′,5′,6′-hexamethoxy- (**18**)	2	3

a) Dihydrochalcone.

TABLE II. Inhibition of Bovine Lens Aldose Reductase by Flavone Derivatives



	Percentage of inhibition	
	1×10^{-5} M	1×10^{-7} M
3′,4′-Dibenzyloxy-7-methoxy-[a] (**19**)	0	0
6-Benzyloxy-3′,4′-methylenedioxy-5,7-dimethoxy-[a] (**20**)	0	2
6-Benzyloxy-5,7,3′,4′-tetramethoxy- (**21**)	0	0
2′,5′-Diisopropyloxy-5,7,8-trimethoxy- (**22**)	32	0
6-Hydroxy-5,7,3′,4′-tetramethoxy-[a] (**23**)	1	0
6,4′-Dihydroxy-5,7,3′-trimethoxy-[a] (**24**)	0	0
5,2′,4′-Trihydroxy-6,7-dimethoxy- (**25**)	32	1
5,2′,4′-Trihydroxy-6,8-dimethoxy- (**26**)	21	3
5,2′,5′-Trihydroxy-6,7-dimethoxy- (**27**)	79	6
5, 2′,5′-Trihydroxy-7,8-dimethoxy- (**28**)	72	4
5, 4′-Dihydroxy-6,7,3′,5′-tetramethoxy- (**29**)	6	0
4′-Hydroxy-5,6,7,3′,5′-pentamethoxy- (**30**)	1	0
3,5,2′,4′-Tetrahydroxy-7,8,5′-trimethoxy- (**31**)	58	0
5,2′,4′-Trihydroxy-3,7,8,5′-tetramethoxy- (**32**)	65	3
3,7,2′-Trihydroxy-5,6,8,4′,5′-pentamethoxy- (**33**)	20	0
5,7,2′-Trihydroxy-3,6,8,4′,5′-pentamethoxy- (**34**)	34	0
5,7,5′-Trihydroxy-3,6,8,2′,4′-pentamethoxy- (**35**)	34	0
5,7,3′,4′-Tetrahydroxy-3-*O*-rhamnoside (quercitrin)	47	4

a) Dihydroderivative, that is, flavanone.

activity. Saturation of the α,β double bond did not necessarily increase the activity, as observed in **4** in comparison with **3**. This is seen in the relation between a flavone and the corresponding flavanone (**12** *vs.* **13**); they show a similar activity so long as the substitution pattern is the same. The chalcones di- or tri-substituted by methoxyl groups on the A ring, such as **5**, **14**, **17** and **18** showed rather high activities in spite of the absence of hydroxyl groups on the B ring; this can be explained by the torsion of the A ring cause by the electron-withdrawing effect of the methoxyl groups. In the above chalcones, the substituents on the B ring play a significant role in the activity. The chalcones possessing a methylenedioxy group at C-3 and **4**, such as **9** and **11**, or a benzyloxyl group at C-2 or C-4, such as **15** and **16**, showed no activity.

TABLE III. Inhibition of Bovine Lens Aldose Reductase by Some Phenolic Compounds

	Percentage of inhibition	
	1×10^{-5} M	1×10^{-7} M
2-Benzyloxy-3,5-dimethoxybenzaldehyde (**36**)	0	1
4-Benzyloxy-2,5-dimethoxybenzaldehyde (**37**)	0	0
4-Benzyloxy-3,5-dimethoxybenzoic acid (**38**)	3	0
2,4,5-Trimethoxybenzoic acid (**39**)	2	1
8-Dihydrocinnamoyl-5,7-dihydroxy-dihydroneoflavone (**40**)	45	0
6-Cinnamoyl-5-hydroxy-7-methoxy-dihydroneoflavone (**41**)	16	2
6,8-Di(γ,γ-dimethylallyl)genistein (**42**)	60	1
Warangalone (**43**)	57	14
1-Maackiain (**44**)	10	0
Trifolirhizin (**45**)	7	0
Derrone-4′-*O*-methyl ether (**46**)	0	0
5,7,8,2′,5′-Pentamethoxyaurone (**47**)	45	0
3,3-Tetramethyleneglutaric acid (TMGA)	52	5

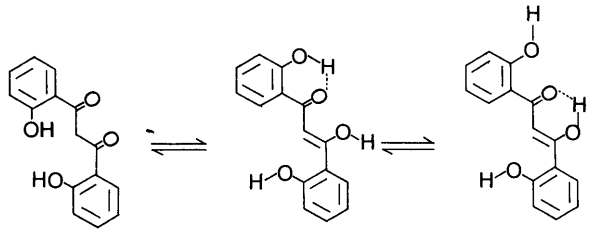


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Chart 2

In the case of flavanones and flavones, the number of methoxyl groups on the A ring was closely related with the activity. Substitutional isomerism, for example, of trimethoxylated flavones, 5,6,7- (**25** and **27**), 5,6,8- (**26**) and 5,7,8- (**28**), seemed not to influence the inhibitory activity. A hydroxyl group located at C-6 (**23**) and/or C-7 (**33**) on the A ring did not decrease the activity so long as it was surrounded by other methoxyl groups. As mentioned above, methylation, benzylation and isopropylation of hydroxyl groups on the B ring, in other words, the presence of methoxyl, benzyloxyl and isopropyloxyl groups on the ring, greatly diminished the activity and sometimes resulted in inactivity. The location of hydroxyl groups on the B ring is another important factor determining the activity. In the previous papers,[2,3] 4′-hydroxylated and 3′,4′-dihydroxylated B ring compounds were mainly discussed. The flavones hydroxylated at C-2′,4′ (**25**, **26** and **32**) and C-2′,5′ (**27** and **28**) were found to possess the activity. When the

number of methoxyl groups is greater than that of hydroxyl groups on the B ring, such as in **29**, **30** and **33**, the activity was clearly attenuated. Introduction of an O-function at C-3, which converts a flavone into a flavonol, does not always increase the activity, nor does a methoxyl group at the same position decrease it. From the results described above, it appears that suitable substituents on flavones or flavanones for high inhibitory activity against aldose reductase are as follows: the A ring must be hydrophobic, which will be satisfied by the presence of methoxyl groups or a γ,γ-dimethylallyl group(s), and the B ring must be hydrophilic, *e.g.*, substituted with hydroxyl groups. The rather high inhibitory activities of axillarin (5,6,3′,4′-tetrahydroxy-3,6-dimethoxyflavone),[3] LAR-1 (6,3′,4′-trihydroxy-5,7,8-trimethoxyflavone)[3] and LAR-2 (4′-hydroxy-5,6,7,8-tetramethoxyflavone),[3] the last of which was isolated from the peel of *Citrus reticulata* (Rutaceae)[15] can be explained by the above structure–activity correlations.

Introduction of lipophilic residues such as dihydrocinnamoyl or cinnamoyl groups on the A ring, as in **40** and **41**, conferred moderate activity. Furthermore, introduction of a γ,γ-dimethylallyl group(s) into the A ring of isoflavone nucleus such as **42** and **43** resulted in a much stronger inhibitory effect as compared with the isoflavones previously reported.[2] As observed in **46**, the presence of a methoxyl group at C-4′ attenuated the activity. The pterocarpans (**44** and **45**) used in the present experiment exhibited very low activity because of the presence of a methylenedioxy group on the B ring and a hydroxyl or a glycoxyl group on the A ring. In the structure–activity correlation of flavonoid compounds for inhibitory activity against aldose reductase, the important factors determining the activity are the number and kind of hydrophobic substituents on the A ring and those of hydrophilic substituents on the B ring.

The crude extracts of three plants were examined to see whether they contain active principles. These plants contain flavones and flavonols with highly methoxylated A ring. As for the B ring, the flavone in *Scutellaria baicalensis* (Labiatae) is characterized by a 2′,3′,6′-trioxygenated moiety,[16,17] the flavones and flavonols in *Gutierrezia microcephala* (Compositae) by a 2′,4′,5′-trioxygenated moiety,[17,18] and the flavones in *Andrographis paniculata* (Acanthaceae) by a 2′,3′,4′-trioxygenated moiety.[17,19] The extracts of *S. baicalensis* and *A. paniculata* did not show high activities (Table IV), but the extract of *C. microcephala*, in particular, its *n*-hexane extract, showed a rather strong inhibitory effect in comparison with that of quercitrin.

The aldose reductase-inhibitory principle(s) contained in the *n*-hexane extract of *G. microcephala* are now being researched.

TABLE IV. Inhibition of Bovine Lens Aldose Reductase by Extracts of Some Plants

	Percentage of inhibition	
	1×10^{-5} M	1×10^{-7} M
Scutellaria baicalensis		
n-Hexane ext.	25	0
Acetone ext.	14	1
Methanol ext.	42	1
Gutierrezia microcephala		
n-Hexane ext.	84	2
Acetone ext.	45	3
Methanol ext.	59	1
Andrographys paniculata		
n-Hexane ext.	10	0
Acetone ext.	0	0
Quercitrin	43	0

References

1) S. D. Varma, I. Mikuni and J. H. Kinoshita, *Science*, **188**, 1251 (1975).
2) S. D. Varma and J. H. Kinoshita, *Biochem. Pharmacol.*, **25**, 2505 (1976).
3) J. Okuda, I. Miwa, K. Inagaki, T. Horie and M. Nakayama, *Biochem. Pharmacol.*, **31**, 3807 (1982).
4) J. Okuda, I. Miwa, K. Inagaki, T. Horie and M. Nakayama, *Chem. Pharm. Bull.*, **32**, 767 (1984).
5) E. Wollenweber, K. Mann, M. Iinuma, T. Tanaka and M. Mizuno, *Phytochemistry*, **28**, 295 (1989).
6) M. Iinuma, S. Matsuura and T. Tanaka, *Chem. Pharm. Bull.*, **33**, 3982 (1985).
7) M. Iinuma, T. Tanaka, M. Mizuno and Z. Min, *Chem. Pharm. Bull.*, **33**, 3982 (1985).
8) M. Iinuma, T. Tanaka and S. Matsuura, *Chem. Pharm. Bull.*, **32**, 2296 (1984).
9) M. Iinuma, K. Iwashima and S. Matsuura, *Chem. Pharm. Bull.*, **32**, 4935 (1984).
10) M. Iinuma, T. Tanaka, M. Mizuno and T. J. Mabry, *Chem. Pharm. Bull.*, **34**, 2228 (1986).
11) M. Iinuma, S. Matsuura and F. Asai, *Heterocycles*, **20**, 1923 (1983).
12) Y. Shirataki, A. Manaka, I. Yokoe and M. Komatsu, *Phytochemistry*, **21**, 2959 (1982).
13) K. Inagaki, I. Miwa and J. Okuda, *Arch. Biochem. Biophys.*, **216**, 337 (1982).
14) K. Inagaki, I. Miwa, T. Yashiro and J. Okuda, *Chem. Pharm. Bull.*, **30**, 3244 (1982).
15) M. Iinuma, S. Matsuura, K. Kurogochi and T. Tanaka, *Chem. Pharm. Bull.*, **28**, 717 (1980).
16) T. Tomimori, Y. Miyaichi, Y. Imoto, H. Kizu and T. Tanabe, *Yakugaku Zasshi*, **104**, 524 (1984); Y. Kimura, H. Okuda, Z. Taira, N. Shoji, T. Takemoto and S. Arichi, *Planta Medica*, **51**, 290 (1984).
17) M. Iinuma and M. Mizuno, *Phytochemistry*, **28**, 681 (1989).
18) N. Fang, M. Leidig, T. J. Mabry and M. Iinuma, *Phytochemistry*, **24**, 3029 (1985); N. Fang, M. Leidig and T. J. Mabry, *ibid.*, **25**, 927 (1986).
19) M.Kuroyanagi, M. Sato, A. Ueno and K. Kishi, *Chem. Pharm. Bull.*, **35**, 4429 (1987).