Medicinal Flowers. VI.[1] Absolute Stereostructures of Two New Flavanone Glycosides and a Phenylbutanoid Glycoside from the Flowers of *Chrysanthemum indicum* L.: Their Inhibitory Activities for Rat Lens Aldose Reductase

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Received January 7, 2002; accepted March 20, 2002

Two new flavanone glycosides, (2*S*)- and (2*R*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acids, and a new phenylbutanoid glycoside, (2*S*,3*S*)-1-phenyl-2,3-butanediol 3-*O*-*β*-D-glucopyranoside, were isolated from the flowers of *Chrysanthemum indicum* L. cultivated in China together with eight flavonoids. The absolute stereostructures of the new compounds were determined on the basis of chemical and physicochemical evidence. Both of the new flavanone glycosides were found to show inhibitory activity for rat lens aldose reductase.

Key words aldose reductase inhibitor; *Chrysanthemum indicum*; medicinal flower; (2*S*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid; (2*R*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid; (2*S*,3*S*)-1-phenyl-2,3-butanediol 3-*O*-*β*-D-glucopyranoside

The Compositae plant, the flowers of *Chrysanthemum indicum* L. (Chrysanthemi Indici Flos) have been prescribed for antiinflammation, analgesic, and antipyretic purposes and the treatment of eye disease in Chinese traditional preparations. In the course of our characterization studies on the bioactive constituents from medicinal foodstuffs[2—9] and medicinal flowers,[1,10—13] we have reported the isolation and structural elucidation of three eudesmane-type sesquiterpenes, kikkanols A, B, and C, and five germacrane-type sesquiterpenes, kikkanols D, D monoacetate, E, F, and F monoacetate, from the methanolic extract of the flowers of *C. indicum* cultivated in China.[10,11] In addition, the methanolic extract and several constituents were found to show inhibitory activities against rat lens aldose reductase[10] and nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages.[11] As a continuing study on this natural medicine, we recently isolated two new flavanone glycosides, (2*S*)- and (2*R*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acids (**1**, **2**), and a new phenylbutanoid glycoside, (2*S*,3*S*)-1-phenyl-2,3-butanediol 3-*O*-*β*-D-glucopyranoside (**3**). This paper deals with the isolation and absolute stereostructure elucidation of new constituents (**1**—**3**) from the flowers of *C. indicum* as well as the inhibitory activity of **1** and **2** for rat lens aldose reductase.

Isolation of Chemical Constituents from the Dried Flowers of *C. indicum* The methanolic extract from the flowers of *C. indicum* was partitioned into a mixture of ethyl acetate and water to furnish the ethyl acetate-soluble portion and an aqueous phase. The aqueous phase was further extracted with 1-butanol to give a 1-butanol-soluble portion and a water-soluble portion as described.[10] The 1-butanol-soluble portion was subjected to silica gel and octadecyl silica gel (ODS) column chromatography and finally HPLC to furnish three new constituents called (2*S*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid (**1**, 0.0027% from the natural medicine), (2*R*)-eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid (**2**, 0.0023%), and (2*S*,3*S*)-1-phenyl-2,3-butanediol 3-*O*-*β*-D-glucopyranoside (**3**, 0.019%), and three known flavonoids, apigenin 7-*O*-*β*-D-glucopyranoside (=apigetrin, **4**,[14] 0.014%), diosmetin 7-*O*-*β*-D-glucopyranoside (**5**,[14] 0.086%), and quercetin 3,7-di-*O*-*β*-D-glucopyranoside (**6**,[15] 0.0038%), together with luteolin,[10] luteolin 7-*O*-*β*-D-glucopyranoside,[10] luteolin 7-*O*-*β*-D-glucopyranosiduronic acid,[10] acacetin 7-*O*-(6″-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside,[10] and eupatilin.[10]

Absolute Stereostructures of (2*S*)- and (2*R*)-Eriodictyol 7-*O*-*β*-D-Glucopyranosiduronic Acids (1, 2) (2*S*)-Eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid (**1**) was isolated as a yellow powder with negative optical rotation ($[\alpha]_D^{26}$ −35.6°). The positive-ion fast atom bombardment (FAB)-MS of **1** showed a quasimolecular ion peak at *m*/*z* 487 $(M+Na)^+$, while a quasimolecular ion peak was observed at *m*/*z* 463 $(M-H)^-$ together with a fragment ion peak at *m*/*z* 287 $(M-C_6H_9O_6)^-$ in the negative-ion FAB-MS. The molecular formula $C_{21}H_{20}O_{12}$ of **1** was determined from the quasimolecular ion peaks and by high-resolution MS measurement. The IR spectrum of **1** showed absorption bands at 3417, 1719, 1655, 1637, and 1071 cm^{-1} ascribable to hydroxyl, carboxyl, chelated carbonyl, aromatic ring, and ether functions. In the UV spectrum of **1**, absorption maxima were observed at 248 (log *ε* 4.21) and 326 (3.55) nm, suggestive of the flavanone structure. The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra of **1**, which was constructed on the basis of ^{1}H–^{1}H and ^{13}C–^{1}H correlation spectroscopies (H–H and C–H COSY), showed signals assignable to a dihydropyrone moiety in flavanone structure by a characteristic ABX type coupling pattern {[*δ* 2.74 (dd, *J*=3.0, 17.4 Hz), 3.25 (dd, *J*=12.5, 17.4 Hz), 3-H_2], 5.45 (dd, *J*=3.0, 12.5 Hz, 2-H)}, an anomeric proton [*δ* 5.07 (d, *J*=7.3 Hz, 1″-H)], five aromatic protons [*δ* 6.13, 6.16 (both d, *J*=2.1 Hz, 6, 8-H), 6.75 (2H, br s, 5′, 6′-H), 6.89 (s, 2′-H)], and hydroxyl and chelated hydroxyl groups [*δ* 8.96 (2H, br s), 12.01 (br s), –OH].

(2*R*)-Eriodictyol 7-*O*-*β*-D-glucopyranosiduronic acid (**2**) was also isolated as a yellow powder with negative optical rotation ($[\alpha]_D^{24}$ −54.5°) and its IR, UV, MS, and NMR spectra were very similar to those of **1**. Acid hydrolysis of **1** and **2** with 5% sulfuric acid (H_2SO_4) furnished D-glucuronic acid, which was identified by GLC analysis of the trimethylsilyl (TMS) thiazolidine derivative.[16] Enzymatic hydrolysis of the

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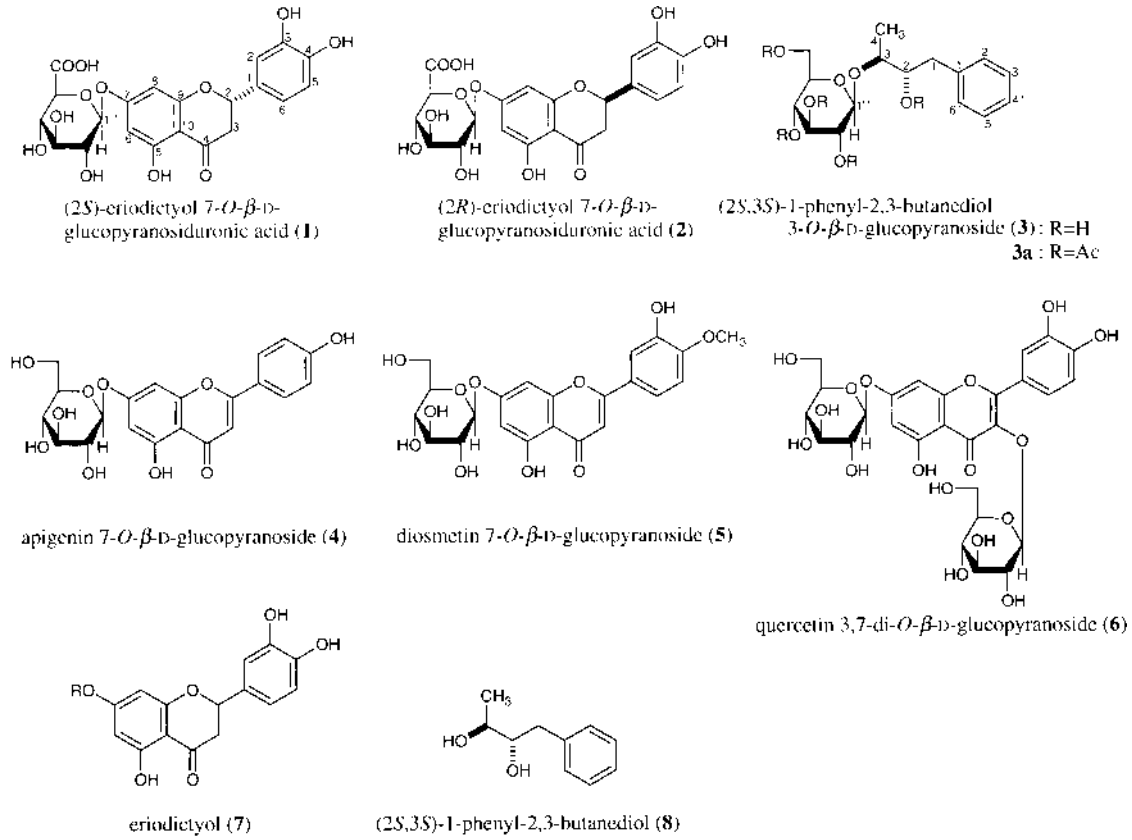


Chart 1

mixture of **1** and **2** with β-glucuronidase liberated eriodictyol (**7**).[17] In the heteronuclear multiple bond correlation (HMBC) experiment on **1** and **2**, a long-range correlation was observed between the anomeric proton and 7-carbon (Fig. 1), so that the connectivities between the aglycon and D-glucuronic acid moiety were elucidated.

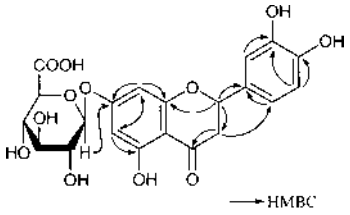
The absolute stereostructures of **1** and **2** were determined on the basis of circular dichronic (CD) spectroscopic analysis. Thus, the CD spectra of **1** showed negative Cotton effects [248 ($\Delta\varepsilon$ +0.24), 287 (−3.59) nm], which indicated the absolute configuration of the 2-position to be *S* orientation, while positive Cotton effects [252 (−1.00), 292 (+3.66)] suggestive of 2*R*-orientation were observed at the CD spectra of **2**.[18—20] On the basis of this evidence, the absolute stereostructures of **1** and **2** were elucidated as shown. The mixture of (2*S*)- and (2*R*)-eriodictyol 7-*O*-β-D-glucopyranosiduronic acids was previously isolated from the rhizome of a fern *Davallia mariesii* MOORE, whereas the separation of these diastereomers was unsuccessful.[21]

Absolute Stereostructure of (2*S*,3*S*)-1-Phenyl-2,3-butanediol 3-*O*-β-D-Glucopyranoside (3) (2*S*,3*S*)-1-Phenyl-2,3-butanediol 3-*O*-β-D-glucopyranoside (**3**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{27}$ −21.9°). The molecular formula $C_{16}H_{24}O_7$ of **3** was determined from the quasimolecular ion peaks at *m/z* 329 $(M+H)^+$ and *m/z* 327 $(M-H)^-$ in the positive- and negative-ion FAB-MS and by high-resolution MS measurement. The IR spectrum of **3** showed absorption bands at 3417, 1605, 1507, and 1075 cm^{-1} ascribable to hydroxyl, aromatic ring, and ether functions. Acetylation of **3** furnished the pentaacetate (**3a**). The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **3** and **3a** showed signals assignable to a secondary methyl [δ **3**: 1.24 (d, J=4.0 Hz, 4-H_3); **3a**: 1.27 (d, J=6.4 Hz, 4-H_3)], a methylene {δ **3**: [2.59 (br s), 2.79 (br d, J=*ca.* 11 Hz), 1-H_2]; **3a**: [2.76 (dd, J=8.8, 14.0 Hz), 2.89 (dd, J=4.8, 14.0 Hz), 1-

Table 1. ^{13}C-NMR Data for (2*S*)-Eriodictyol 7-*O*-β-D-Glucopyranosiduronic acid (**1**), (2*R*)-Eriodictyol 7-*O*-β-D-Glucopyranosiduronic acid (**2**), and (2*S*,3*S*)-1-Phenyl-2,3-butanediol 3-*O*-β-D-Glucopyranoside (**3**), and Its Acetate (**3a**)

	1[a]	**2**[a]	**3**[b]	**3a**[b]		**3a**[b]
C-1			39.1	35.8	–OAc	20.6
C-2	78.6	78.6	76.5	71.5		20.6
C-3	42.1	42.1	81.6	76.3		20.6
C-4	197.0	197.0	14.3	17.5		20.7
C-5	162.8	162.8				21.0
C-6	96.4	96.4				169.3
C-7	165.0	164.8				169.4
C-8	95.3	95.4				170.3
C-9	162.7	162.6				170.4
C-10	103.3	103.3				170.6
C-1′	129.2	129.2	138.5	137.2		
C-2′	114.4	114.3	129.5	129.2		
C-3′	145.2	145.2	128.3	128.5		
C-4′	145.7	145.7	126.3	126.7		
C-5′	115.3	115.3	128.3	128.5		
C-6′	117.9	117.8	129.5	129.2		
C-1″	99.2	99.0	104.2	101.3		
C-2″	72.8	72.8	73.7	76.5		
C-3″	75.8	75.9	75.5	73.0		
C-4″	71.4	71.4	69.2	68.5		
C-5″	74.8	74.8	76.3	71.7		
C-6″	170.3	170.2	61.2	62.0		

a) Measured in DMSO-d_6, *b*) $CDCl_3$ at 125 MHz.



1, 2

Fig. 1

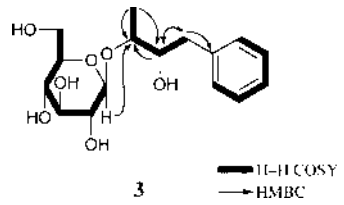


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Fig. 2

Table 2. Inhibitory Activity of Constituents from *C. indicum* for Rat Lens Aldose Reductase

Compound	IC_{50} (μM)
(2*S*)-Eriodictyol 7-*O*-β-D-glucopyranosiduronic acid (**1**)	2.1
(2*R*)-Eriodictyol 7-*O*-β-D-glucopyranosiduronic acid (**2**)	1.5
(2*S*,3*S*)-1-phenyl-2,3-butanediol 3-*O*-β-D-glucopyranoside (**3**)	>100 (9.7)
3a	>100 (16.8)
Apigenin 7-*O*-β-D-glucopyranoside (**4**)	23
Diosmetin 7-*O*-β-D-glucopyranoside (**5**)	23
Quercetin 3,7-di-*O*-β-D-glucopyranoside (**6**)	84
Eriodictyol (**7**)	7.7
(2*S*,3*S*)-1-Phenyl-2,3-butanediol (**8**)	>100 (0.4)
Epalrestat	0.072

Values in parentheses represent the inhibition (%) at 100 μM.

H_2]}, two methines bearing an oxygen function [δ **3**: 3.55 (br s, 3-H), 3.68 (br d, J=*ca.* 11 Hz, 2-H); **3a**: 3.83 (dd, J=4.9, 6.4 Hz, 3-H), 5.06 (ddd, J=4.8, 4.9, 8.8 Hz, 2-H)], an anomeric proton [δ **3**: 4.44 (br d, J=*ca.* 7 Hz, 1″-H); **3a**: 4.65 (d, J=7.9 Hz, 1″-H)], and aromatic protons [δ **3**: 7.14—7.25 (5H, m); **3a**: 7.16—7.31 (5H, m)]. Acid hydrolysis of **3** with 5% H_2SO_4 furnished D-glucose, which was identified by GLC analysis.[16] Enzymatic hydrolysis of **3** with β-glucosidase yielded (2*S*,3*S*)-1-phenyl-2,3-butanediol (**8**).[22] In the HMBC experiment of **3**, long-range correlation was observed between the 1″-proton and 3-carbon. Consequently, the absolute stereostructure of **3** was clarified as shown.

Inhibitory Activity of 1—6 for Rat Lens Aldose Reductase Aldose reductase as a key enzyme in the polyol pathway is reported to catalyze the reduction of glucose to sorbitol. 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. Previously, the methanolic extract of the flowers of *C. indicum* and isolated flavonoids exhibited potent inhibitory activity against rat lens aldose reductase.[10] In the present study, inhibitory effects of additional isolated compounds (**1**—**6**) and their derivatives were examined as shown in Table 2. Among them, **1** (IC_{50}=2.1 μM) and **2** (1.5 μM) showed the potent inhibitory activity for rat lens aldose reductase. However, the inhibitory activities of **1** and **2** were weaker than those of luteolin (0.45 μM) and luteolin 7-*O*-β-D-glucopyranoside (0.99 μM), which were also isolated from the flowers of *C. indicum*.[10]

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; CD spectra, JASCO J-720WI spectropolarimeter; IR spectra, Shimadzu FTIR-8100 spectrometer; MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; ^{1}H-NMR spectra, JEOL JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JEOL JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC detector, Shimadzu SPD-10A UV-VIS detector (254 nm).

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; Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh). 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.

Extraction and Isolation Dried flowers of *C. indicum* L. (5.8 kg, cultivated in China and purchased from Koshiro Co., Ltd., Osaka) were finely cut and extracted with methanol under reflux. Evaporation of the solvent under reduced pressure gave the MeOH extract (1650 g, 28.4%). The MeOH extract (1600 g) was partitioned in an AcOEt–H_2O (1 : 1) mixture, and the aqueous layer was further extracted with 1-BuOH. Removal of the solvent under reduced pressure from the AcOEt- and 1-BuOH-soluble portions yielded 438 g (7.8%) and 226 g (4.0%) of residues, respectively. The 1-BuOH-soluble portion (182 g) was subjected to ordinary-phase silica gel column chromatography [3.0 kg, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→6 : 4 : 1)→MeOH] to give seven fractions [Fr. 1 (7.5 g), Fr. 2 (38.6 g), Fr. 3 (21.9 g), Fr. 4 (28.2 g), Fr. 5 (14.2 g), Fr. 6 (22.2 g), Fr. 7 (32.3 g)], as reported previously.[10] Fraction 2 (7.0 g) was subjected to reversed-phase silica gel column chromatography [140 g, MeOH–H_2O (30 : 70→50 : 50, v/v)→MeOH] and finally HPLC [YMC-Pack ODS-A, 20×250 mm, i.d., MeOH–H_2O (40 : 60 or 50 : 50, v/v)] to give (2*S*,3*S*)-1-phenyl-2,3-butanediol 3-*O*-β-D-glucopyranoside (**3**, 153 mg, 0.019%), apigenin 7-*O*-β-D-glucopyranoside (**4**, 113 mg, 0.014%) and diosmetin 7-*O*-β-D-glucopyranoside (**5**, 693 mg, 0.086%). Fraction 6 (20.0 g) was also subjected to reversed-phase silica gel column chromatography [600 g, MeOH–H_2O (30 : 70)→MeOH] and finally HPLC [YMC-Pack ODS-A, 20×250 mm, i.d., MeOH–H_2O (30 : 70, v/v)] to furnish the mixture fraction of (2*S*)- and (2*R*)-eriodyctyol 7-*O*-β-D-glucopyranosiduronic acids (205 mg) and quercetin 3,7-di-*O*-β-D-glucopyranoside (**6**, 156 mg, 0.0038%). The above mixture (20 mg) was further purified by HPLC [YMC-Pack ODS-A, 10×250 mm, i.d., isopropanol–H_2O–AcOH (10 : 90 : 1, v/v)] to yield (2*S*)-eriodictyol 7-*O*-β-D-glucopyranosiduronic acid (**1**, 11 mg, 0.0027%) and (2*R*)-eriodictyol 7-*O*-β-D-glucopyranosiduronic acid (**2**, 9 mg, 0.0023%). The known constituents were identified by comparison of their physical data (IR, UV, and ^{1}H- and ^{13}C-NMR) with those of authentic samples (**4**, **5**)[14] or with reported values.[15]

(2*S*)-Eriodictyol 7-*O*-β-D-Glucopyranosiduronic Acid (**1**): A yellow powder, $[\alpha]_D^{26}$ −35.6° (c=0.20, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{20}O_{12}Na$ $(M+Na)^+$: 487.0852. Found: 487.0867. CD [MeOH, nm ($\Delta\varepsilon$)]: 248 (+0.24), 287 (−3.59), 340 (+0.24). UV [MeOH, nm (log ε)]: 284 (4.21), 326 (3.55). IR (KBr): 3417, 1719, 1655, 1637, 1458, 1071 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: [2.74 (1H, dd, J=3.0, 17.4 Hz), 3.25 (1H, dd, J=12.5, 17.4 Hz), 3-H_2], 5.07 (1H, d, J=7.3 Hz, 1″-H), 5.45 (1H, dd, J=3.0, 12.5 Hz, 2-H), 6.13, 6.16 (1H each, both d, J=2.1 Hz, 6, 8-H), 6.75 (2H, br s, 5′, 6′-H), 6.89 (1H, br s, 2′-H), [8.96 (2H, br s), 12.01 (1H, br s), –OH]. ^{13}C-NMR (125 MHz, DMSO-d_6) δ_c: given in Table 1. Positive-ion FAB-MS: m/z 487 $(M+Na)^+$. Negative-ion FAB-MS: m/z 463 $(M-H)^-$, 287 $(M-C_6H_9O_6)^-$.

(2*R*)-Eriodictyol 7-*O*-β-D-Glucopyranosiduronic Acid (**2**): A yellow powder, $[\alpha]_D^{24}$ −54.5° (c=0.10, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{20}O_{12}Na$ $(M+Na)^+$: 487.0852. Found: 487.0871. CD [MeOH, nm ($\Delta\varepsilon$)]: 252 (−1.00), 292 (+3.66), 334 (−1.80). UV [MeOH, nm (log ε)]: 285 (4.14), 332 (3.48). IR (KBr): 3436, 1719, 1655, 1638, 1458, 1069 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: [2.75 (1H, dd, J=3.4,

17.1 Hz), 3.25 (1H, dd, J=12.2, 17.1 Hz), 3-H_2], 5.10 (1H, d, J=7.6 Hz, 1″-H), 5.44 (1H, dd, J=3.4, 12.2 Hz, 2-H), 6.13, 6.18 (1H each, both d, J=2.1 Hz, 6, 8-H), 6.74 (2H, br s, 5′, 6′-H), 6.90 (1H, br s, 2′-H), [8.95 (2H, br s), 12.00 (1H, br s), –OH]. ^{13}C-NMR (125 MHz, DMSO-d_6) δ_c: given in Table 1. Positive-ion FAB-MS: m/z 487 $(M+Na)^+$. Negative-ion FAB-MS: m/z 463 $(M-H)^-$ 287 $(M-C_6H_9O_6)^-$.

(2*S*,3*S*)-1-Phenyl-2,3-butanediol 3-*O*-β-D-Glucopyranoside (**3**): A white powder, $[\alpha]_D^{27}$ −21.9° (c=2.00, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{16}H_{25}O_7$ $(M+H)^+$: 329.1600. Found: 329.1615. IR (KBr): 3417, 1605, 1507, 1075 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.24 (3H, d, J=4.0 Hz, 4-H_3), [2.59 (1H, br s), 2.79 (1H, br d, J=*ca.* 11 Hz), 1-H_2], 3.20 (1H, m, 3″-H), 3.42 (1H, m, 2″-H), 3.49 (1H, m, 5″-H), 3.55 (1H, m, 4″-H), 3.55 (1H, br s, 3-H), 3.68 (1H, br d, J=*ca.* 11 Hz, 2-H), 3.73 (1H, m, 6″-H_2), 4.44 (1H, br d, J=*ca.* 7 Hz, 1″-H), 7.14—7.25 (5H, m, ph-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_c: given in Table 1. Positive-ion FAB-MS: m/z 329 $(M+H)^+$. Negative-ion FAB-MS: m/z 327 $(M-H)^-$.

Acid Hydrolysis of 1—3 A solution of **1**—**3** (2 mg each) in 5% aqueous H_2SO_4–1,4-dioxane (0.5 ml, 1 : 1, v/v) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was transferred to a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucuronic acid (**i**) and D-glucose (**ii**). GLC conditions: column, Supeluco STB^{TM}-1, 30 m×0.25 mm (i.d.) capillary column; injector temperature, 230 °C, detector temperature, 230 °C; column temperature, 230 °C; He flow rate, 15 ml/min; t_R, **i**, 26.5 min (from **1** and **2**); **ii**, 24.4 min (from **3**).

Enzymatic Hydrolysis of 1 and 2 A solution of the mixture fraction of **1** and **2** (10.0 mg, 0.022 mmol) in 0.2 M acetate buffer (pH 5.0, 5.0 ml) was treated with β-glucuronidase (1.0 ml, Sigma) and the solution was stirred at 37 °C for 14 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 [1.0 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give eriodictyol (**7**, 5.4 mg, 87%).

Acetylation of 3 A solution of **3** (5.0 mg, 0.015 mmol) in pyridine (1.0 ml) was treated with Ac_2O (0.8 ml) and the mixture was stirred at room temperature for 24 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, 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 ordinary-phase silica gel column chromatography [0.5 g, *n*-hexane–AcOEt (1 : 1)] to furnish the pentaacetate (**3a**, 8.3 mg, quant.).

3a: A white powder, $[\alpha]_D^{27}$ −7.8° (c=0.10, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{34}O_{12}Na$ $(M+Na)^+$: 561.1948. Found: 561.1963. IR (KBr): 1755, 1456, 1375, 1229, 1038 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.27 (3H, d, J=6.4 Hz, 4-H_3), 1.98, 2.01, 2.03, 2.03, 2.08 (3H each, all s, Ac-H_3), [2.76 (1H, dd, J=8.8, 14.0 Hz), 2.89 (1H, dd, J=4.8, 14.0 Hz), 1-H_2], 3.69 (1H, m, 5″-H), 3.83 (1H, dd, J=4.9, 6.4 Hz, 3-H), [4.14 (1H, dd, J=2.5, 12.2 Hz), 4.23 (1H, dd, J=4.9, 12.2 Hz), 6″-H_2], 4.65 (1H, d, J=7.9 Hz, 1″-H), 5.04 (1H, dd, J=7.9, 9.8 Hz, 2″-H), 5.06 (1H, ddd, J=4.8, 4.9, 8.8 Hz, 2-H), 5.08 (1H, dd like, 4″-H), 5.20 (1H, dd, J=9.2, 9.8 Hz, 3″-H), 7.16—7.31 (5H, m, ph-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_c: given in Table 1. Positive-ion FAB-MS: m/z 561 $(M+Na)^+$. Negative-ion FAB-MS: m/z 537 $(M-H)^-$.

Enzymatic Hydrolysis of 3 A solution of **3** (20.0 mg, 0.061 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 24 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 [1.0 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give (2*S*,3*S*)-1-phenyl-2,3-butanediol (**8**, 9.8 mg, 97%). Compound **8** was identified by comparison of the physical data (IR, $[\alpha]_D$, and ^{1}H- and ^{13}C-NMR) with those of reported values.[22]

Bioassay Aldose reductase activity was assayed by the method described in a previous paper.[10,20] 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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