Structural Requirements of Flavonoids and Related Compounds for Aldose Reductase Inhibitory Activity

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The methanolic extracts of several natural medicines and medicinal foodstuffs were found to show an inhibitory effect on rat lens aldose reductase. In most cases, flavonoids were isolated as the active constituents by bioassay-guided separation, and among them, quercitrin (IC_{50}=0.15 μM), guaijaverin (0.18 μM), and desmanthin-1 (0.082 μM) exhibited potent inhibitory activity. Desmanthin-1 showed the most potent activity, which was equivalent to that of a commercial synthetic aldose reductase inhibitor, epalrestat (0.072 μM). In order to clarify the structural requirements of flavonoids for aldose reductase inhibitory activity, various flavonoids and related compounds were examined. The results suggested the following structural requirements of flavonoid: 1) the flavones and flavonols having the 7-hydroxyl and/or catechol moiety at the B ring (the 3′,4′-dihydroxyl moiety) exhibit the strong activity; 2) the 5-hydroxyl moiety does not affect the activity; 3) the 3-hydroxyl and 7-*O*-glucosyl moieties reduce the activity; 4) the 2–3 double bond enhances the activity; 5) the flavones and flavonols having the catechol moiety at the B ring exhibit stronger activity than those having the pyrogallol moiety (the 3′,4′,5′-trihydroxyl moiety).

Key words aldose reductase inhibitor; flavonoid; structural requirement; desmanthin-1; quercitrin; guaijaverin

Aldose reductase as a key enzyme in the polyol pathway is reported to catalyze the reduction of glucose to sorbitol. In normal tissue, aldose reductase has low substrate affinity to glucose, so that the conversion of glucose to sorbitol is little catalyzed. However, in diabetes mellitus, the increased availability of glucose in insulin-insensitive tissues such as lens, nerve, and retina leads to the increased formation of sorbitol through the polyol pathway. Sorbitol does not readily diffuse across cell membranes and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataract, neuropathy, and retinopathy. These findings suggest that aldose reductase inhibitor prevents the conversion of glucose to sorbitol and may have the capacity of preventing and/or treating several diabetic complications.[1)]

In the course of our characterization studies on antidiabetic principles of natural medicines,[2—6)] we have found that the methanolic extracts of several natural medicines and medicinal foodstuffs, such as *Chrysanthemum* (*C.*) *indicum*,[7—9)] *C. morifolium*,[10)] *Prunus mume*,[10)] *Myrcia multiflora*,[3,6)] *Centella asiatica*,[11)] and *Salacia* (*S.*) *reticulata*, *S. oblonga*, and *S. chinensis*[12,13)] exhibited potent inhibitory activity against rat lens aldose reductase. By bioassay-guided separation on the above extracts, several flavonoids were isolated as active components. Since the mid.-1970's, several studies on the inhibition of aldose reductase by flavonoids have been reported.[14—16)] However, their structure–activity relationships were not discussed satisfactorily because of the limited number of compounds. In the present study, 94 flavonoids and stilbenes were examined to clarify the further structural requirements of flavonoids for aldose reductase inhibitory activity.

Inhibitory Effects of Several Methanolic Extracts on Rat Lens Aldose Reductase The methanolic extracts of several natural medicines and medicinal foodstuffs were examined for rat lens aldose reductase inhibitory activity. Among them, the flowers of *Chrysanthemum* (*C.*) *indicum*,[7—9)] *C. morifolium* (Compositae),[10)] and *Prunus mume* (Rosaceae),[10)] the leaves of *Myrcia multiflora* (Myrtaceae),[3,6)] the aerial parts of *Centella asiatica* (Umbelliferae),[11)] and the stems of *Salacia* (*S.*) *reticulata*, *S. oblonga*, and *S. chinensis* (Hippocrateaceae)[12,13)] exhibited potent inhibitory activity as shown in Table 1.

Preparation of Flavonoids and Related Compounds From the above-mentioned active extracts, various flavonoid constituents were commonly isolated as active compounds. To clarify the structural requirements of flavonoids for rat lens aldose reductase inhibitory activity, the following flavonoids and related compounds were prepared: tectochrysin (**4**) and izalpinin (**21**) from the fruit of *Alpinia oxyphylla*;[17,18)] apigenin 7-*O*-β-D-glucopyranoside (**10**), acacetin 7-*O*-(6″-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**11**), luteolin (**12**), luteolin 7-*O*-β-D-glucopyranoside (**17**), luteolin 7-*O*-β-D-glucopyranosiduronic acid (**18**), diosmetin 7-*O*-β-D-glucopyranoside (**19**), quercetin 3,7-di-*O*-β-D-glucopyranoside (**37**), and (2*S*)- and (2*R*)-eriodictyol 7-*O*-β-D-glucopyranosiduronic acids (**63**, **64**) from the flowers of *Chrysanthemum indicum*;[7—9)] kaempferol 3-*O*-β-D-glucopyranosiduronic acid (**23**) from the aerial parts of *Centella asiatica*;[11)] isoquercitrin (**33**) and quercitrin (**35**) from the roasted leaves of *Apocynum venetum*;[19)] rutin (**38**) from the flowers of *Sophora japonica*;[20)] guaijaverin (**36**), mearncitrin (**51**), and desmanthin-1 (**56**) from the leaves of *Myrcia multiflora*;[3,6)]

Table 1. Inhibitory Activity of Methanolic Extracts of Several Natural Medicines and Medicinal Foodstuffs on Rat Lens Aldose Reductase

MeOH ext.	IC_{50} (μg/ml)
Chrysanthemum indicum (Flowers)	3.5[7)]
Chrysanthemum morifolium (Flowers)	2.6
Prunus mume (Flowers)	3.0
Myrcia multiflora (Leaves)	1.1[3)]
Centella asiatica (Aerial parts)	0.8[11)]
Salacia reticulata (Stems)[a)]	36[13)]
Salacia oblonga (Stems)[a)]	3.46[13)]
Salacia chinensis (Stems)[a)]	3.66[13)]

The above natural medicines and medicinal foodstuffs were extracted with methanol under reflux 3 h×3 times. *a*) Extracted with 80% aqueous methanol.

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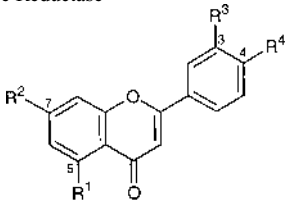
Table 2. Inhibitory Activity of Flavones for Rat Lens Aldose Reductase



	R^1	R^2	R^3	R^4	IC_{50} (μM)
Flavone (**1**)	H	H	H	H	>100 (16)##
7-Hydroxyflavone (**2**)	H	OH	H	H	10
Chrysin (**3**)	OH	OH	H	H	8.5
Tectochrysin (**4**)	OH	OCH_3	H	H	>100 (34)##
4′,7-Dihydroxyflavone (**5**)	H	OH	H	OH	3.8
3′,4′-Dihydroxyflavone (**6**)	H	H	OH	OH	0.37
3′,4′,7-Trihydroxyflavone (**7**)	H	OH	OH	OH	0.30
Apigenin (**8**)	OH	OH	H	OH	2.2
9	OH	OCH_3	H	OCH_3	>30 (39)#
Apigenin 7-*O*-Glc (**10**)	OH	O-Glc	H	OH	23[9]
Acacetin 7-*O*-Rut (**11**)	OH	O-Glc(6→1)Rha	H	OCH_3	4.7[7]
Luteolin (**12**)	OH	OH	OH	OH	0.45[7]
Diosmetin (**13**)	OH	OH	OH	OCH_3	8.5
Pilloin (**14**)	OH	OCH_3	OH	OCH_3	12
15	OH	OCH_3	OCH_3	OCH_3	72
16	OCH_3	OCH_3	OCH_3	OCH_3	>30 (30)#
Luteolin 7-*O*-Glc (**17**)	OH	O-Glc	OH	OH	0.99[7]
Luteolin 7-*O*-GlcA (**18**)	OH	O-GlcA	OH	OH	3.1[7]
Diosmetin 7-*O*-Glc (**19**)	OH	O-Glc	OH	OCH_3	23[9]

Glc: β-D-glucopyranosyl; GlcA: β-D-glucopyranosiduronic acid; Rha: α-L-rhamnopyranosyl Rut: Glc(6→1)Rha. Values in parentheses represent the inhibition (%) at # 30 μM and ## 100 μM.

myricetin (**43**) and myricitrin (**50**) from the stems of *Myrica rubra*;[21] liquiritigenin (**58**) and liquiritin (**60**) from the roots of *Glycyrrhiza uralensis*;[22] daidzein (**66**), daidzin (**67**), genistein (**68**), and genistin (**69**) from the seeds of *Glycine max*[23]; tectorigenin (**70**), tectoridin (**71**), tectorigenin 7-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**72**), glycitein (**73**), glycitin (**74**), glycitein 7-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**75**), and puerarin (**76**) from the flowers of *Pueraria thunbergiana*;[24] (+)-catechin (**78**) and (−)-epicatechin (**79**) from the flowers of *Camellia japonica*;[25] and (−)-epigallocatechin (**80**) from *Salacia reticulata*.[12]

Stilbenes were obtained from the rhizome of *Rheum undulatum*.[26—28]

Apigenin (**8**),[29] diosmetin (**13**),[29] kaempferol (**22**),[29] quercetin (**24**),[29] **30**,[30] and **48**[31] were derived by methanolysis of **10**, **19**, **23**, **38**, **41**,[32] and **54**, respectively.

The following derivatives were prepared by diazomethane methylation: **9**[33] was prepared from **8**; pilloin (**14**)[34] and **15**[35] from luteolin (**12**); rhamnetin (**25**),[36] tamarixetin (**26**),[37] **27**,[38] ombuine (**28**),[36,37] ayanin (**29**),[37] and **31**[39] from **24**; rhamnetin 3-*O*-rutinoside (**39**),[40] ombuine 3-*O*-rutinoside (**40**),[41] and **41**[32] from **38**; mearnsetin (**44**),[42] **45**,[43] **46**,[44] and **47**[45] from **43**; **52**—**55** from **50**; **61** from **60**.

Compounds **16**,[46] **32**,[47] and **49**[43] were derived by methyl iodide (CH_3I) methylation of **12**, **24**, and **43**, respectively.

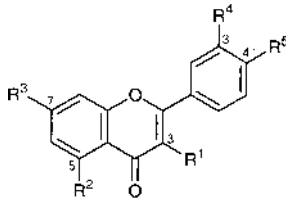
Compounds **59**[48] and **62**[9] were prepared by enzymatic hydrolysis of **61** and the mixture of **63** and **64** (*ca.* 1 : 1) with β-glucuronidase.

The following flavonoids were purchased from Funakoshi Co., Ltd., (Tokyo, Japan): flavone (**1**), 7-hydroxyflavone (**2**), chrysin (**3**), 4′,7-dihydroxyflavone (**5**), 3′,4′-dihydroxyflavone (**6**), 3′,4′,7-trihydroxyflavone (**7**), 3-hydroxyflavone (**20**), hyperin (**34**), flavanone (**57**), fustin (**65**), and biochanin A (**77**); from Wako Pure Industries Ltd., (Osaka, Japan): fisetin (**42**).

Structural Requirement of Flavonoids and Related Compounds for Rat Lens Aldose Reductase Inhibitory Activity As shown in Table 2, 3′,4′-dihydroxyflavone (**6**, IC_{50}=0.37 μM), 3′,4′,7-trihydroxyflavone (**7**, 0.30 μM), luteolin (**12**, 0.45 μM), and luteolin 7-*O*-β-D-glucopyranoside (**17**, 0.99 μM) were potent inhibitors among the flavone constituents, while flavone (**1**, >100 μM) and tectochrysin (**4**, >100 μM) lacked the activity. The activities of flavones lacking the 5-hydroxyl group were equipotent to those of 5-hydroxyl flavones [ex. 7-hydroxyflavone (**2**, 10 μM)≒chrysin (**3**, 8.5 μM); 4′,7-dihydroxyflavone (**5**, 3.8 μM)≒apigenin (**8**, 2.2 μM)]. The activities of 7-*O*-glucosyl flavones were weaker than those of aglycons [ex. apigenin 7-*O*-β-D-glucopyranoside (**10**, 23 μM)<**8**; **17**<**12**; diosmetin 7-*O*-β-D-glucopyranoside (**19**, 23 μM)<diosmetin (**13**, 8.5 μM)]. In addition, the activities of the flavones having a catechol moiety at the B ring (the 3′,4′-dihydroxyl moiety) were stronger than those of monohydroxyl, mono- or dimethylated compounds [ex. **6** and **7**>**5**; **12**>**8** and **13**; **17**>**19**].

Next, various flavonols, flavanones, and a dihydroflavonol were examined, and the results are shown in Tables 3—5. Compounds **27** (0.82 μM), quercitrin (**35**, 0.15 μM), guaijaverin (**36**, 0.18 μM), and desmanthin-1 (**56**, 0.082 μM) were found to show potent inhibition, and these results also support the above suggestion. In addition, the activities of the flavonols (having the 3-hydroxyl group) and flavanones (single bond at the 2–3 position) were reduced as compared with their corresponding flavones [ex. **8**>kaempferol (**22**, 10 μM);

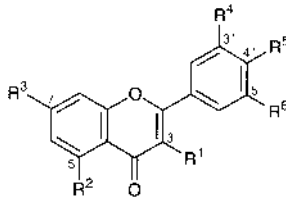
Table 3. Inhibitory Activity of Flavonols for Rat Lens Aldose Reductase-1



	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)
3-Hydroxyflavone (**20**)	OH	H	H	H	H	>30 (14)[#]
Izalpinin (**21**)	OH	OH	OCH_3	H	H	>100 (38)[##]
Kaempferol (**22**)	OH	OH	OH	H	OH	10
Kaempferol 3-*O*-GlcA (**23**)	O-GlcA	OH	OH	H	OH	5.1[11]
Quercetin (**24**)	OH	OH	OH	OH	OH	2.2
Rhamnetin (**25**)	OH	OH	OCH_3	OH	OH	2.7
Tamarixetin (**26**)	OH	OH	OH	OH	OCH_3	11
27	OCH_3	OH	OCH_3	OH	OH	0.82
Ombuine (**28**)	OH	OH	OCH_3	OH	OCH_3	6.0
Ayanin (**29**)	OCH_3	OH	OCH_3	OH	OCH_3	34
30	OH	OH	OCH_3	OCH_3	OCH_3	73
31	OCH_3	OH	OCH_3	OCH_3	OCH_3	25
32	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	>100 (24)[##]
Isoquercitrin (**33**)	O-Glc	OH	OH	OH	OH	4.5
Hyperin (**34**)	O-Gal	OH	OH	OH	OH	3.0
Quercitrin (**35**)	O-Rha	OH	OH	OH	OH	0.15[3]
Guaijaverin (**36**)	O-Ara	OH	OH	OH	OH	0.18[3]
Quercitrin 3,7-di-*O*-Glc (**37**)	O-Glc	OH	O-Glc	OH	OH	84[9]
Rutin (**38**)	O-Glc(6→1)Rha	OH	OH	OH	OH	9.0
Rhamnetin 3-*O*-Rut (**39**)	O-Glc(6→1)Rha	OH	OCH_3	OH	OH	21
Ombuine 3-*O*-Rut (**40**)	O-Glc(6→1)Rha	OH	OCH_3	OH	OCH_3	41
41	O-Glc(6→1)Rha	OH	OCH_3	OCH_3	OCH_3	88
Fisetin (**42**)	OH	H	OH	OH	OH	3.7

Glc: β-D-glucopyranosyl; Gal: β-D-galactopyranosyl; GlcA: β-D-glucopyranosiduronic acid; Rha: α-L-rhamnopyranosyl; Ara: α-L-arabinopyranosyl; Rut: Glc(6→1)Rha. Values in parentheses represent the inhibition (%) at #30 μM and ## 100 μM.

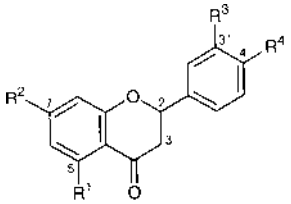
Table 4. Inhibitory Activity of Flavonols for Rat Lens Aldose Reductase-2

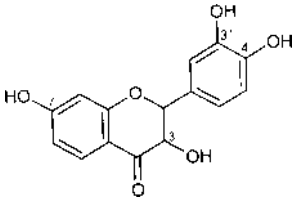


	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} (μM)
Myricetin (**43**)	OH	OH	OH	OH	OH	OH	29
Mearnsetin (**44**)	OH	OH	OH	OH	OCH_3	OH	19
45	OH	OH	OCH_3	OH	OH	OH	21
46	OCH_3	OH	OCH_3	OH	OH	OH	12
47	OH	OH	OCH_3	OH	OCH_3	OH	24
48	OH	OH	OCH_3	OCH_3	OCH_3	OH	44
49	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	>100 (12)[##]
Myricitrin (**50**)	O-Rha	OH	OH	OH	OH	OH	3.8
Mearncitrin (**51**)	O-Rha	OH	OH	OH	OCH_3	OH	3.8[3]
52	O-Rha	OH	OCH_3	OH	OCH_3	OH	48
53	O-Rha	OH	OH	OCH_3	OCH_3	OH	21
54	O-Rha	OH	OCH_3	OCH_3	OCH_3	OH	71
55	O-Rha	OH	OCH_3	OCH_3	OCH_3	OCH_3	71
Desmanthin-1 (**56**)	O-(2′-galloyl)-Rha	OH	OH	OH	OH	OH	0.082[3]

Rha: α-L-rhamnopyranosyl. Values in parentheses represent the inhibition (%) at ## 100 μM.

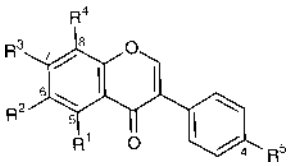
Table 5. Inhibitory Activity of Flavanones and Dihydroflavonol for Rat Lens Aldose Reductase



	R^1	R^2	R^3	R^4	IC_{50} (μM)
Flavanone (**57**)	H	H	H	H	>100 (38)[##]
Liquiritigenin (**58**)	H	OH	H	OH	3.4
59	H	OCH_3	H	OH	1.9
Liquiritin (**60**)	H	OH	H	O-Glc	9.5
61	H	OCH_3	H	O-Glc	30
Eriodictyol (**62**)	OH	OH	OH	OH	7.7[9]
(2*S*)-Eriodictyol 7-*O*-GlcA (**63**)	OH	O-GlcA	OH	OH	2.1[9]
(2*R*)-Eriodictyol 7-*O*-GlcA (**64**)	OH	O-GlcA	OH	OH	1.5[9]
 Fustin (**65**)					14

Glc: β-D-glucopyranosyl; GlcA: β-D-glucopyranosiduronic acid. Values in parentheses represent the inhibition (%) at ## 100 μM.

Table 6. Inhibitory Activity of Isoflavones for Rat Lens Aldose Reductase



	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)
Daidzein (**66**)	H	H	OH	H	OH	24
Daidzin (**67**)	H	H	O-Glc	H	OH	>100 (40)[##]
Genistein (**68**)	OH	H	OH	H	OH	20
Genistin (**69**)	OH	H	O-Glc	H	OH	37
Tectorigenin (**70**)	OH	OCH_3	OH	H	OH	14
Tectoridin (**71**)	OH	OCH_3	O-Glc	H	OH	29
Tectorigenin 7-*O*-Glc-Xyl (**72**)	OH	OCH_3	O-Glc(6→1)Xyl	H	OH	29
Glycitein (**73**)	H	OCH_3	OH	H	OH	93
Glycitin (**74**)	H	OCH_3	O-Glc	H	OH	65
Glycitein 7-*O*-Glc-Xyl (**75**)	H	OCH_3	O-Glc(6→1)Xyl	H	OH	100
Puerarin (**76**)	H	H	OH	Glc	OH	>100 (37)[##]
Biochanin A (**77**)	OH	H	OH	H	OCH_3	22

Glc; β-D-glucopyranosyl, Xyl; β-D-xylopyranosyl. Values in parentheses represent the inhibition (%) at ## 100 μM.

12>quercetin (**24**, 2.2 μM) and eriodictyol (**62**, 7.7 μM)]. Various flavonols having 3-*O*-methyl or 3-*O*-monosaccharide showed stronger inhibition than free flavonols at the 3-position [ex. kaempferol 3-*O*-β-D-glucuronide (**23**, 5.1 μM)>**22**; **27** (0.82 μM)>rhamnetin (**25**, 2.7 μM); **31** (25 μM)>**30** (73 μM); **35** and **36**>**24**; myricitrin (**50**, 3.8 μM)>myricetin (**43**, 29 μM); **46** (12 μM)>**45** (21 μM)], while opposite examples were found [ex. **24**>isoquercitrin (**33**, 4.5 μM) and hyperin (**34**, 3.0 μM); ombuine (**28**, 6.0 μM)>ayanin (**29**, 34 μM); **47** (24 μM)>**52** (48 μM); **48** (44 μM)>**54** (71 μM)]. The compounds having a catechol moiety at the B ring showed stronger activities than those having a pyrogallol moiety (the 3′,4′,5′-trihydroxyl moiety) [ex. **24**>**43**; **25**>**45**; **27**>**46**; **35**>**50**]. Finally, we carried out the screening for other type polyphenols, such as isoflavones (**66**—**77**, Table 6), flavan-3-ols (**78**—**80**, Table 7), and stilbenes (**81**—**94**, Table 8). Their inhibitory activities were weaker than flavone, flavonol, and flavanone type compounds.

Previous study demonstrated the possible relationships of structure to the inhibitory activities of flavonoids: 1) flavones and flavonols having a catechol moiety at the B ring (the 3′,4′-dihydroxyl moiety) show stronger activity; 2) the 2–3 double bond enhances the activity; and 3) isoflavones and catechins show less activity than flavones.[14—16] In the pre-

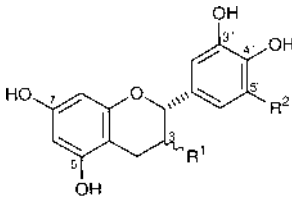
sent study, our results indicated some additional structural requirements of flavonoids for aldose reductase inhibitory activity: 1) the 5-hydroxyl moiety does not affect the activity; 2) 3-hydroxyl and 7-*O*-glucosyl moieties reduce the activity; 3) the flavones and flavonols having a catechol moiety at the B ring exhibit stronger activities than pyrogallol type compounds.

In conclusion, quercitrin (**35**, 0.15 μM), guaijaverin (**36**, 0.18 μM), and desmanthin-1 (**56**, 0.082 μM) were found to show potent inhibitory activity. Especially, the activity of **56** was equivalent to that of a commercial synthetic aldose reductase inhibitor, epalrestat (0.072 μM). Some additional structural requirements of flavonoids for the activity were also clarified.

Experimental

The following instruments were used to obtain physical data: electron impact (EI)-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) spectrometer; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC detector, Shimadzu SPD-10A UV-VIS detector (254 nm).

Table 7. Inhibitory Activity of Flavan-3-ols for Rat Lens Aldose Reductase



	R^1	R^2	IC_{50} (μM)
(+)-Catechin (**78**)	β-OH	H	>30 (38)#
(−)-Epicatechin (**79**)	α-OH	H	>30 (41)#
(−)-Epigallocatechin (**80**)	α-OH	OH	>30 (19)#

Values in parentheses represent the inhibition (%) at # 30 μM.

The following experimental conditions were used for chromatography: ordinary-phase column chromatography; Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh), reversed-phase silica gel column chromatography. Packed column for HPLC: YMC-Pack ODS-A (10×250 mm, i.d. and 20×250 mm, i.d.). TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm) (reversed-phase). Detection was done by spraying with 1% $Ce(SO_4)_2$-10% aqueous H_2SO_4 followed by heating.

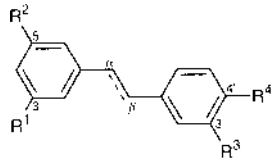
Methanolysis of 10, 19, 23, 38, 41, and 54 A solution of apigenin 7-*O*-β-D-glucopyranoside (**10**, 30 mg, 0.069 mmol), diosmetin 7-*O*-β-D-glucopyranoside (**19**, 30 mg, 0.065 mmol), or kaempferol 3-*O*-β-D-glucuronide (**23**, 30 mg, 0.069 mmol) in 9% HCl–dry MeOH (3.0 ml) was heated under reflux for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [1 g, *n*-hexane–AcOEt (1 : 1)] to give apigenin (**8**, 18 mg, 95%), diosmetin (**13**, 20 mg, quant.), and kaempferol (**22**, 20 mg, quant.), respectively. Through a similar procedure, a solution of rutin (**38**, 2.0 g, 3.28 mmol) in 9% HCl–dry MeOH (30.0 ml) was heated under reflux for 3 h. The reaction mixture was treated in the usual manner to give a residue, which was purified by silica gel column chromatography [50 g, *n*-hexane–AcOEt (1 : 1)] to give quercetin (**24**, 950 mg, 96%). A solution of **41** (30 mg, 0.048 mmol) in 9% HCl–dry MeOH (3.0 ml) was heated under reflux for 2 h. The reaction mixture was treated in the usual manner to give a residue, which was purified by silica gel column chromatography [500 mg, *n*-hexane–AcOEt (2 : 1)] to give **30** (13 mg, 87%). A solution of **54** (30 mg, 0.059 mmol) in 9% HCl–dry MeOH (3.0 ml) was heated under reflux for 1 h. Work-up of the reaction mixture yielded a residue, which was purified by silica gel column chromatography [1 g, *n*-hexane–AcOEt (1 : 1)] to give **48** (18 mg, 84%).

Compounds **8**, **13**, **22**, and **24** were identified by comparison of the physical data with reported values.[29] The structures of **30**[30] and **48**[31] were confirmed by the following physical data.

30: High-resolution EI-MS: Calcd for $C_{18}H_{16}O_7$ (M^+): 344.0896. Found: 344.0892. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 3.85, 3.85, 3.88 (3H each, all s, 3′, 4′, 7-OCH_3), 6.35 (1H, br s, 6-H), 6.77 (1H, br s, 8-H), 7.13 (1H, d, J=8.5 Hz, 5′-H), 7.78 (1H, br s, 2′-H), 7.84 (1H, br d, J=*ca.* 9 Hz, 6′-H), 12.41 (1H, br s, –OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 55.5, 55.7, 55.9 (–OCH_3), 91.4 (C-8), 97.3 (C-6), 103.9 (C-10), 111.1 (C-5′), 111.4 (C-2′), 121.4 (C-6′), 123.1 (C-1′), 136.3 (C-3), 148.2 (C-3′), 150.3, 150.5 (C-2, 4′), 155.8 (C-5), 160.1 (C-9), 164.7 (C-7), 175.8 (C-4). EI-MS: *m/z* 344 (M^+, 100).

48: High-resolution EI-MS: Calcd for $C_{18}H_{16}O_8$ (M^+): 360.0845. Found:

Table 8. Inhibitory Activity of Stilbenes for Rat Lens Aldose Reductase



	α–β	R^1	R^2	R^3	R^4	IC_{50} (μM)
Rhapontigenin (**81**)	C=C	OH	OH	OH	OCH_3	>100 (39)##
Rhaponticin (**82**)	C=C	OH	O-Glc	OH	OCH_3	80
Dihydrorhaponticin (**83**)	C–C	OH	OH	OH	OCH_3	>100 (39)##
Piceatannol (**84**)	C=C	OH	OH	OH	OH	36
Dihydropiceatannol (**85**)	C–C	OH	OH	OH	OH	32
3,3′,4′-Trimethylpiceatannol (**86**)	C=C	OCH_3	OH	OCH_3	OCH_3	>100 (22)##
3,4′,5-Trimethylpiceatannol (**87**)	C=C	OCH_3	OCH_3	OH	OCH_3	>100 (23)##
Piceatannol 3′-*O*-Glc (**88**)	C=C	OH	OH	O-Glc	OH	85
Dihydropiceatannol 3′-*O*-Glc (**89**)	C–C	OH	OH	O-Glc	OH	>100 (41)##
Isorhapontigenin (**90**)	C=C	OH	OH	OCH_3	OH	80
Desoxyrhapontigenin (**91**)	C=C	OH	OH	H	OCH_3	>100 (45)##
Resveratrol (**92**)	C=C	OH	OH	H	OH	25
93	C=C	OCH_3	OCH_3	H	OCH_3	>100 (13)##
trans-Stilbene (**94**)	C=C	H	H	H	H	>100 (6)##

Glc; β-D-glucopyranosyl. Values in parentheses represent the inhibition (%) at ## 100 μM.

360.0834. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 3.77, 3.86, 3.87 (3H each, all s, 4′, 3′, 7-OCH$_3$), 6.35 (1H, br s, 6-H), 6.72 (1H, br s, 8-H), 7.33 (1H, br s, 2′-H), 7.45 (1H, br s, 6′-H), 9.46, 12.35 (1H each, both br s, –OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 55.9, 55.9, 59.9 (–OCH$_3$), 91.8 (C-8), 97.3 (C-6), 103.3 (C-10), 103.9 (C-2′), 109.5 (C-6′), 125.6 (C-1′), 136.7 (C-3), 138.1 (C-4′), 146.0 (C-2), 150.1 (C-5′), 152.6 (C-3′), 155.9 (C-9), 160.1 (C-5), 164.8 (C-7), 175.9 (C-4). EI-MS: *m/z* 360 (M$^+$, 100).

Diazomethane Methylation of 8, 12, 24, 38, 43, 50, and 60 A solution of apigenin (**8**, 20 mg, 0.074 mmol) in MeOH (2.0 ml) was treated with ethereal diazomethane (CH$_2$N$_2$–Et$_2$O) until the yellow color persisted. The reaction solution was stirred at room temperature for 30 min. Removal of the solvent under reduced pressure furnished a residue, which was purified by silica gel column chromatography [500 mg, *n*-hexane–AcOEt (2 : 1)] to give **9** (21 mg, 94%). A solution of luteolin (**12**, 100 mg, 0.35 mmol) in MeOH (5.0 ml) was treated with CH$_2$N$_2$–Et$_2$O and the whole mixture was stirred at room temperature for 1 h. The reaction mixture was purified by HPLC [YMC-Pack ODS-A, 20×250 mm, i.d., MeOH–1% aqueous AcOH (80 : 20, v/v)] to give pilloin (**14**, 36 mg, 33%) and **15** (63 mg, 55%). Through a similar procedure, rhamnetin (**25**, 81 mg, 22%), tamarixetin (**26**, 7 mg, 2%), **27** (50 mg, 13%), ombuine (**28**, 64 mg, 16%), ayanin (**29**, 127 mg, 32%), and **31** (59 mg, 14%) were obtained from quercetin [**24**, 350 mg, 1.16 mmol, HPLC conditions: YMC-Pack ODS-A, 20×250 mm, i.d., MeOH–1% aqueous AcOH (75 : 25, v/v)]. From rutin (**38**, 400 mg, 0.66 mmol), rhamnetin 3-*O*-rutinoside (**39**, 71 mg, 17%), ombuin 3-*O*-rutinoside (**40**, 132 mg, 32%), and **41** (143 mg, 33%) were obtained [HPLC conditions: YMC-Pack ODS-A, 20×250 mm, i.d., MeOH–1% aqueous AcOH (70 : 30, v/v)]. From myricetin (**43**, 125 mg, 0.39 mmol), mearnsetin (**44**, 41 mg, 31%), **45** (33 mg, 25%), **46** (8 mg, 6%), and **47** (12 mg, 9%) were obtained [HPLC conditions: YMC-Pack ODS-A, 20×250 mm, i.d., MeOH–1% aqueous AcOH (70 : 30, v/v)]. From myricitrin (**50**, 400 mg, 0.86 mmol), **52** (107 mg, 25%), **53** (61 mg, 14%), **54** (114 mg, 26%), and **55** (85 mg, 19%) were obtained [HLPC conditions: YMC-Pack ODS-A, 20×250 mm, i.d., MeOH–1% aqueous AcOH (70 : 30, v/v)]. A solution of liquiritin (**60**, 100 mg, 0.24 mmol) was treated with a similar procedure, then the reaction mixture was removed and purified by silica gel column chromatography [2 g, *n*-hexane–AcOEt (1 : 1)] to give **61** (104 mg, quant.).

Compounds **9**, **14**, **15**, **25**, **27**, **28**, **39**, and **44** were identified by comparison of the physical data with reported values.[33—38,40,42] The structures of **26**,[37] **29**,[37] **31**,[39] **40**,[41] **41**,[32] **45**,[43] **46**,[44] and **47**[45] were confirmed by the following physical data.

26: High-resolution EI-MS: Calcd for $C_{16}H_{12}O_7$ (M$^+$): 316.0583. Found: 316.0575. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 3.85 (3H, s, 4′-OCH$_3$), 6.19 (1H, d, *J*=1.8 Hz, 6-H), 6.42 (1H, d, *J*=1.8 Hz, 8-H), 7.08 (1H, d, *J*=8.4 Hz, 5′-H), 7.67 (1H, d, *J*=2.1 Hz, 2′-H), 7.65 (1H, dd, *J*=2.1, 8.4 Hz, 6′-H). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 55.5 (–OCH$_3$), 93.2 (C-8), 98.0 (C-6), 102.8 (C-10), 111.7 (C-5′), 114.4 (C-2′), 119.5 (C-6′), 123.2 (C-1′), 135.8 (C-3), 145.9 (C-3′), 146.0 (C-2), 149.0 (C-4′), 155.9 (C-5), 160.4 (C-9), 163.6 (C-7), 175.6 (C-4). EI-MS: *m/z* 316 (M$^+$, 100).

Ayanin (**29**): High-resolution EI-MS: Calcd for $C_{18}H_{16}O_7$ (M$^+$): 344.0896. Found: 344.0891. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 3.81, 3.87, 3.87 (3H each, all s, 3, 4′, 7-OCH$_3$), 6.36 (1H, br s, 6-H), 6.70 (1H, br s, 8-H), 7.10 (1H, d, *J*=8.8 Hz, 5′-H), 7.58 (1H, br s, 2′-H), 7.58 (1H, br d, *J*=*ca.* 9 Hz, 6′-H), 9.33, 12.62 (1H each, both br s, –OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 55.6, 55.9, 59.6 (–OCH$_3$), 92.1 (C-8), 97.5 (C-6), 105.0 (C-10), 111.7 (C-5′), 114.9 (C-2′), 120.1 (C-6′), 122.0 (C-1′), 138.0 (C-3), 146.1 (C-3′), 150.0 (C-2), 155.3 (C-4′), 156.0 (C-5), 160.7 (C-9), 164.8 (C-7), 177.7 (C-4). EI-MS: *m/z* 344 (M$^+$, 100).

31: High-resolution EI-MS: Calcd for $C_{19}H_{18}O_7$ (M$^+$): 358.1052. Found: 358.1046. ^{1}H-NMR (270 MHz, CDCl$_3$) δ: 3.86, 3.87, 3.97, 3.97 (3H each, all s, 4′, 7, 3, 3′, –OCH$_3$), 6.35 (1H, br s, 6-H), 6.44 (1H, br s, 8-H), 6.99 (1H, d, *J*=8.4 Hz, 5′-H), 7.69 (1H, br s, 2′-H), 7.71 (1H, dd like, 6′-H), 12.63 (1H, br s, –OH). ^{13}C-NMR (68 MHz, CDCl$_3$) δ_c: 55.8, 56.0, 56.0, 60.2 (–OCH$_3$), 92.1 (C-8), 97.7 (C-6), 105.9 (C-10), 110.7 (C-5′), 111.1 (C-2′), 122.0 (C-6′), 122.8 (C-1′), 138.8 (C-3), 148.6 (C-3′), 151.2 (C-2), 155.6 (C-4′), 156.5 (C-5), 161.8 (C-9), 165.2 (C-7), 178.5 (C-4). EI-MS: *m/z* 358 (M$^+$, 100).

Ombuine 3-*O*-Rutinoside (**40**): High-resolution positive-ion FAB-MS: Calcd for $C_{29}H_{35}O_{16}$ (M+H)$^+$: 639.1925. Found: 639.1918. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 0.98 (3H, d, *J*=6.1 Hz, 6—H), 3.87, 3.87 (3H each, both s, 4′, 7-OCH$_3$), 4.50 (1H, br s, 1—H), 5.39 (1H, d, *J*=7.1 Hz, 1″-H), 6.37 (1H, d, *J*=2.1 Hz, 6-H), 6.67 (1H, d, *J*=2.1 Hz, 8-H), 7.04 (1H, d, *J*=8.5 Hz, 5′-H), 7.55 (1H, d, *J*=2.2 Hz, 2′-H), 7.72 (1H, dd, *J*=2.2, 8.5 Hz, 6′-H). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 17.6 (C-1‴), 55.6, 56.0 (–OCH$_3$), 66.8 (C-6″), 68.1 (C-5‴), 69.8 (C-4″), 70.3 (C-2‴), 70.5 (C-3‴), 71.8 (C-4‴), 74.0 (C-2″), 75.8 (C-5″), 76.4 (C-3″), 92.2 (C-8), 97.8 (C-6), 100.7 (C-1‴), 101.1 (C-1″), 105.0 (C-10), 111.4 (C-2′), 115.8 (C-5′), 121.4 (C-1′), 122.4 (C-6′), 133.8 (C-3), 145.8 (C-3′), 150.1 (C-4′), 156.3 (C-2), 156.6 (C-9), 160.8 (C-5), 165.1 (C-4), 177.5 (C-7). Positive-ion FAB-MS: *m/z* 639 (M+H)$^+$.

41: High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{37}O_{16}$ (M+H)$^+$: 653.2081. Found: 653.2078. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 0.97 (3H, d, *J*=6.1 Hz, 6—H), 3.84, 3.86, 3.87 (3H each, all s, 3′, 4′, 7-OCH$_3$), 4.41 (1H, br s, 1—H), 5.44 (1H, d, *J*=7.4 Hz, 1″-H), 6.37 (1H, d, *J*=1.8 Hz, 6-H), 6.72 (1H, d, *J*=1.8 Hz, 8-H), 7.10 (1H, d, *J*=8.6 Hz, 5′-H), 7.71 (1H, dd, *J*=1.8, 8.6 Hz, 6′-H), 7.82 (1H, d, *J*=1.8 Hz, 2′-H), 12.51 (1H, br s, –OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 17.6 (C-1‴), 55.6, 55.6, 56.0 (–OCH$_3$), 66.7 (C-6″), 68.2 (C-5‴), 70.0 (C-4″), 70.2 (C-2‴), 70.6 (C-3‴), 71.7 (C-4‴), 74.2 (C-2″), 75.9 (C-5″), 76.4 (C-3″), 92.4 (C-8), 97.9 (C-6), 100.8 (C-1‴), 101.2 (C-1″), 105.0 (C-10), 111.2 (C-2′), 112.6 (C-5′), 121.4 (C-1′), 122.3 (C-6′), 133.6 (C-3), 148.0 (C-3′), 151.1 (C-4′), 156.4 (C-2), 156.5 (C-9), 160.8 (C-5), 165.2 (C-7), 177.5 (C-4). Positive-ion FAB-MS: *m/z* 653 (M+H)$^+$.

45: High-resolution EI-MS: Calcd for $C_{16}H_{12}O_8$ (M$^+$): 332.0532. Found: 332.0533. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 3.87 (3H, s, 7-OCH$_3$), 6.34 (1H, br s, 6-H), 6.63 (1H, br s, 8-H), 7.30 (2H, br s, 2′, 6′-H). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 55.8 (–OCH$_3$), 91.5 (C-8), 97.2 (C-6), 103.8 (C-10), 107.2 (C-2′, 6′), 120.5 (C-1′), 135.8, 135.9 (C-3, 4′), 145.5 (C-2), 147.1 (C-3′, 5′), 155.7 (C-9), 160.1 (C-5), 164.6 (C-7), 175.5 (C-4). EI-MS: *m/z* 332 (M$^+$, 100).

46: High-resolution EI-MS: Calcd for $C_{17}H_{14}O_8$ (M$^+$): 346.0688. Found: 346.0682. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 3.80, 3.87 (3H each, both s, 3, 7-OCH$_3$), 6.36 (1H, d, *J*=2.2 Hz, 6-H), 6.64 (1H, d, *J*=2.2 Hz, 8-H), 7.16 (2H, br s, 2′, 6′-H). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 55.9, 59.5 (–OCH$_3$), 92.0 (C-8), 97.6 (C-6), 105.1 (C-10), 107.7 (C-2′, 6′), 119.5 (C-1′), 136.9, 137.9 (C-3, 4′), 145.8 (C-3′, 5′), 156.0, 156.1 (C-2, 9), 160.9 (C-5), 165.0 (C-7), 177.9 (C-4). EI-MS: *m/z* 346 (M$^+$, 100).

47: High-resolution EI-MS: Calcd for $C_{17}H_{14}O_8$ (M$^+$): 346.0688. Found: 346.0681. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 3.79, 3.87 (3H each, both s, 4′, 7-OCH$_3$), 6.35 (1H, br s, 6-H), 6.64 (1H, br s, 8-H), 7.27 (2H, br s, 2′, 6′-H). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 55.8, 59.6 (–OCH$_3$), 91.6 (C-8), 97.2 (C-6), 103.9 (C-10), 107.2 (C-2′, 6′), 125.5 (C-1′), 136.6, 137.1 (C-3, 4′), 146.2 (C-2), 150.3 (C-3′, 5′), 155.8 (C-9), 160.1 (C-5), 164.7 (C-7), 175.9 (C-4). EI-MS: *m/z* 346 (M$^+$, 100).

52: High-resolution positive-ion FAB-MS: Calcd for $C_{23}H_{25}O_{12}$ (M+H)$^+$: 493.1346. Found: 493.1341. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 0.84 (3H, d, *J*=5.2 Hz, 6″-H), 3.76, 3.87 (3H each, both s, 4′, 7-OCH$_3$), 5.20 (1H, br s, 1″-H), 6.39 (1H, d, *J*=2.0 Hz, 6-H), 6.65 (1H, d, *J*=2.0 Hz, 8-H), 6.85 (2H, br s, 2′, 6′-H), 9.39 (2H, br s, –OH), 12.55 (1H, br s, –OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 17.4 (C-6″), 56.0, 59.6 (–OCH$_3$), 69.9 (C-5″), 70.2 (C-2″), 70.4 (C-3″), 71.1 (C-4″), 92.1 (C-8), 97.7 (C-6), 102.0 (C-1″), 105.0 (C-10), 108.0 (C-2′, 6′), 124.4 (C-1′), 134.8 (C-3), 137.7 (C-4′), 150.3 (C-3′, 5′), 156.2 (C-2), 157.4 (C-9), 160.7 (C-5), 165.0 (C-7), 177.7 (C-4). Positive-ion FAB-MS: *m/z* 493 (M+H)$^+$. Negative-ion FAB-MS: *m/z* 491 (M−H)$^-$, 345 (M−C$_6$H$_{11}$O$_4$)$^-$.

53: High-resolution positive-ion FAB-MS: Calcd for $C_{23}H_{25}O_{12}$ (M+H)$^+$: 493.1346. Found: 493.1349. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 0.79 (3H, d, *J*=5.9 Hz, 6″-H), 3.75, 3.84 (3H each, both s, 4′, 3′-OCH$_3$), 5.21 (1H, br s, 1″-H), 6.22 (1H, d, *J*=2.0 Hz, 6-H), 6.41 (1H, d, *J*=2.0 Hz, 8-H), 6.97 (2H, br s, 2′, 6′-H), 9.48, 10.84, 12.52 (1H each, all br s, –OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 17.4 (C-6″), 55.9, 59.9 (–OCH$_3$), 69.9 (C-5″), 70.3 (C-2″), 70.4 (C-3″), 71.0 (C-4″), 93.6 (C-8), 98.6 (C-6), 101.8 (C-1″), 104.1 (C-6′), 104.7 (C-10), 110.0 (C-2′), 124.7 (C-1′), 134.6 (C-3), 138.6 (C-4′), 150.2 (C-5′), 152.5 (C-3′), 156.3 (C-2), 156.8 (C-9), 161.0 (C-5), 164.0 (C-7), 177.4 (C-4). Positive-ion FAB-MS: *m/z* 493 (M+H)$^+$. Negative-ion FAB-MS: *m/z* 491 (M−H)$^-$, 345 (M−C$_6$H$_{11}$O$_4$)$^-$.

54: High-resolution positive-ion FAB-MS: Calcd for $C_{24}H_{27}O_{12}$ (M+H)$^+$: 507.1502. Found: 507.1496. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 0.82 (3H, d, *J*=5.2 Hz, 6″-H), 3.77, 3.87, 3.88 (3H each, all s, 4′, 3′, 7-OCH$_3$), 5.25 (1H, br s, 1″-H), 6.39 (1H, br s, 6-H), 6.68 (1H, br s, 8-H), 7.00, 7.02 (1H each, both br s, 2′, 6′-H), 9.50, 12.52 (1H each, both br s, –OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 17.4 (C-6″), 55.9, 56.0, 59.9 (–OCH$_3$), 69.9 (C-5″), 70.3 (C-2″), 70.4 (C-3″), 71.0 (C-4″), 92.2 (C-8), 97.8 (C-6), 101.9 (C-1″), 104.7, 110.1 (C-2′, 6′), 105.0 (C-10), 124.6 (C-1′), 134.9 (C-3), 138.7 (C-4′), 150.3 (C-5′), 152.5 (C-3′), 156.2 (C-2), 157.1 (C-9), 160.7 (C-5), 165.0 (C-7), 177.6 (C-4). Positive-ion FAB-MS: *m/z* 507 (M+H)$^+$. Negative-ion FAB-MS: *m/z* 505 (M−H)$^-$, 359 (M−C$_6$H$_{11}$O$_4$)$^-$.

55: High-resolution positive-ion FAB-MS: Calcd for $C_{25}H_{29}O_{12}$ (M+H)$^+$: 521.1659. Found: 521.1652. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 0.81 (3H, d,

J=5.9 Hz, 6″-H), 3.78, 3.88 (3H each, both s, 4′, 7-OCH_3), 3.89 (6H, s, 3′, 5′-OCH_3), 5.30 (1H, br s, 1″-H), 6.38 (1H, br s, 6-H), 6.70 (1H, br s, 8-H), 7.18 (2H, br s, 2′, 6′-H), 12.49 (1H, br s, –OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 17.4 (C-6″), 56.0, 56.1×2, 60.1 (–OCH_3), 69.9 (C-5″), 70.4 (C-2″), 70.5 (C-3″), 71.0 (C-4″), 92.3 (C-8), 97.8 (C-6), 101.7 (C-1″), 105.1 (C-10), 106.8 (C-2′, 6′), 124.7 (C-1′), 134.9 (C-3), 139.9 (C-4′), 152.4 (C-3′, 5′), 156.2 (C-2), 157.0 (C-9), 160.7 (C-5), 165.0 (C-7), 177.5 (C-4). Positive-ion FAB-MS: m/z 521 $(M+H)^+$.

61: High-resolution EI-MS: Calcd for $C_{22}H_{24}O_9$ (M^+): 432.1420. Found: 432.1428. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: [2.73 (1H, br d, J=*ca.* 17 Hz), 3.13 (1H, dd like), 3-H_2), 3.81 (3H, s, 7-OCH_3), 4.88 (1H, d like, 1″-H), 5.57 (1H, br d, J=*ca.* 11 Hz, 2-H), 6.60 (1H, br s, 8-H), 6.65 (1H, br d, J=*ca.* 9 Hz, 6-H), 7.07 (2H, d, J=7.4 Hz, 3′, 5′-H), 7.45 (2H, d, J=7.4 Hz, 2′, 6′-H), 7.71 (1H, d, J=8.9 Hz, 5-H). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_c: 43.0 (3-C), 55.7 (–OCH_3), 60.6 (6″-C), 69.7 (4″-C), 73.1 (2″-C), 76.5 (3″-C), 76.9 (5″-C), 78.7 (2-C), 100.2 (1″-C), 100.8 (8-C), 109.6 (6-C), 114.2 (10-C), 116.0 (3′, 5′-C), 127.7 (5-C), 127.7 (2′, 6′-C), 131.9 (1′-C), 157.2 (4′-C), 162.8 (9-C), 165.3 (7-C), 189.7 (4-C). EI-MS: m/z 432 (M^+, 1), 270 (100).

Complete Methylation of 12, 24, and 43 A solution of luteolin (**12**, 30 mg, 0.10 mmol) in *N*,*N*-dimethylformamide (DMF, 2.0 ml) was treated with methyl iodide (CH_3I, 0.1 ml) in the presence of sodium hydride (NaH, 10 mg) and the mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder and the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [1.0 g, *n*-hexane–AcOEt (1 : 1)] to give **16** (30 mg, 84%). Through a similar procedure of methylation, quercetin (**24**, 30 mg, 0.10 mmol) in DMF (2.0 ml) using CH_3I (0.1 ml) and NaH (10 mg) was stirred at room temperature for 12 h. Work-up and the residue was purified by silica gel column chromatography [1.0 g, *n*-hexane–AcOEt (1 : 1)] to give **32** (31 mg, 84%). A solution of myricetin (**43**, 40 mg, 0.13 mmol) in DMF (2.0 ml) using CH_3I (0.1 ml) and NaH (20 mg) was stirred at room temperature for 12 h. Work-up and the residue was purified by silica gel column chromatography [1.0 g, *n*-hexane–AcOEt (3 : 1)] to give **49** (48 mg, 95%). Compounds **16**, **32**, and **49** were identified by comparison of the physical data with reported values.[43,46,47]

Enzymatic Hydrolysis of 61 A solution of **61** (50 mg, 0.12 mmol) in 0.2 M acetate buffer (pH 5.0, 5.0 ml) was treated with β-glucosidase (20.0 mg, Sigma) and the solution was stirred at 37 °C for 12 h. After treatment of the reaction mixture with EtOH, the mixture was evaporated to dryness under reduced pressure and the residue was purified by ordinary-phase silica gel column chromatography [2 g, *n*-hexane–AcOEt (1 : 1)] to give **59** (29 mg, 93%), which was identified by comparison of its physical data with those of reported values.[48]

Bioassay

Aldose reductase activity was assayed by the method described in a previous paper.[3,6,7,9,11] The supernatant fluid of rat lens homogenate was used as the crude enzyme. The incubation mixture contained 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM Li_2SO_4, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 100 μl of enzyme fraction, with or without 25 μl of sample solution, in a total volume of 0.5 ml. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min, the reaction was stopped by the addition of 150 μl 0.5 M HCl. Then, 0.5 ml 6 M NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. Fluorescence was measured using a fluorophotometer (Luminescence Spectrometer LS50B, Perkin Elmer, England) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

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