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Synthesis and Aldose Reductase Inhibitory Activity of Thiazolidinecarboxylic Acids Containing a Disulfide Bond

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Symmetrical disulfides (**2a**—**m**, **9**, **13**) were synthesized by oxidation or by a new reaction using ethyl α-bromomalonate from the corresponding thiazolidinecarboxylic acids containing a sulfhydryl group (**1a**—**m**, **8**, **12**). Mixed disulfides (**14a**—**g**) were synthesized by reaction of thiols (**1a**, **c**) with Bunte salt. Stereoselective acylation of the thiazolidinecarboxylic acid **5a** gave the symmetrical disulfide **2c** or the dicarboxylic acid **15**, depending on the conditions. The absolute configurations of these disulfides were decided to be (2*R*,2′*R*,4*R*,4′*R*) by comparison of nuclear magnetic resonance spectra and specific rotation with those of the (2*S*,2′*S*,4*R*,4′*R*)-disulfide (**4**). The disulfides were tested for aldose reductase inhibitory activity *in vitro*. The symmetrical disulfide **2j** and dicarboxylic acid **15** showed remarkably high potency.

Keywords——symmetrical disulfide; mixed disulfide; thiol; ethyl α-bromomalonate; Bunte salt; 4-thiazolidinecarboxylic acid; stereoselective acylation; absolute configuration; aldose reductase inhibitor; (2*R*,2′*R*,4*R*,4′*R*)-3,3′-(3,3′-dithiodipropionyl)bis[2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid]

We reported previously that (2*R*,4*R*)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (SA 446) (**1c**) showed potent inhibitory activity against angiotensin-converting enzyme (ACE).[1] Synthesis of related compounds and various screening tests thereof were carried out, and it was found that the disulfide **2c**, an oxidized form of **1c**, showed an entirely different kind of activity: inhibition of aldose reductase (AR). In this paper, we describe the AR inhibitory activity of this compound and related disulfides. This enzyme reduces aldoses to the corresponding sugar alcohols and is involved in the initiation of cataracts associated with diabetes and galactosemia.[2] Abnormally high levels of glucose and galactose in blood lead to the accumulation of sorbitol and galactitol in tissues. It is considered that this accumulation causes swelling of cells to induce complications such as cataract, retinopathy, nephropathy and neuropathy.[3] Therefore, Kinoshita and co-workers suggested that effective AR inhibitors might be useful for the prevention or treatment of these complications.[2]

Drugs found during initial research on AR inhibitors include tetramethylene glutaric acid,[4] alrestatin,[5] flavonoids such as quercitrin,[6] chromone-2-carboxylic acids,[7] sorbinil[8] and 7-sulfamoylxanthene-2-carboxylic acids.[9] More recent reports have dealt with isoxazolidine-3,5-diones,[10] spiro oxazolidine-diones,[11] thiazolidine-2,4-diones[12] and compounds having hydantoin structure[13] as AR inhibitors. We are interested in the activity of **2c**, which differs from the above compounds in structure.

Chemistry

The symmetrical disulfides in Table I were synthesized by the following method: these disulfides (**2a**—**m**) were obtained by oxidation of the corresponding thiols (**1a**—**m**)[1,14] with potassium triiodide (method A, Chart 1). The disulfide **2c** was also obtained by acylation of the thiazolidinecarboxylic acid **5a**[15] (prepared by reaction of salicylaldehyde with (*R*)-cysteine) with half the molar quantity of dithiodipropionyl chloride in aqueous sodium carbonate solution (method B, Chart 1). Compound **2c** isolated by this method was identical with that isolated by method A. On the other hand, the (2*S*,2′*S*,4*R*,4′*R*)-disulfide **4**, a diastereoisomer of **2c**, was obtained by method A from the (2*S*,4*R*)-thiol **3**.[14] Both the specific rotations and proton nuclear magnetic resonance (^{1}H-NMR) spectral patterns of **2a**—**m** differed from those of **4**: **2a**—**m** were dextrorotatory, whereas **4** was levorotatory (−295.0°, Table I): **2a**—**m** possessed larger coupling constants ($J_{AX}+J_{BX}$) of the ABX system consisting of C_4-H, C_5-H_A and C_5-H_B on the thiazolidine ring as compared with **4** ($J_{AX}+J_{BX}=5.4$ Hz, Table III).[16] On the basis of these results, the absolute configurations of **2a**—**m** were decided to be (2*R*,2′*R*,4*R*,4′*R*). It is, therefore, possible to conclude that the absolute configurations of the thiols (**1a**—**m**) used as starting materials are (2*R*,4*R*), although those of **1a** and **1c** have been already determined.[14]

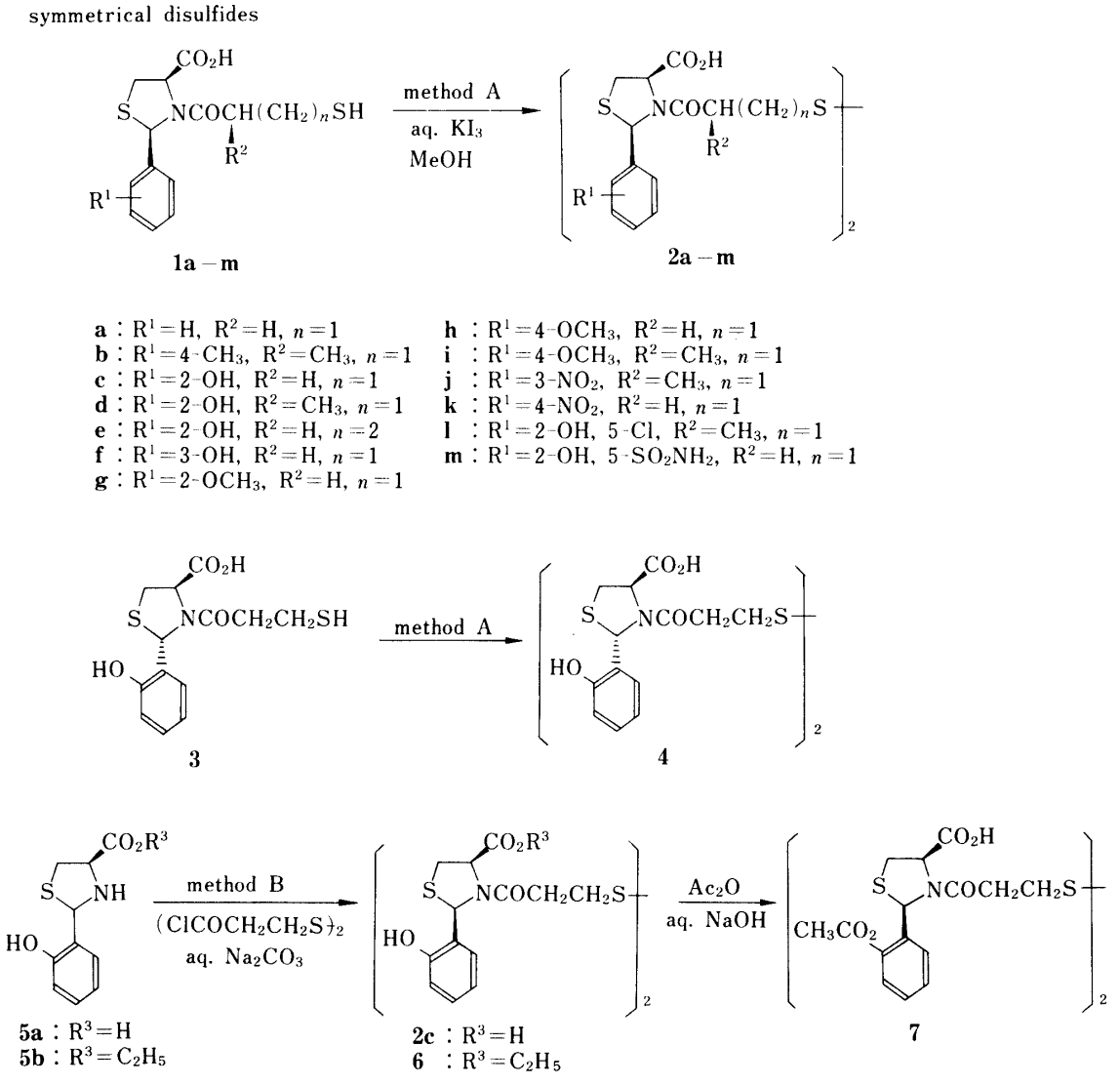

Chart 1

The acylation of **5b** (prepared by reaction of salicylaldehyde with (*R*)-cysteine ethyl ester) by method B in aqueous solution gave **6** in high yield, whereas **6** could not be isolated in this acylation in methylene chloride because of the formation of diastereomeric mixtures of (*R*)- and (*S*)-aryl group (Chart 1). Since **6** was dextrorotatory (+ 162.7 °, Table I) and possessed a large coupling constant ($J_{AX}+J_{BX}=15$ Hz, Table III), its absolute configurations were decided to be (2*R*,2′*R*,4*R*,4′*R*). Consequently, it is clear that the acylation in aqueous solution was stereoselective.

The acetate **7** was obtained by treatment of **2c** with acetic anhydride (Chart 1).

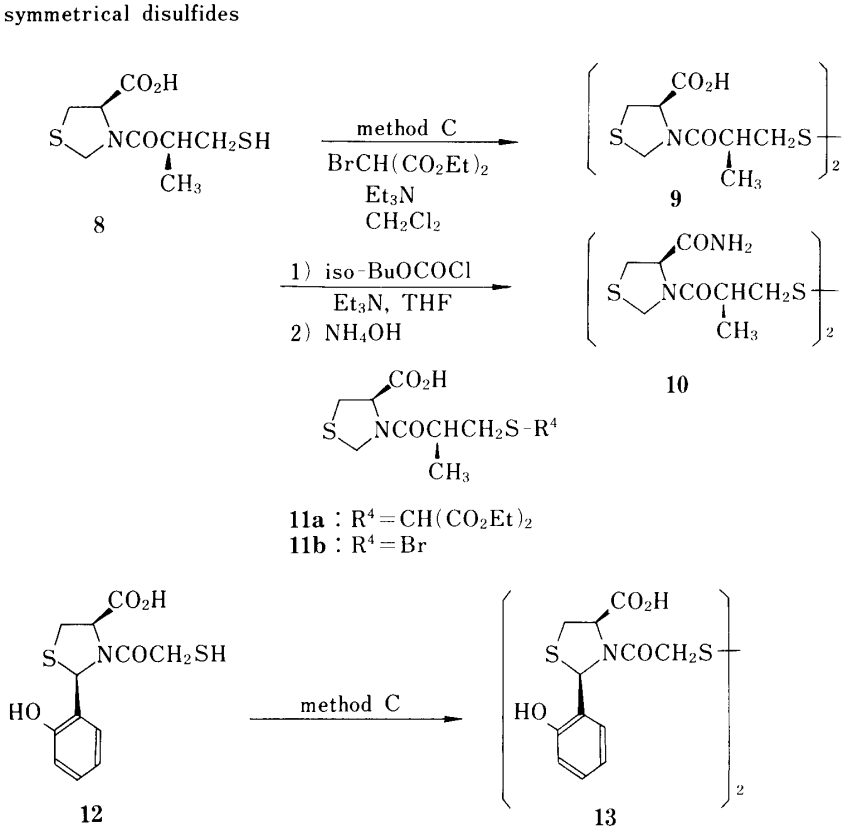

Chart 2

The disulfides **9** and **13** were synthesized by reaction of the thiols **8** and **12** with ethyl α-bromomalonate in the presence of triethylamine in methylene chloride (method C, Chart 2). This reaction was developed on the basis of the following result: only **9** was unexpectedly obtained when reaction of **8** with the bromide was carried out in order to obtain **11a**. It is considered that the reaction proceeded by either path a or b: (a) **8** reacted with the bromide to give the intermediate **11a**, which reacted with **8** to give **9** and ethyl malonate; (b) **8** reacted with the bromide to give ethyl malonate and the intermediate **11b**, which reacted with **8** to give **9**. Various disulfides are now being synthesized by this method. The acid **9** was treated with isobutyl chloroformate, followed by treatment with aqueous ammonia to give the amide **10** in 62% yield (Chart 2). The physicochemical properties of the above compounds are shown in Tables I and III.

The mixed disulfides (**14a—g**) in Table II were synthesized by reaction of the thiols (**1a, c**) with Bunte salt (*S*-alkyl thiosulfate) in an aqueous alkaline solution (method D, Chart 3).[17] Compound **5a** reacted with dithiodipropionic anhydride in the presence of triethylamine to

TABLE I. Symmetrical Disulfides and Their Inhibitory Activity towards Aldose Reductase

Compd. No.	Prepn. method	Yield (%)	mp (°C) (Recrystn. solv.)	$[\alpha]_D$ deg. (*c*, MeOH, °C)	IR $\nu_{C=O}^{Nujol}$ cm^{-1} CO_2	IR $\nu_{C=O}^{Nujol}$ cm^{-1} CON	Activity[a] IC_{50} (M)
2a	A	61	97—102 (EtOH–H_2O)	+109.0 (0.5, 26)	1730	1640	1.4×10^{-7}
2b	A	97	99—105[b]	+114.2 (0.5, 23)	1725	1620	8.9×10^{-8}
2c	A	93	187 (dec.)	+165.1	1722	1622	1.8×10^{-7}
	B	85	(AcOEt)	(1.1, 25)			
	C	56					
2d	A	87	154—157 (dec.) (MeOH–H_2O)	+76.9 (1.0, 23)	1723	1634	7.8×10^{-8}
2e	A	70	110—124[b] (MeOH–H_2O)	+141.4 (0.5, 26)	1723	1610	7.9×10^{-8}
2f	A	87	88—116[b]	+113.6 (0.5, 23)	1720	1625	3.0×10^{-7}
2g	A	72	90—92.5[b]	+161.3 (1.2, 24)	1740 1720	1625	2.2×10^{-7}
2h	A	98	85—104[b]	+127.5 (1.0, 26)	1740	1635	4.3×10^{-8}
2i	A	94	102—121[b]	+32.6 (0.5, 25)	1737	1610	1.9×10^{-7}
2j	A	93	120—125 (dec.)[b]	+38.2 (0.6, 25)	1738	1635[c]	5.8×10^{-9}
2k	A	87	93—123[b]	+130.1 (0.5, 25)	1720	1625	5.5×10^{-8}
2l	A	98	147—155[b]	+24.3 (0.5, 23)	1735	1650	2.2×10^{-7}
2m	A	85	141—150 (dec.)[b]	+166.8 (1.0, 26)	1725	1625[d]	6.2×10^{-8}
4	A	58	132—143[b]	−295.0 (0.3, 24)	1723	1625	39%[e]
6	B	68	195—201 (DMF–H_2O)	+162.7[f] (0.5, 24)	1745	1625	—[g]
7		88[h]	119—120 (dec.)[b]	+116.9 (1.0, 24)	1765 1738	1645	3.2×10^{-7}
9	C	91	208—209 (dec.) (EtOH–H_2O)	−202.0[i] (0.5, 24)	1753 1721	1607	5.8×10^{-7}
10		62[j]	179—180 (EtOH–H_2O)	−258.3 (0.5, 25)		1682[k] 1656	19%[e]
13	C	71	128—129 (dec.) (MeOH–H_2O)	+231.4 (0.5, 26)	1723	1629	1.3×10^{-7}

a) Molar concentration that causes 50% inhibition of partially purified rat lens aldose reductase activity towards glyceraldehyde. *b*) Amorphous solid. *c*) $\nu_{N=O}^{Nujol}$ 1523 cm^{-1}. *d*) $\nu_{S=O}^{Nujol}$ 1310 cm^{-1}. *e*) Inhibition (%) at 10^{-6} M. *f*) Measured in dimethylformamide (DMF). *g*) Could not be measured because **6** was insoluble in the medium. *h*) Yield from **2c**. *i*) Measured in EtOH. *j*) Yield from **9**. *k*) Measured by the KBr disk method.

give the dicarboxylic acid **15** in low yield (Chart 3). The absolute configurations of **14a**—**g** and **15** were decided to be (2*R*,4*R*), because they were dextrorotatory (Table II) and showed a large coupling constant (Table III).

Biological Results and Discussion

The disulfides listed in Tables I and II were examined for aldose reductase (AR) inhibitory activity *in vitro*. The assay was carried out in the manner of Varma and Kinoshita[6]

Table II. Mixed Disulfides and Their Inhibitory Activity towards Aldose Reductase

Compd. No.	Yield (%)	mp (°C) (Recrystn. solv.)	$[\alpha]_D$ deg. (*c*, MeOH, °C)	IR $\nu_{C=O}^{Nujol}$ cm^{-1} CO_2H	CON	Activity[a] IC_{50} (M)
14a	67	98—100 (Ether)	+88.9 (0.6, 24)	1743	1617	4.3×10^{-7}
14b	51	92—94 (AcOEt)	+119.5 (1.0, 25)	1738	1685 1625	2.3×10^{-7}
14c	31	Amorph.[b]	+127.6 (0.5, 25)	1720	1625	1.4×10^{-7}
14d	29	Oil[b]	+93.8 (0.5, 24)	1722	1625[c]	4.7×10^{-7}
14e	60	Amorph.[b]	+132.0 (0.5, 24)	1723	1622[c]	4.7×10^{-7}
14f	22	168—168.5 (dec.) (EtOH–AcOEt)	+133.9 (1.0, 26)	1745	1620	4.8×10^{-7}
14g	21	154.5—155 (dec.) (EtOH–isopropyl ether)	+78.4 (0.6, 26)	1710	1615	27%[d]
15	7	150.5—151.5 ($AcOEt-CHCl_3$)	+83.8 (0.7, 25)	1710	1625	1.4×10^{-8}
1c[e]						1.6×10^{-6}
Quercitrin						1.4×10^{-7}[f]

a) See the corresponding footnote in Table I.
b) These compounds were purified by column chromatography on silica gel.
c) Measured by the film method. *d*) Inhibition (%) at 10^{-6} M.
e) Reference 1. *f*) Mean of IC_{50} (S.D. $\pm0.9\times10^{-7}$; $n=13$).

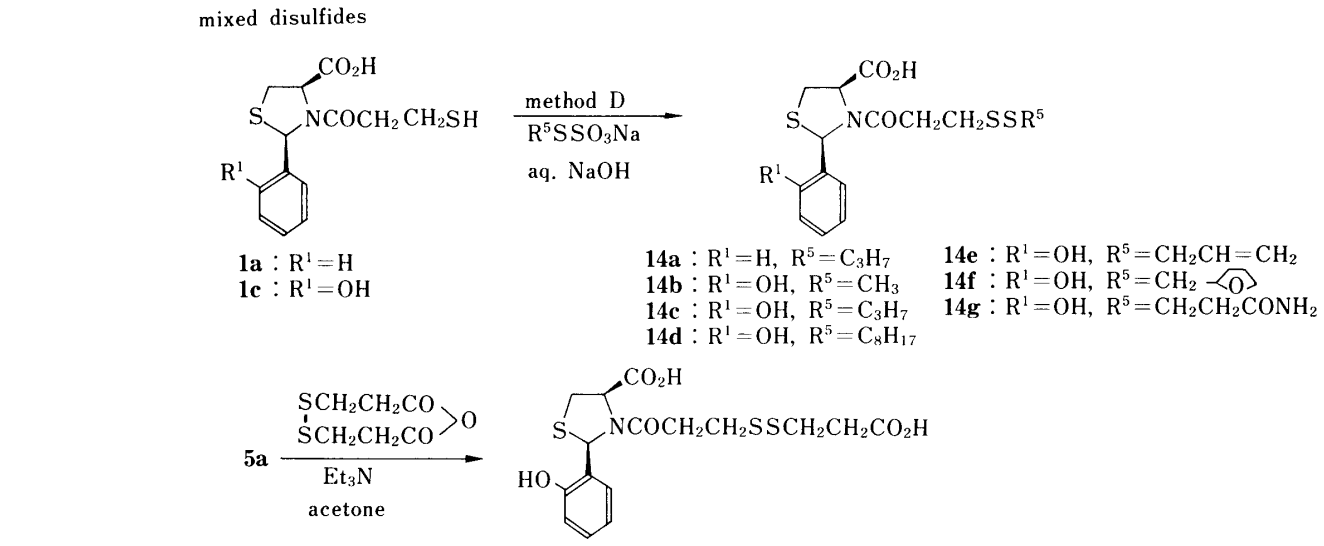

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Chart 3

with AR partially purified from rat lenses mainly according to the procedure of Hayman and Kinoshita[18] without the column chromatography. Quercitrin was used as a control drug.[6]

We found that conversion of the thiol **1c** to the disulfide **2c** resulted in marked inhibitory activity against AR: **2c** showed activity equal to that of quercitrin. Therefore, the symmetrical disulfides (**2a—m**, **9**, **10**, **13**) were initially examined for this type of activity. Substitution of an

(*R*)-aryl group for the thiazolidine ring increased the activity (compounds **2a**—**m** *vs.* compound **9**), whereas the substitution of an (*S*)-aryl group decreased the activity (compound **4**). In this group, the 3-nitrophenyl disulfide (**2j**) showed the most potent activity (IC_{50}: 5.8×10^{-9} M). On the other hand, conversion of the carboxyl group into an amide led to low activity (compound **9** *vs.* compound **10**). These results suggest that carboxyl and 3-nitrophenyl groups in *cis*-configuration are necessary for strong inhibitory activity against AR.

Next, we tested the activity of unsymmetrical mixed disulfides in order to check the effect of the disulfide bond. Mixed disulfides (**14a**—**f**) showed a higher potency than the original thiol (**1c**). Introduction of a carboxyl group into the disulfide moiety increased the activity: the dicarboxylic acid **15** showed a high activity (IC_{50}: 1.4×10^{-8} M) and was ten times more active than the symmetrical disulfide **2c**. Amidation of one carboxyl group of **15** was accompanied by a loss of potency (compound **14g**). Consequently, the disulfide bond and carboxyl group are important functional groups for the activity.

The above structure–activity relationship suggests that a dicarboxylic acid which has a disulfide bond between a (2*R*,4*R*)-2-(3-nitrophenyl)-4-thiazolidinecarboxylic acid moiety and another carboxylic acid moiety should show effective inhibitory activity against AR.

Experimental

Melting points were determined on a Yamato MP-21 melting point apparatus and are uncorrected. Specific rotations were measured with a JASCO DIP-4 polarimeter. Infrared (IR) spectra were recorded on a JASCO IR-E spectrophotometer. NMR spectra were measured on a JEOL PMX-60 spectrometer using tetramethylsilane as an internal standard.

Method A: (2*R*,2′*R*,4*R*,4′*R*)-3,3′-[3,3′-Dithiobis[(2*S*)-2-methylpropionyl]]bis[2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic Acid] (2d)——A stirred solution of (2*R*,4*R*)-2-(2-hydroxyphenyl)-3-[(2*S*)-3-mercapto-2-methylpropionyl]-4-thiazolidinecarboxylic acid (**1d**) (326 mg, 1.0 mmol) in MeOH (15 ml) was treated in a dropwise manner with 0.1 N KI_3 (9.9 ml). The resulting mixture was continuously stirred for 15 min at room temperature and then the MeOH was removed *in vacuo*. The crystals were collected and recrystallized from MeOH–H_2O to give **2d** (296 mg, 87%).

Method B: (2*R*,2′*R*,4*R*,4′*R*)-3,3′-(3,3′-Dithiodipropionyl)bis[2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic Acid] (2c)——Dithiodipropionyl chloride (3.0 g, 0.012 mol) was added dropwise to a stirred solution of (4*R*)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid (**5a**) (4.5 g, 0.02 mol) and Na_2CO_3 (3.0 g, 0.028 mol) in H_2O (70 ml), with cooling in an ice-water bath. The resulting mixture was continuously stirred for 1 h and then acidified with 2 N HCl. The crystals were collected and recrystallized from AcOEt to give **2c** (5.3 g, 85%).

The disulfide **6** was obtained from **5b** by this method in the presence of tetrahydrofuran (THF).

Method C: (4*R*,4′*R*)-3,3′-[3,3′-Dithiobis[(2*S*)-2-methylpropionyl]]bis(4-thiazolidinecarboxylic Acid) (9)——Ethyl α-bromomalonate[19] (1.6 g, 6.7 mmol) in CH_2Cl_2 (10 ml) was added dropwise to a stirred solution of (4*R*)-3-[(2*S*)-3-mercapto-2-methylpropionyl]-4-thiazolidinecarboxylic acid (**8**) (3.1 g, 13.2 mmol) and triethylamine (2.9 ml, 21 mmol) in CH_2Cl_2 (40 ml) with cooling in an ice-salt bath. The mixture was stirred for 0.5 h, the ice-salt bath was removed, and the reaction mixture was continuously stirred for 0.5 h followed by extraction with 10% aq. $NaHCO_3$ (20 ml). The aqueous layer was washed with ether (20 ml) and then acidified with 4 N HCl. The crystals were collected and recrystallized from EtOH–H_2O to give **9** (2.8 g, 91%).

The CH_2Cl_2 layer was washed with water, dried over Na_2SO_4 and evaporated *in vacuo* to give ethyl malonate (1.0 g, 93%). Its identity was checked by ^{1}H-NMR spectral comparison with an authentic sample.

Method D: (2*R*,4*R*)-2-(2-Hydroxyphenyl)-3-(3-methyldithiopropionyl)-4-thiazolidinecarboxylic Acid (14b)——Sodium methylthiosulfate[17] (1.8 g, 12 mmol) in H_2O (5 ml) was added to a stirred solution of (2*R*,4*R*)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (**1c**) (1.0 g, 3.2 mmol) in 1 N NaOH (10 ml). The resulting solution was vigorously stirred for 1 min at room temperature, acidified with 3 N HCl and extracted with AcOEt (20 ml). The organic layer was washed with water, dried over $MgSO_4$, and evaporated *in vacuo*. The residual oil was purified by column chromatography on silica gel ($CHCl_3$–AcOEt system) and recrystallized from AcOEt to give **14b** (0.7 g, 51%).

Ethyl (4*R*)-2-(2-Hydroxyphenyl)-4-thiazolidinecarboxylate (5b)——Triethylamine (10.1 g, 0.1 mol) and then salicylaldehyde (12.2 g, 0.1 mol) were added to a stirred suspension of L-cysteine ethyl ester hydrochloride (18.6 g, 0.1 mol) in $CHCl_3$ (100 ml). The resulting mixture was continuously stirred for 2 h at room temperature, then washed with H_2O (100 ml) and evaporated *in vacuo*. The residual solid was recrystallized from isopropyl ether to give **5b** (21.7 g, 86%): mp 75—77 °C; $[\alpha]_D^{25}$ −105.1° (c=0.9, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1730 (CO_2). ^{1}H-NMR ($CDCl_3$) δ: 1.26

TABLE III. ^{1}H-NMR Spectral Data [δ (ppm), J = Hz in DMSO-d_6] for the Disulfides[a)]

Compd. No.	C_4-H (1H)	$J_{AX}+J_{BX}$	C_2-H (1H)	Others
2b	4.73 (t, J=6.0) and 4.97—5.30 (m) (3:1)	12.0	6.12 (s) and 6.28 (s) (1:3)	0.33—0.92 (br) and 0.92—1.33 (br) (3:1) (6H, $CH_3\times2$), 2.27 (6H, s, $CH_3\times2$), 2.40—2.97 (6H, m, $COCHCH_2S\times2$), 3.07 (2H, dd, J=6.0, 12.0, C_5-$H_B\times2$), 3.37 (2H, dd, J=6.0, 12.0, C_5-$H_A\times2$), 7.13 (4H, d, J=8.0, aromatic H), 7.57 (4H, d, J=8.0, aromatic H), 9.50—14.00 (2H, br, $CO_2H\times2$)
2c	4.63 (dd, J=6.0, 8.0) and 4.87—5.27 (m) (5:2)	14.0	6.30 (s)	1.80—2.97 (8H, m, $COCH_2CH_2S\times2$), 3.02 (2H, dd, J=8.0, 11.0, C_5-$H_B\times2$), 3.35 (2H, dd, J=6.0, 11.0, C_5-$H_A\times2$), 6.47—7.30 (6H, m, aromatic H), 7.50 (br d, J=7.0) and 7.82 (d, J=6.0) (2:5) (2H, aromatic H), 9.30—9.57 (br) and 9.57—10.13 (br) (2:5) (2H, $OH\times2$), 11.80—14.30 (2H, br, $CO_2H\times2$)
2d	4.63 (dd, J=6.0, 9.0)	15.0	6.30 (s) and 6.45 (s) (1:5)	0.33—1.00 (br) and 1.00—1.30 (br) (5:1) (6H, $CH_3\times2$), 2.23—2.90 (6H, m, $COCHCH_2S\times2$), 3.03 (2H, dd, J=9.0, 11.0, C_5-$H_B\times2$), 3.40 (2H, dd, J=6.0, 11.0, C_5-$H_A\times2$), 3.93—6.20 (3H, br, $3/2H_2O$), 6.57—7.43 (6H, m, aromatic H), 7.62 (br d, J=6.0) and 7.88 (d, J=7.0) (1:5) (2H, aromatic H), 9.37—9.65 (br) and 9.87 (br s) (1:5) (2H, $OH\times2$), 10.30—14.00 (2H, br, $CO_2H\times2$)
2h	4.73 (t, J=7.0) and 4.95—5.23 (m) (3:1)	14.0	6.17 (s) and 6.30 (s) (1:3)	1.53—2.97 (8H, m, $COCH_2CH_2S\times2$), 3.00—3.60 (4H, m, C_5-$H\times4$), 3.72 (6H, s, $OCH_3\times2$), 6.53—7.08 (4H, m, aromatic H), 7.13—7.88 (4H, m, aromatic H), 9.53—12.53 (2H, br, $CO_2H\times2$)
2j	4.77 (dd, J=7.0, 8.0) and 4.98—5.38 (m) (1:1)	15.0	6.30 (s) and 6.58 (s) (1:1)	0.43—0.95 (br) and 0.95—1.40 (br) (1:1) (6H, $CH_3\times2$), 2.10—3.90 (10H, m, C_5-$H\times4$, $COCHCH_2S\times2$), 5.60—7.43 (2H, br, $CO_2H\times2$), 7.45—8.97 (8H, m, aromatic H)
2k	4.77 (dd, J=7.0, 8.0) and 5.03—5.40 (m) (1:1)	15.0	6.28 (s) and 6.57 (s) (1:1)	1.70—2.97 (8H, m, $COCH_2CH_2S\times2$), 3.00—3.82 (4H, m, C_5-$H\times4$), 6.85—9.75 (2H, br, $CO_2H\times2$), 7.60—8.47 (8H, m, aromatic H)
2l	4.65 (dd, J=6.0, 9.0) and 4.95—5.33 (m) (5:1)	15.0	6.23 (s) and 6.40 (s) (1:5)	0.40—0.98 (br) and 0.98—1.43 (br) (5:1) (6H, $CH_3\times2$), 2.10—2.92 (6H, m, $COCHCH_2S\times2$), 3.05 (2H, dd, J=9.0, 12.0, C_5-$H_B\times2$), 3.40 (2H, dd, J=6.0, 12.0, C_5-$H_A\times2$), 6.82 (2H, d, J=9.0, aromatic H), 7.12 (2H, dd, J=2.0, 9.0, aromatic H), 8.02 (2H, d, J=2.0, aromatic H), 9.40—10.60 (2H, br, $OH\times2$), 10.60—13.80 (2H, br, $CO_2H\times2$)
4b[b)]	5.14 (d, J=5.4) and 5.24—5.48 (m) (3:2)	5.4	6.24 (s) and 6.29 (s) (3:1)	1.80—2.98 (8H, m, $COCH_2CH_2S\times2$), 2.98—3.88 (14H, m, C_5-$H\times4$, $H_2O\times5$), 6.44—7.22 (8H, m, aromatic H), 9.70 (br s) and 9.80—10.30 (m) (1:1) (2H, $OH\times2$), 11.70—14.40 (2H, br, $CO_2H\times2$)
6	4.69 (dd, J=6.0, 9.0) and 4.97—5.30 (m) (5:1)	15.0	6.33 (s)	1.26 (6H, t, J=7.0, $CH_3\times2$), 1.90—2.80 (8H, m, $COCH_2CH_2S\times2$), 3.03 (2H, dd, J=9.0, 12.0, C_5-$H_B\times2$), 3.35 (2H, dd, J=6.0, 12.0, C_5-$H_A\times2$), 4.21 (4H, q, J=7.0, $CH_2\times2$),

				6.47—7.28 (6H, aromatic H), 7.43 (d, $J=7.0$) and 7.80 (d, $J=7.0$) (1 : 5) (2H, aromatic H), 9.53 (br s) and 9.78 (br s) (1 : 5) (2H, OH × 2)
7	4.70 (dd, $J=6.0$, 9.0) and 4.90—5.22 (m) (3 : 1)	15.0	6.25 (br s) and 6.33 (br s)	2.28 (6H, s, CH_3CO_2 × 2), 2.38—2.92 (8H, m, $COCH_2CH_2S$ × 2), 3.03 (2H, dd, $J=9.0$, 12.0, C_5-H_B × 2), 3.40 (2H, dd, $J=6.0$, 12.0, C_5-H_A × 2), 4.20—6.50 (6H, br, H_2O × 3), 6.60—7.40 (6H, m, aromatic H), 7.65—8.30 (2H, m, aromatic H), 10.00—14.00 (2H, br, CO_2H × 2)
9	4.08—5.27 (6H, m)[c]			1.13 (6H, d, $J=5.5$, CH_3 × 2), 2.38—3.63 (10H, m, C_5-H × 4, $COCHCH_2S$ × 2), 11.62—13.60 (2H, br, CO_2H × 2)
10	4.10—5.02 (6H, m)[c]			1.13 (6H, d, $J=5.0$, CH_3 × 2), 2.20—3.67 (10H, m, C_5-H × 4, $COCHCH_2S$ × 2), 6.65—7.57 (4H, m, $CONH_2$ × 2)
13	4.58 (dd, $J=6.0$, 8.0) and 4.92—5.30 (m) (3 : 1)	14.0	6.28 (br s) and 6.37 (br s)	2.10—8.20 (9H, br, OH, CO_2H × 2, H_2O × 3), 3.02 (2H, dd, $J=8.0$, 11.0, C_5-H_B × 2), 3.15 (3H, s, CH_3OH), 3.33 (2H, dd, $J=6.0$, 11.0, C_5-H_A × 2), 3.45 and 3.78 (4H, ABq, $J=8.0$, $COCH_2S$ × 2), 6.52—7.28 (6H, m, aromatic H), 7.43 (d, $J=7.0$) and 7.80 (d, $J=7.0$) (1 : 3) (2H, aromatic H), 9.20—10.35 (2H, br, OH × 2)
14b[d]	4.78 (t, $J=7.0$)	14.0	6.37 (s)	1.88 (3H, s, CH_3), 2.27—2.88 (4H, m, $COCH_2CH_2S$), 3.23 (2H, d, $J=7.0$, C_5-H × 2), 6.57—7.17 (3H, m, aromatic H), 7.90 (1H, d, $J=7.0$, aromatic H), 9.43—11.40 (2H, br, OH, CO_2H)
14e	4.60 (dd, $J=6.5$, 9.0)	15.5	6.30 (s)	1.87—3.67 (8H, m, C_5-H × 2, $COCH_2CH_2SSCH_2$), 4.80—5.32 (2H, m, $CH=CH_2$), 5.32—6.03 (1H, m, $CH=CH_2$), 6.50—7.33 (3H, m, aromatic H), 7.50 (br d, $J=6.0$) and 7.83 (d, $J=7.0$) (1 : 3) (1H, aromatic H), 9.50 (br s) and 9.78 (br s) (1 : 3) (1H, OH), 10.33—14.33 (1H, br, CO_2H)
14f	4.62 (dd, $J=6.0$, 9.0) and 4.90—5.28 (m) (4 : 1)	15.0	6.32 (s)	1.23—2.37 (4H, m, CH_2CH_2), 2.53—3.08 (4H, m, $COCH_2CH_2S$), 3.02 (1H, dd, $J=9.0$, 12.0, C_5-H_B), 3.35 (1H, dd, $J=6.0$, 12.0, C_5-H_A), 3.43—4.20 (3H, m, $CHOCH_2$), 6.45—7.28 (3H, m, aromatic H), 7.50 (d, $J=7.0$) and 7.83 (d, $J=7.0$) (1 : 4) (1H, aromatic H), 9.45 (br s) and 9.75 (br s) (1 : 4) (1H, OH), 12.28—13.38 (1H, br, CO_2H)
14g	4.68 (dd, $J=6.0$, 9.0) and 4.93—5.30 (m) (4 : 1)	15.0	6.38 (s)	2.07—2.95 (8H, m, $COCH_2CH_2S$ × 2), 3.07 (1H, dd, $J=9.0$, 11.5, C_5-H_B), 3.38 (1H, dd, $J=6.0$, 11.5, C_5-H_A), 6.53—7.47 (5H, m, aromatic H, $CONH_2$), 7.58 (d, $J=7.0$) and 7.92 (d, $J=7.0$) (1 : 4) (1H, aromatic H), 9.27—10.40 (1H, OH), 11.60—14.00 (1H, br, CO_2H)
15	4.61 (dd, $J=6.0$, 9.0) and 4.90—5.20 (m) (5 : 1)	15.0	6.31 (s)	2.07—2.99 (8H, m, CH_2CH_2S × 2), 3.03 (1H, dd, $J=9.0$, 11.5, C_5-H_B), 3.33 (1H, dd, $J=6.0$, 11.5, C_5-H_A), 6.57—7.27 (3H, m, aromatic H), 7.53 (d, $J=7.0$) and 7.83 (d, $J=7.0$) (1 : 5) (1H, aromatic H), 9.00—10.70 (1H, br, OH), 11.30—13.70 (1H, br, CO_2H)

a) Typical NMR data are listed in this Table. *b*) Measured on a JEOL FX-100 spectrometer.
c) Signals of C_2-H and C_4-H overlapped each other. *d*) Measured in $CDCl_3$–DMSO-d_6.

Table IV. Elemental Analyses

Compd. No.	Formula	Analysis (%) Calcd (Found)		
		C	H	N
2c	$C_{26}H_{28}N_2O_8S_4$	49.99	4.52	4.48
		(50.10	4.52	4.54)
2d	$C_{28}H_{32}N_2O_8S_4 \cdot 3/2H_2O$	49.47	5.19	4.12
		(49.75	5.01	4.15)
6	$C_{30}H_{36}N_2O_8S_4$	52.92	5.33	4.11
		(52.55	5.35	4.33)
9	$C_{16}H_{24}N_2O_6S_4$	41.01	5.16	5.98
		(41.04	5.16	5.98)
10	$C_{16}H_{26}N_4O_4S_4 \cdot 1/2H_2O$	40.40	5.72	11.78
		(40.30	5.71	11.62)
13	$C_{24}H_{24}N_2O_8S_4 \cdot 3H_2O \cdot CH_4O$[a]	43.98	5.02	4.10
		(44.09	4.83	4.20)
14b	$C_{14}H_{17}NO_4S_3 \cdot C_4H_8O_2$[b]	48.30	5.63	3.13
		(48.46	5.62	3.12)
14f	$C_{18}H_{23}NO_5S_3$	50.33	5.40	3.26
		(50.39	5.41	3.32)
14g	$C_{16}H_{20}N_2O_5S_3$	46.13	4.85	6.73
		(46.06	4.85	6.67)
15	$C_{16}H_{19}NO_6S_3$	46.02	4.60	3.36
		(45.89	4.59	3.33)

a) CH_4O is MeOH. b) $C_4H_8O_2$ is AcOEt.

(3H, t, J=7.5 Hz, CH_3), 3.13 (1H, dd, J=6.0, 10.5 Hz, C_5-H_B), 3.50 (1H, dd, J=7.0, 10.5 Hz, C_5-H_A), 3.97 (1H, dd, J=6.0, 7.0 Hz, C_4-H), 4.18 (2H, q, J=7.5 Hz, CH_2), 4.95—7.47 (2H, br, OH, NH), 5.53 (s) and 5.82 (s) (1 : 3) (1H, C_2-H), 6.55—7.37 (4H, m, aromatic H).

(2*R*,2′*R*,4*R*,4′*R*)-3,3′-(3,3′-Dithiodipropionyl)bis[2-(2-acetoxyphenyl)-4-thiazolidinecarboxylic Acid] (7)——Acetic anhydride (0.42 ml) was added dropwise to a stirred solution of **2c** (1.3 g, 2.1 mmol) (prepared according to method B) in 1 N NaOH (8.6 ml). The resulting mixture was vigorously stirred for 2 h at room temperature and acidified with conc. HCl. The precipitate was collected to give **7** (1.3 g, 88%).

(4*R*,4′*R*)-3,3′-[3,3′-Dithiobis[(2*S*)-2-methylpropionyl]]bis(4-thiazolidinecarboxamide) (10)——A stirred solution of **9** (1.9 g, 4.05 mmol) and triethylamine (1.15 ml, 8.25 mmol) in anhydrous THF (20 ml) was treated dropwise with isobutyl chloroformate (1.05 ml, 8.07 mmol) with cooling in an ice-salt bath. The resulting mixture was continuously stirred for 15 min and, after addition of 30% aqueous ammonia solution (2 ml), was further stirred for 1 h at room temperature followed by addition of H_2O (100 ml). The crystals were collected and recrystallized from EtOH–H_2O to give **10** (1.2 g, 62%).

(2*R*,4*R*)-3-[3-(2-Carboxyethyldithio)propionyl]-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic Acid (15)——Dithiodipropionic anhydride[20] (2.0 g, 10.4 mmol) was added to a stirred solution of **5a** (2.0 g, 8.9 mmol) and triethylamine (1.7 ml, 12.2 mmol) in anhydrous acetone (15 ml). The resulting mixture was continuously stirred for 40 min at room temperature and extracted with AcOEt (30 ml) after addition of H_2O (50 ml) containing a small amount of K_2CO_3. The aqueous layer was acidified with 1 N HCl and extracted with AcOEt (30 ml). The second organic layer was washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residual oil was purified by column chromatography on silica gel ($CHCl_3$–AcOEt system) and recrystallized from AcOEt–$CHCl_3$ to give **15** (250 mg, 7%).

Assay of Aldose Reductase Activity——Aldose reductase was prepared from rat lenses in the following manner. Lenses (100 to 150) were homogenized in 3 volumes of distilled water, followed by centrifugation at 10000 × ***g*** for 30 min to remove insoluble material. Saturated ammonium sulfate was added to the supernatant fluid to 30% saturation. The suspension was centrifuged after 15 min and the supernatant was recovered. Aldose reductase was precipitated from the 30% saturated solution by the addition of powdered ammonium sulfate to 75% saturation, and was obtained by centrifugation. The precipitated enzyme was dissolved in 0.05 M NaCl and dialyzed overnight.

The enzyme solution was assayed by following the ultraviolet (UV) absorbance at 340 nm on a Shimadzu UV-190 spectrophotometer equipped with a temperature-controlled cuvette chamber. The solution (0.1 ml) was added to

a cuvette containing phosphate buffer (1.6 ml, pH 6.2, 0.1 M final concentration) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) (0.1 ml, 0.25 mM final concentration), and the cuvette was inserted into the spectrophotometer. The reaction was started by the addition of DL-glyceraldehyde (0.1 ml, 5.0 mM final concentration) to the cuvette, and the decrease of absorbance at 340 nm in 5 min at 25 °C was determined. The reaction was linear for at least 8 min. Disulfides to be tested were dissolved in water with a minimal amount of 0.1 N NaOH or EtOH and diluted to the desired concentration with water. The resulting solution (0.1 ml) was added to the cuvette. The reference blank (to correct for non-specific oxidation of NADPH and absorption of the disulfides) was prepared by using water instead of DL-glyceraldehyde solution.

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