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Synthesis and Antihypertensive Activity of 5-(2-Hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic Acids[1)]

EISHIN KATO,* KOJI YAMAMOTO, YOICHI KAWASHIMA, TOSHIO WATANABE, MASAYUKI OYA, TADASHI ISO, and JUN-ICHI IWAO

Research Laboratory, Santen Pharmaceutical Co., Ltd., 9–19, Shimoshinjo 3-chome, Higashi Yodogawa-ku, Osaka 533, Japan

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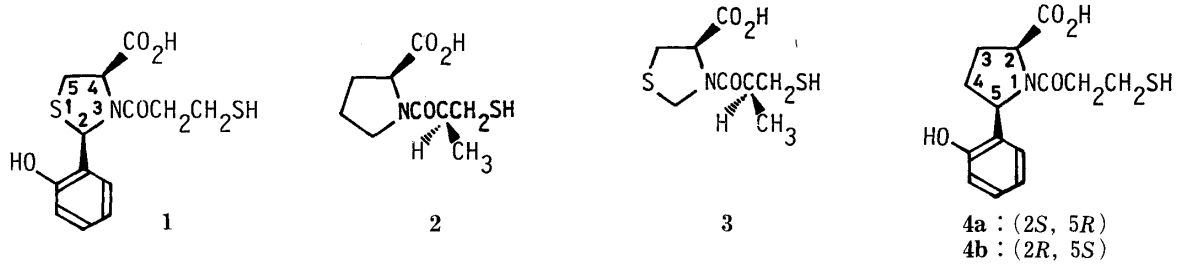
The (2*S*,5*R*)-(+)- or (2*R*,5*S*)-(−)-thiol (**4a** or **4b**) was synthesized by catalytic hydrogenation of the corresponding (*S*)-(−)- or (*R*)-(+)-pyrrolinecarboxylic acid (**8a** or **8b**) resolved with (*R*)-(−)-1,2-diphenylethylamine, followed by acylation with 3-(benzoylthio)propionyl chloride and ammonolysis. The thiols were converted into the corresponding *O*,*S*-diacetates (**16a** and **16b**), which were transformed into (2*R*,5*R*)-(+)- and (2*S*,5*S*)-(−)-thiols (**18a** and **18b**) *via* their *O*,*S*-diacetates (**17a** and **17b**) by epimerization and then ammonolysis. The stereochemistry of these thiols was elucidated on the basis of synthesis from *tert*-butoxycarbonyl-L-glutamic acid γ-benzyl ester (**9**) and proton nuclear magnetic resonance (^{1}H-NMR) analysis. The thiols were tested for inhibitory activity against angiotensin-converting enzyme *in vitro*. (2*S*,5*R*)-5-(2-Hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic acid (**4a**) showed the most potent activity among them.

Keywords——(2*S*,5*R*)-(+)-5-(2-hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic acid; (*S*)-Δ^1-2-(2-hydroxyphenyl)-5-pyrrolinecarboxylic acid; L-glutamic acid; (*R*)-1,2-diphenylethylamine; optical resolution; absolute configuration; conformation; epimerization; angiotensin-converting enzyme inhibitor

As a part of our search for new antihypertensive agents, we have synthesized a series of *N*-(mercaptoacyl)amino acids and screened them for inhibitory activities against angiotensin-converting enzyme (ACE).[2,3)] In this research, (2*R*,4*R*)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (SA 446) (**1**) was found to show higher activity than captopril (**2**) and 2-unsubstituted 4-thiazolidinecarboxylic acid (YS 980) (**3**) (Chart 1). In an attempt to obtain a more active compound, we synthesized (2*S*,5*R*)-5-(2-hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic acid (**4a**), in which the sulfur atom in the thiazolidine ring of **1** was changed into methylene. This paper describes the synthesis and the ACE inhibitory activities of **4a** and its stereoisomers.



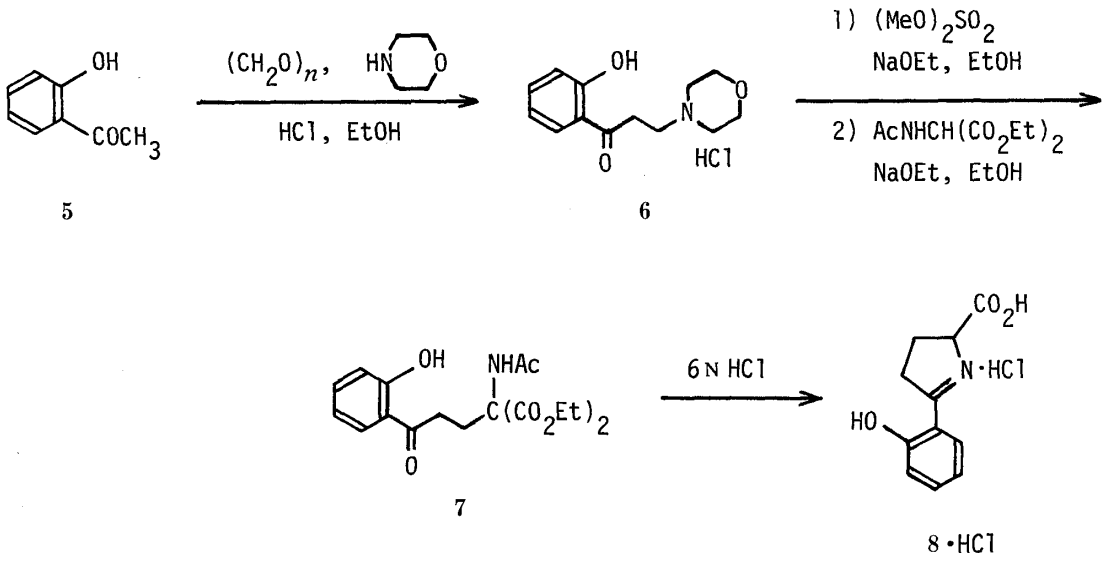
1 **2** **3** **4a** : (2*S*, 5*R*) **4b** : (2*R*, 5*S*)

The structures of the **b**-series compounds are the mirror images of those depicted for the **a**-series compounds in the charts below.

Chart 1

Chemistry

Synthesis and Resolution of (±)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylic Acid (8) ——The hydrochloride of **8** was prepared from *o*-hydroxyacetophenone (**5**) *via* diethyl 2-acetamido-2-[3-(2-hydroxyphenyl)-3-oxopropyl]malonate (**7**) in 22% overall yield by a modification of the method described in the patent literature (Chart 2).[4]



5 6 7 8·HCl

$(CH_2O)_n$: paraformaldehyde

Chart 2

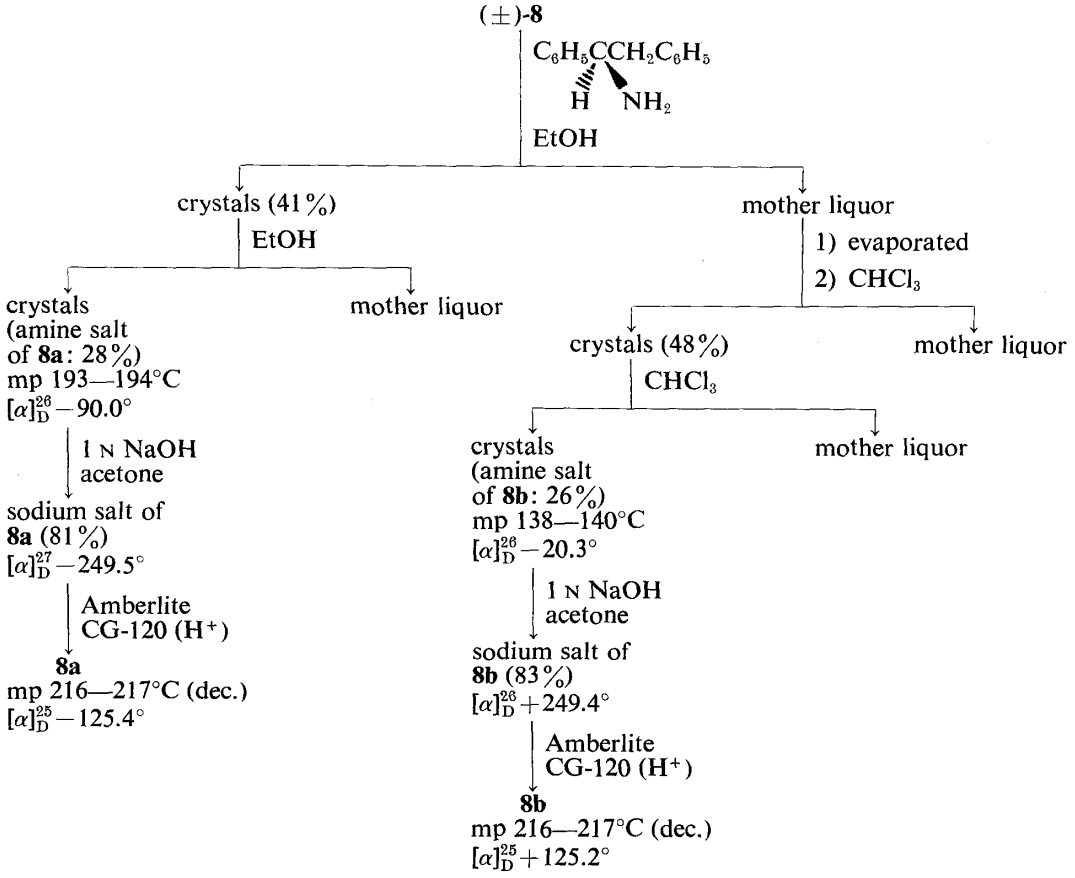


Chart 3

The resolution of **8** was achieved by fractional recrystallization of the diastereoisomeric salt with (*R*)-(−)-1,2-diphenylethylamine[5] as illustrated in Chart 3. These salts were converted into (−)-acid **8a** and (+)-acid **8b** *via* their sodium salts by treatment with sodium hydroxide followed by desalting with cation-exchange resin.

Determination of Absolute Configuration of (−)- and (+)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylic Acid (8a and 8b)——In order to determine the absolute configurations of **8a** and **8b**, we aimed to derive its asymmetric center from an L-amino acid and chose the available *tert*-butoxycarbonyl-L-glutamic acid γ-benzyl ester (**9**) as a starting material (Chart 4). Compound **9** was transformed into the α-methyl ester **11** *via* **10** in quantitative yield by treatment with diazomethane in ether and subsequent catalytic reduction using 10% Pd–carbon in *tert*-butanol. The ester (**11**) was converted into the thioester **12** by a mixed

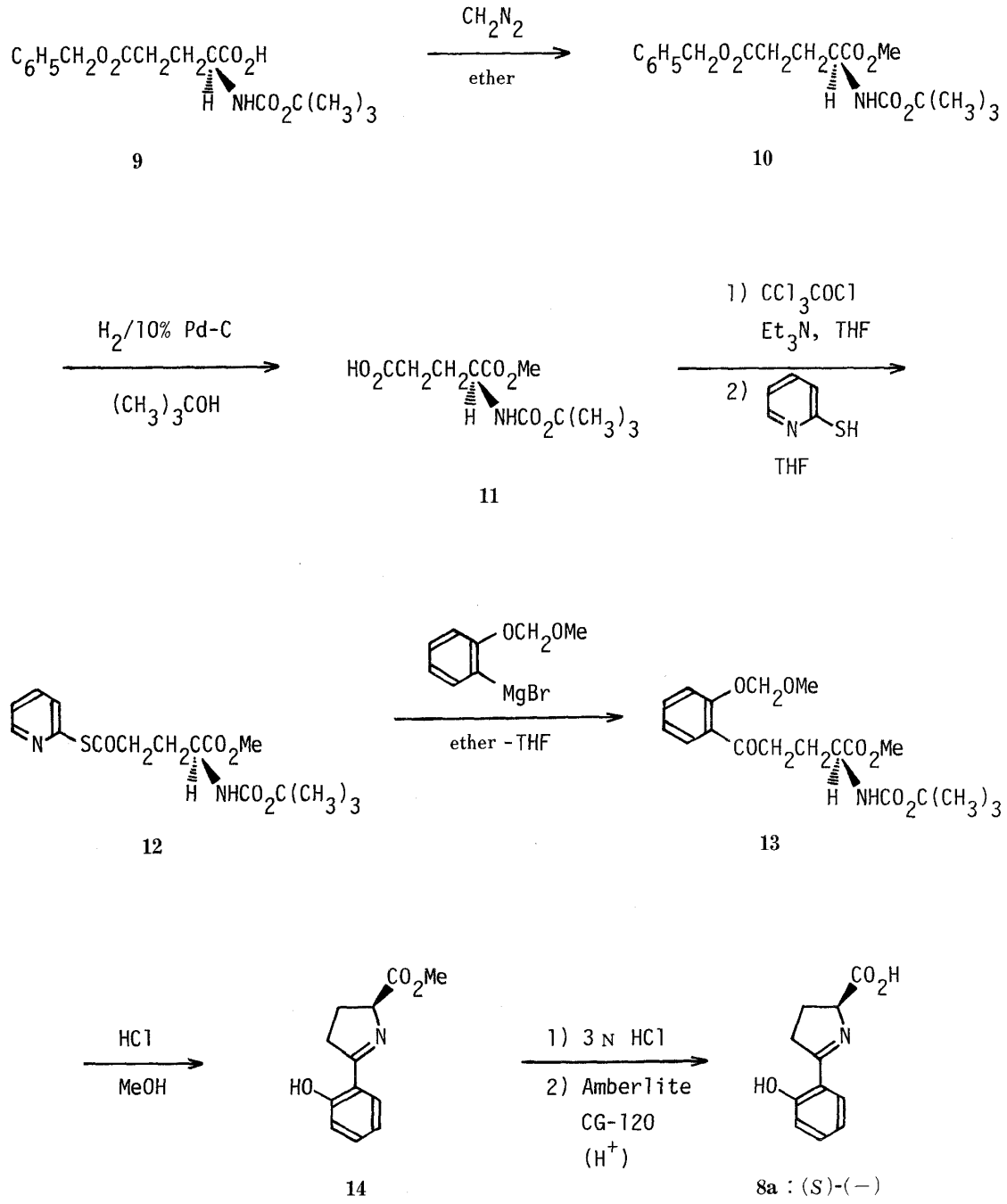
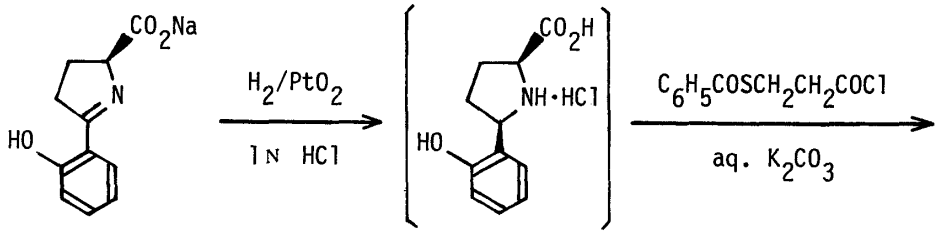


Chart 4

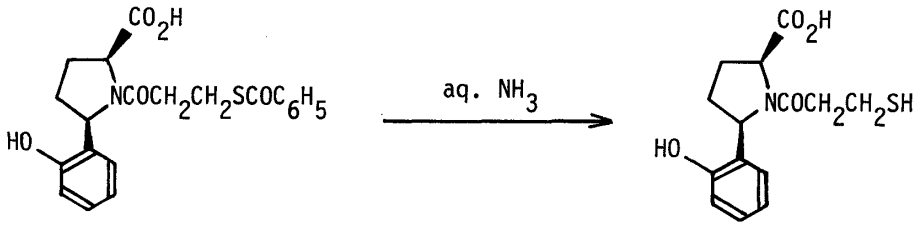
anhydride method[6] using trichloroacetyl chloride in tetrahydrofuran (THF). Reaction[7] of **12** with 1.5 eq of Grignard reagent in ether–THF at 2 °C gave the ketone **13**, which was treated with hydrogen chloride in methanol at room temperature to give the (*S*)-(−)-methyl ester **14**. This ester was refluxed in 3 N hydrochloric acid, followed by treatment with cation-exchange resin to give the (*S*)-(−)-acid **8a**, in 10% overall yield from **11**. The (*S*)-(−)-acid (**8a**) was identical with the (−)-acid obtained by the optical resolution previously described. Consequently, the absolute configuration of **8b** was determined to be (*R*).

Synthesis of (2*S*,5*R*)-(+)- and (2*R*,5*S*)-(−)-5-(2-Hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic Acids (4a and 4b) and Their Isomers (18a and 18b)——The sodium salt of **8a** or **8b** was hydrogenated with platinum oxide in 1 N hydrochloric acid, followed by treatment with 3-(benzoylthio)propionyl chloride in aqueous potassium carbonate to give compound **15a** or **15b** in 37% or 50% yield, respectively (Chart 5). These compounds were treated with aqueous ammonia to give the thiols **4a** and **4b** in 82% and 77% yield, respectively.

The (±)-thiol (**4**) was also obtained from the hydrochloride of **8** in a similar manner.



8a (Na) : (*S*)-(−)
8b (Na) : (*R*)-(+)



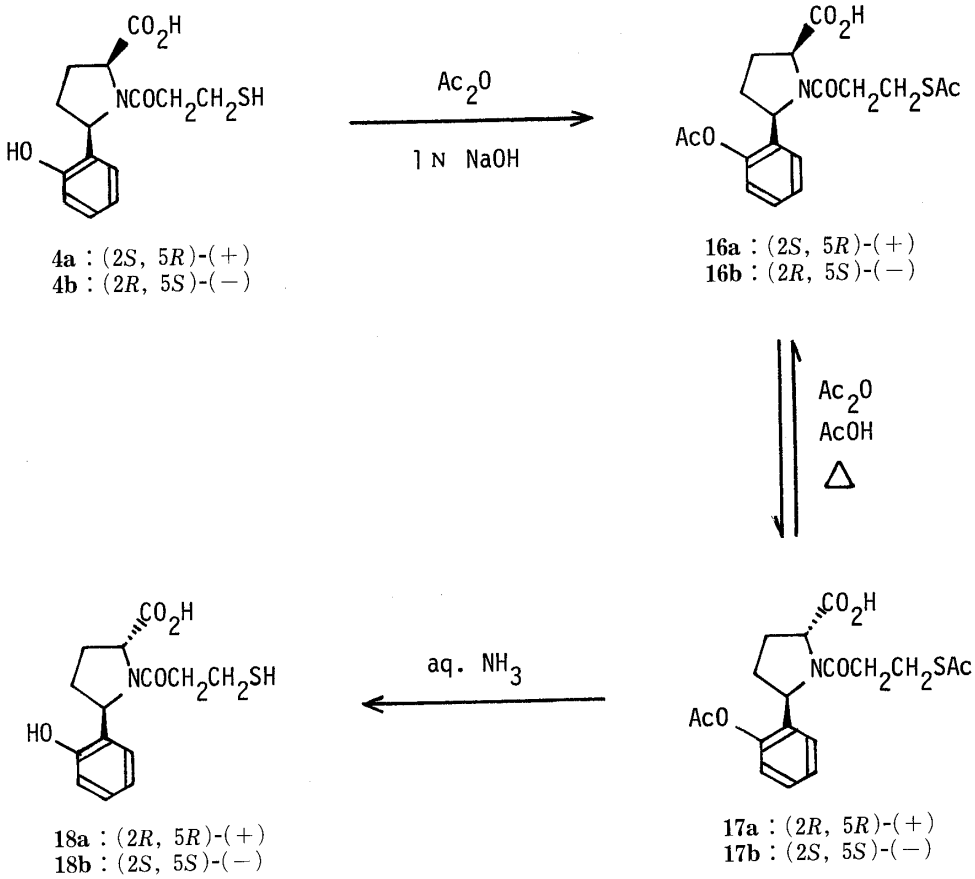
15a : (2*S*, 5*R*)-(+)
15b : (2*R*, 5*S*)-(−)

4a : (2*S*, 5*R*)-(+)
4b : (2*R*, 5*S*)-(−)

Chart 5

In order to obtain the *trans*-isomers of **4a** and **4b**, they were acetylated with acetic anhydride to give the *O*,*S*-diacetates **16a** and **16b** in 83% and 91% yield, respectively (Chart 6). The acetate (**16a** or **16b**) was epimerized at the C_2-position in the pyrrolidine ring by heating in acetic anhydride–acetic acid solution[8] to give the *O*,*S*-diacetate **17a** or **17b** in 15% or 18% yield, respectively. The diacetate (**17a** or **17b**) was deacetylated with aqueous ammonia to give the thiol **18a** or **18b** in 89% or 94% yield, respectively.

Stereochemistry——The stereochemistry at the C_2- and C_5-position in the diacetates (**16** and **17**) is discussed below. The absolute configuration at the C_2-position of **4a** was determined to be (2*S*) by derivation from L-glutamic acid as previously described, whereas the absolute configuration at the C_5-position was concluded to be (5*R*) on the basis of the general concept that the less hindered side (surface opposite the carboxyl group) of **8a** is liable to be attacked by hydrogen.[9,10] These conclusions are also supported by the following results



4a : (2*S*, 5*R*)-(+)
4b : (2*R*, 5*S*)-(−)

16a : (2*S*, 5*R*)-(+)
16b : (2*R*, 5*S*)-(−)

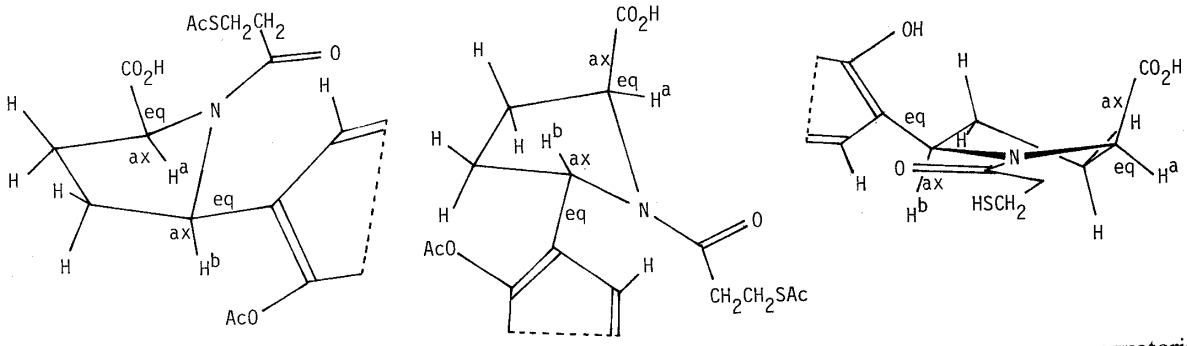
18a : (2*R*, 5*R*)-(+)
18b : (2*S*, 5*S*)-(−)

17a : (2*R*, 5*R*)-(+)
17b : (2*S*, 5*S*)-(−)

Chart 6

16a : X=CH_2
19 : X=S

17b : X=CH_2
20 : X=S



16a envelope **17b** envelope **4a** half-chair

eq = equatorial
ax = axial

Fig. 1. Conformations of the *O*,*S*-Diacetates **16a** and **17b** and Thiol **4a**

TABLE I. ^{1}H-NMR Spectral Data [δ (ppm), J=Hz in $CDCl_3$] for the *O*,*S*-Diacetates

Compd. No.	H^a	H^b	Aromatic H	Others
16a	5.10 (dd, J=6.5, 4.5)	4.62 (t, J=6.5)	6.92—7.48 (3H, m) 7.57—7.95 (1H, m)	1.72—2.82 (6H, m, $CH_2\times3$), 2.23 (3H, s, CH_3COS), 2.33 (3H, s, CH_3CO_2), 3.03 (2H, t, J=6.5, CH_2S), 10.72 (1H, s, CO_2H)
17b	5.25 (d, J=7.0)	4.83 (dd, J=5.5, 3.0)	6.83—7.50 (4H, m)	1.70—2.88 (6H, m, $CH_2\times3$), 2.20 (3H, s, CH_3COS), 2.33 (3H, s, CH_3CO_2), 3.05 (2H, t, J=6.5, CH_2S), 10.33 (1H, br s, CO_2H)
19[a]	4.97 (t, J=9.5)	6.20 (s)	7.00—7.53 (3H, m) 7.77—8.17 (1H, m)	2.27 (3H, s, CH_3COS), 2.37 (3H, s, CH_3CO_2), 2.53 (2H, t, J=7.0, $COCH_2$), 3.08 (2H, t, J=7.0, CH_2S), 3.30 (2H, d, J=9.5, CH_2), 10.13 (1H, s, CO_2H)
20[a]	5.30 (dd, J=4.5, 3.0)	6.25 (s) and 6.47 (s)	6.88—7.58 (4H, m)	2.21 (3H, s, CH_3COS), 2.35 (3H, s, CH_3CO_2), 2.45—3.12 (4H, m, $CH_2\times2$), 3.28 (1H, dd, J=8.0, 3.0, CH), 3.36 (1H, dd, J=8.0, 4.5, CH), 9.98 (1H, s, CO_2H)

a) Reference 11.

(Fig. 1 and Table I): the proton nuclear magnetic resonance (^{1}H-NMR) spectral patterns (H^a and aromatic H) of **16a** and **17b** were comparable to those of the *O*,*S*-diacetate **19** of **1**, the absolute configuration of which was established by X-ray crystallographic analysis,[11] and its C_2-epimer **20**, respectively.

Next, we attempted to decide the conformations of **16a** and **17b** on the basis of the Karplus equation, which relates coupling constants between vicinal protons in the ^{1}H-NMR spectra and the dihedral angle between the protons.[12] In the case of **16a**, the proton (H^a) at the C_2-position appeared as a double doublet signal (J=6.5 and 4.5 Hz) at 5.10 ppm, which was attributable to an ABX system, and the proton (H^b) at the C_5-position gave a triplet signal (J=6.5 Hz) of the same ABX system at 4.62 ppm. Thus, it can be concluded that both the carboxyl group and the benzene nucleus on the pyrrolidine ring have equatorial orientations in an envelope conformation[13] (Fig. 1). On the other hand, we concluded that a favorable conformation of **17b** is an envelope form in which the carboxyl group and the benzene nucleus are axial and equatorial, respectively, because its spectrum exhibited a doublet (J=7.0 Hz) at 5.25 ppm and a double doublet (J=5.5 and 3.0 Hz) at 4.83 ppm attributable to H^a and H^b, respectively.

The proton at the *o*-position of the phenyl group in **16a** appeared at lower field than that in **17b** (Table I). This phenomenon is accounted for by a deshielding of the proton by the amide bond carbonyl in **16a**, the oxygen of which is located on the phenyl side. The *O*,*S*-diacetates **19** and **20**, which showed the same phenomenon, are also considered to be similar in conformation to **16a** and **17b**.

The thiol **4a** without a bulky *O*-acetyl group was concluded to have a half-chair conformation with axial carboxyl and equatorial phenyl groups because of the broad doublet signal (J=6.0 Hz) of the C_2-proton (H^a) at 5.22 ppm and the double doublet (J=8.0 and 6.0 Hz) of the C_5-proton (H^b) at 4.27 ppm (see Experimental). It was concluded that the thiol **18b** takes a half-chair conformation and has axial carboxyl and phenyl groups: the signals of the C_2- and C_5-protons appeared as a broad doublet (J=6.0 Hz) at 5.32 ppm and a broad doublet (J=5.5 Hz) at 4.55 ppm, respectively. Furthermore, the spectral pattern of aromatic protons in **4a** was the same as in **16a**, so its amide oxygen is located on the phenyl side. On the other hand, since the thiol **1** showed the same spectral pattern as the diacetates **16a** and **19** (the

TABLE II. Inhibitory Activities of 5-(2-Hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic Acids against ACE[a]

Compd. No.	AI pI_{50}	ACE pI_{50}	BK pA_{50}
(±)-**4**	6.77	6.25	8.74
4a	7.12	7.19	9.02
4b	4.06	4.00	5.85
18a	4.96	—	7.11
18b	4.08	—	5.80
1	7.55	7.15	9.15

a) AI, angiotensin I; BK, bradykinin. pI_{50}: $-\log$ of the molar concentration of compound which gives 50% inhibition of the enzyme activity or agonist effect. pA_{50}: $-\log$ of the molar concentration of compound which gives 50% enhancement of the agonist effect.

proton on the carbon joined to the carboxyl group and the aromatic protons),[11] it must be different from **4a** and have an envelope conformation.[14]

Biological Results and Discussion

The thiols listed in Table II were examined for inhibitory activities against angiotensin-converting enzyme (ACE) *in vitro*. The assay was carried out according to the method described in the previous report.[3]

The inhibitory activity of **4a** was twice that of (±)-**4**, but somewhat less than that of the thiol **1** having the thiazolidine ring. The enantiomer (**4b**) and the diastereoisomers (**18a** and **18b**) showed low activity.

These results demonstrate that a thiol which has both a (2*S*)-carboxyl group (the same stereochemistry as natural L-amino acids) and a (5*R*)-phenyl group shows effective inhibitory activity against ACE. The small difference in activity between **1** and **4a** may be due to slight differences in their conformations.

Experimental

Melting points were determined on a Yamato MP-21 melting point apparatus and are uncorrected. Specific rotations were measured with a JASCO DIP-140 polarimeter. Infrared (IR) spectra were recorded on a JASCO A-302 spectrophotometer. ^{1}H-NMR spectra were measured on a JEOL PMX-60 spectrometer using tetramethylsilane or sodium 3-(trimethylsilyl)propanesulfonate as an internal standard.

1-(2-Hydroxyphenyl)-3-morpholinopropanone Hydrochloride (6)——Paraformaldehyde (192 g, 6.39 mol) and **5** (582 g, 4.27 mol) were added to a stirred solution of morpholine (560 g, 6.43 mol) and hydrogen chloride (330 g, 9.05 mol) in EtOH (800 ml). The resulting mixture was stirred under reflux for 6 h and allowed to stand in a refrigerator overnight. The crystals were collected by filtration and recrystallized from 92% EtOH to give **6** (533.9 g, 46%): mp 194—196 °C. IR ν_{max}^{KBr} cm^{-1}: 1636 (CO). ^{1}H-NMR (DMSO-d_6–D_2O) δ: 3.03—4.39 (12H, m, $CH_2 \times 6$), 6.85—7.23 (2H, m), 7.58 (1H, dt, J=7.8, 2.0 Hz) and 7.93 (1H, dd, J=8.4, 2.0 Hz, aromatic H).

Hydrochloride of (±)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylic Acid (8)——A solution of sodium ethoxide in EtOH (650 ml) [containing sodium (38 g, 1.65 mol)] was added to a well-stirred slurry of powdered ketone (**6**) (448.4 g, 1.65 mol) and EtOH (1 l). The resulting suspension was stirred for 30 min, followed by addition of dimethyl sulfate (285 ml, 3.01 mol), then further stirred for 3 h and allowed to stand overnight. To this mixture was added a slurry of diethyl acetaminomalonate (358.4 g, 1.65 mol) and EtOH (1.3 l) containing sodium (75.9 g, 3.3 mol) with stirring. The whole was stirred for 1 h, then refluxed for 2.5 h, and poured onto ice (4 kg) after cooling. The solution was acidified with conc. HCl and extracted with AcOEt (4 l). The organic layer was washed with satd. NaCl solution, dried over $MgSO_4$ and evaporated *in vacuo* to give crude oily **7** (558.4 g, 93%).

This oil in 6 N HCl (6 l) was refluxed for 4 h, filtered and concentrated *in vacuo*, then the residue was allowed to stand in a refrigerator. The resulting crystals were collected by filtration and recrystallized from water to give the hydrochloride of **8** (192 g, 52%): mp 126—128 °C. IR ν_{max}^{KBr} cm^{-1}: 1736 (CO_2H), 1627 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 2.10—3.05 (2H, m, C_4-H_2), 3.65 (2H, t, J=7.5 Hz, C_3-H_2), 5.08 (1H, dd, J=9.0, 5.5 Hz, C_5-H), 6.83—8.03 (4H, m, aromatic H), 11.18—12.58 (3H, br, OH, CO_2H, NH).

(±)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylic Acid (8)——The above hydrochloride (123 g, 0.509 mol) was dissolved in hot water (250 ml) and the solution was adjusted to pH 4 with 3 N NaOH. The precipitate was collected to give **8** (93.9 g, 90%): mp 219—220 °C. IR ν_{max}^{KBr} cm^{-1}: 1730 (CO_2H), 1623 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 1.63—2.65 (2H, m, C_4-H_2), 3.12 (2H, ddd, J=8.5, 7.0, 1.8 Hz, C_3-H_2), 4.88 (1H, tt, J=7.5, 1.8 Hz, C_5-H), 6.68—7.72 (4H, m, aromatic H), 10.63—14.00 (2H, br, OH, CO_2H).

Resolution of (±)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylic Acid (8)——The (±)-acid (**8**) (35.8 g, 0.174 mol) and (*R*)-(−)-1,2-diphenylethylamine (34.5 g, 0.175 mol) were dissolved in boiling EtOH (2.34 l) and the mixture was allowed to stand for 1 d. The resulting yellow needles were filtered off; yield 28.5 g (41%). Fractional recrystallization of the crystals from EtOH gave the diastereoisomeric salt of **8a** (20 g, 28%): mp 193—194 °C, $[\alpha]_D^{26}$ −90.0° (c=1.0, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1611 (C=N), 1574 (CO_2). ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.37 (2H, m, C_4-H_2), 2.70—3.53 (4H, m, C_3-H_2, CH_2), 4.27 (1H, dd, J=8.0, 6.0 Hz, CH), 4.73 (1H, t, J=7.3 Hz, C_5-H), 6.27—9.27 (4H, br, CO_2H, NH_2, OH), 6.60—7.60 (14H, m, aromatic H). *Anal.* Calcd for $C_{11}H_{11}NO_3 \cdot C_{14}H_{15}N$: C, 74.60; H, 6.51; N, 6.96. Found: C, 74.41; H, 6.53; N, 6.87.

The mother liquor of the first crystals was evaporated *in vacuo*, then the residue was dissolved in boiling $CHCl_3$ (285 ml) and allowed to stand in a refrigerator overnight. The resulting yellow fine needles were collected; yield 33.4 g (48%). Fractional recrystallization of the crystals from $CHCl_3$ gave the diastereoisomeric salt of **8b** (18 g, 26%): mp 138—140 °C, $[\alpha]_D^{26}$ −20.3° (c=1.2, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1611 (C=N), 1571 (CO_2). ^{1}H-NMR ($CDCl_3$–DMSO-d_6) δ: 1.88—2.45 (2H, m, C_4-H_2), 2.67—3.48 (4H, m, C_3-H_2, CH_2), 4.22 (1H, dd, J=8.2, 6.2 Hz, CH), 4.75 (1H, t, J=7.3 Hz, C_5-H), 6.60—7.43 (14H, m, aromatic H), 9.10 (4H, brs, CO_2H, NH_2, OH). *Anal.* Calcd for $C_{11}H_{11}NO_3 \cdot C_{14}H_{15}N \cdot 1/4CHCl_3$: C, 70.15; H, 6.12; N, 6.48. Found: C, 70.45; H, 6.12; N, 6.54.

Sodium Salt of (*S*)-(−)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylic Acid (8a)——A stirred solution of the pulverized diastereoisomeric salt of **8a** (51 g, 0.127 mol) was treated with 1 N NaOH (140 ml), then the mixture was stirred for 2 h and filtered to give the sodium salt (23.3 g, 81%) as a yellow powder of $[\alpha]_D^{27}$ −249.5° (c=0.6, water).

Sodium Salt of (*R*)-(+)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylic Acid (8b)——The sodium salt was prepared by the above method from the diastereoisomeric salt of **8b** (16.1 g, 0.037 mol) in 83% (7.0 g) yield. $[\alpha]_D^{26}$ +249.4° (c=0.6, water).

***N*-(*tert*-Butoxycarbonyl)-L-glutamic Acid α-Methyl Ester (11)**——A solution of diazomethane in ether was added to a cooled solution of *N*-(*tert*-butoxycarbonyl)-L-glutamic acid γ-benzyl ester (**9**) (25 g, 0.0778 mol) in AcOEt (200 ml) with stirring. After being stirred for 1 h, the resulting solution was evaporated *in vacuo* to give **10** (26.4 g) as a colorless oil of $[\alpha]_D^{25}$ −18.0° (c=5.2, MeOH).

A solution of the oil in *tert*-BuOH (250 ml) was stirred in the presence of 10% Pd–C (3.0 g) under a hydrogen atmosphere for 4.7 h, then filtered and evaporated *in vacuo*. After addition of hexane, the residue was filtered to give **11** (19.3 g, quantitative) as an amorphous solid of $[\alpha]_D^{25}$ −27.7° (c=4.8, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1685 (br, CO_2H, CO_2CH_3, OCONH), 1508 (OCONH). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s, $CH_3 \times 3$), 1.68—2.67 (4H, m, $COCH_2CH_2$), 3.75 (3H, s, CO_2CH_3), 4.10—4.63 (1H, m, CHN), 4.95—5.50 (1H, br, NH), 8.00 (1H, s, CO_2H).

Methyl (*S*)-2-(*tert*-Butoxycarbonylamino)-5-[2-(methoxymethoxy)phenyl]-5-oxopentanoate (13)——Trichloroacetyl chloride (8.4 ml, 0.075 mol) was added dropwise to a stirred solution of **11** (21.2 g, 0.075 mol) and triethylamine (11.5 ml, 0.083 mol) in anhydrous THF (200 ml) at −13 °C, and the resulting suspension was stirred at −15 °C for 20 min. Then 2-pyridinethiol (8.3 g, 0.075 mol) and triethylamine (11.5 ml, 0.083 mol) in anhydrous THF (80 ml) were added dropwise at −13 °C. The mixture was stirred at −13 °C for 45 min and at room temperature for 1 h, then evaporated *in vacuo*. The residue was dissolved in ether (300 ml), and the solution was washed with water and satd. NaCl, dried over $MgSO_4$ and evaporated *in vacuo*. The residual oil was purified by column chromatography on silica gel (benzene–AcOEt system) to give oily **12** (20 g, 76%). IR ν_{max}^{film} cm^{-1}: 3350 (NH), 1739 (CO_2), 1707 (OCONH, COS), 1513 (OCONH). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, $CH_3 \times 3$), 1.87—2.53 (2H, m, CH_2), 2.78 (2H, t, J=6.5 Hz, CH_2COS), 3.70 (3H, s, CO_2CH_3), 4.10—4.55 (1H, m, CHN), 5.15 (1H, br d, J=8.0 Hz, NH), 7.07—7.75 (3H, m) and 8.55 (1H, dd, J=4.0, 1.0 Hz, aromatic H).

A solution of the oil (**12**) in anhydrous THF (50 ml) was added dropwise to a stirred solution of 2-methoxymethoxyphenylmagnesium bromide [prepared from Mg (2.5 g, 0.10 mol) and 2-bromo(methoxymethoxy)-benzene (18.5 g, 0.085 mol), which was obtained from 2-bromophenol and chloromethyl methyl ether according to the procedure of Winkle and Ronald[15]] in anhydrous ether (50 ml) and anhydrous THF (20 ml) at −13 °C. The resulting solution was allowed to warm to 0 °C and stirred at 2 °C for 1 h, then quenched with satd. NH_4Cl (80 ml), and extracted with ether (300 ml). The organic layer was washed with 1 N NaOH (60 ml × 2) and satd. NaCl, dried over $MgSO_4$, and evaporated *in vacuo*. The residual oil was purified by column chromatography on silica gel (hexane–ether system) to give **13** (4.7 g, 22%) as an oil of $[\alpha]_D^{25}$ −5.8° (c=3.5, MeOH). IR ν_{max}^{film} cm^{-1}: 3360 (NH), 1740 (CO_2CH_3), 1708 (OCONH), 1678 (CO), 1508 (OCONH). ^{1}H-NMR ($CDCl_3$) δ: 1.40 (9H, s, $CH_3 \times 3$), 1.85—2.60 (2H, m, CH_2), 3.08 (2H, t, J=7.5 Hz, CH_2CO), 3.48 (3H, s, OCH_3), 3.70 (3H, s, CO_2CH_3), 4.32 (1H, dt, J=8.0, 6.0 Hz, CHN), 5.08 (1H, d, J=8.0 Hz, NH), 5.22 (2H, s, OCH_2O), 6.80—7.75 (4H, m, aromatic H).

Methyl (*S*)-(−)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylate (14)——The ketone (**13**) (3.6 g, 9.4 mmol) was dissolved in 35% HCl in anhyd. MeOH (40 ml), and the solution was allowed to stand overnight, then evaporated *in vacuo*. $CHCl_3$ (50 ml) and satd. $NaHCO_3$ (20 ml) were added to the residue. The $CHCl_3$ layer was washed with satd.

NaCl, dried over $MgSO_4$, and evaporated *in vacuo*. The residual crystals were recrystallized from isopropyl ether–cyclohexane to give **14** (1.7 g, 82%) as yellow prisms of mp 58.5—59.5 °C, $[\alpha]_D^{25}$ −134.7° (*c* = 1.0, 1 N HCl). IR ν_{max}^{KBr} cm^{-1}: 1740 (CO_2), 1601 (C=N, C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.02—2.55 (2H, m, C_4-H_2), 2.93—3.35 (2H, m, C_3-H_2), 3.74 (3H, s, CO_2CH_3), 4.93 (1H, tt, *J* = 7.5, 2.0 Hz, C_5-H), 6.65—7.55 (4H, m, aromatic H), 13.13 (1H, br s, OH).

(*S*)-(−)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylic Acid (8a)——i) From **14**: A solution of the ester (0.28 g, 1.3 mmol) in 3 N HCl (5 ml) was refluxed for 30 min. After addition of water (20 ml), the solution was passed through Amberlite CG-120 (H^+-Form) (3 ml), and eluted with 0.5 M pyridine (60 ml). The eluate was evaporated *in vacuo* and the residual crystals were recrystallized from MeOH–ether to give **8a** (0.19 g, 72%) as yellow prisms of mp 216—217 °C (dec.), $[\alpha]_D^{25}$ −125.4° (*c* = 1.0, 1 N HCl). IR ν_{max}^{KBr} cm^{-1}: 1647 (CO_2H), 1607 (C=N). *Anal.* Calcd for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.16; H, 5.42; N, 6.81. The ^{1}H-NMR spectrum of **8a** was identical with that of **8**.

ii) From the Sodium Salt of **8a**: A solution of the salt (3.4 g, 0.015 mol) in 1 N HCl (25 ml) was treated as described above to give **8a** (2.41 g, 78%) as yellow prisms. IR and ^{1}H-NMR spectra, melting point and specific rotation of these crystals were identical with those of the product obtained by procedure (i). The mixed melting point of both crystals was not depressed.

(*R*)-(+)-Δ^1-2-(2-Hydroxyphenyl)-5-pyrrolinecarboxylic Acid (8b)——This compound was prepared by the above procedure from the sodium salt of **8b** (0.57 g, 2.5 mmol) in 84% yield (0.43 g) as yellow prisms of mp 216—217 °C (dec.), $[\alpha]_D^{25}$ +125.2° (*c* = 1.0, 1 N HCl).

The IR and ^{1}H-NMR spectra of **8b** were identical with those of **8a**.

(2*S*,5*R*)-(+)-1-[3-(Benzoylthio)propionyl]-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic Acid (15a)——The sodium salt of **8a** (7.95 g, 0.035 mol) and platinic oxide (0.35 g) were added to a mixture of water (40 ml) and 1 N HCl (70 ml). The resulting mixture was stirred under a hydrogen atmosphere until the theoretical amount of hydrogen had been absorbed, then filtered, and the filtrate was cooled in an ice bath. After addition of K_2CO_3 (19.35 g, 0.14 mol), 3-(benzoylthio)propionyl chloride (8.0 g, 0.035 mol) was added dropwise to the cooled solution. The resulting solution was stirred for 1 h with ice-cooling and then acidified with conc. HCl, followed by extraction with AcOEt (150 ml). The organic layer was washed with water and satd. NaCl, dried over $MgSO_4$, and evaporated *in vacuo*. The residual oil was purified by column chromatography on silica gel (benzene–AcOEt system) and recrystallized from AcOEt–benzene to give **15a** (6.2 g, 37%) as colorless prisms (monobenzenate) of mp 89—92 °C (dec.), $[\alpha]_D^{25}$ +47.4° (*c* = 1.0, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1750 (CO_2H), 1660 (COS, CON). ^{1}H-NMR (DMSO-d_6) δ: 1.48—2.88 (6H, m, C_3-H_2, C_4-H_2, CH_2CO), 3.12 (2H, t, *J* = 6.0 Hz, SCH_2), 4.32 (1H, dd, *J* = 7.0, 6.0 Hz, C_5-H), 5.23 (1H, dd, *J* = 6.0, 1.0 Hz, C_2-H), 6.50—8.13 (9H, m, aromatic H), 7.33 (6H, s, C_6H_6), 9.28 (br s) and 9.47 (br s) (1H, OH), 11.43—13.50 (1H, br, CO_2H).

(2*R*,5*S*)-(−)-1-[3-(Benzoylthio)propionyl]-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic Acid (15b)——This compound was prepared by the above procedure from the sodium salt of **8b** (7.88 g, 0.0347 mol) in 50% yield (8.3 g) as colorless prisms of mp 91—93 °C (dec.), $[\alpha]_D^{25}$ −46.8° (*c* = 0.9, MeOH). The IR and ^{1}H-NMR spectra of **15b** were identical with those of **15a**.

(±)-1-[3-(Benzoylthio)propionyl]-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic Acid (15)——This compound was prepared by a procedure similar to that used for **15a** from the hydrochloride of **8** (12.1 g, 0.05 mol) in 47% yield (9.4 g), mp 210—211 °C (MeOH–AcOEt). IR ν_{max}^{Nujol} cm^{-1}: 1742 and 1722 (CO_2H), 1665 (COS), 1615 (CON). *Anal.* Calcd for $C_{21}H_{21}NO_5S$: C, 63.14; H, 5.30; N, 3.51. Found: C, 63.10; H, 5.33; N, 3.40. The ^{1}H-NMR spectrum of **15** was identical with that of **15a** except for the signal based on benzene.

(2*S*,5*R*)-(+)-5-(2-Hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic Acid (4a)——A solution of **15a** (5.5 g, 0.012 mol) in conc. ammonia water (15 ml) and water (30 ml) was stirred at room temperature for 1 h, then extracted with AcOEt (40 ml × 3). The aqueous layer was acidified with conc. HCl and extracted with AcOEt (100 ml). The AcOEt layer was washed with water and satd. NaCl, dried over $MgSO_4$, and evaporated *in vacuo*. Recrystallization of the residual crystals from AcOEt–cyclohexane gave **4a** (2.8 g, 82%) as colorless prisms of mp 197—198 °C (dec.), $[\alpha]_D^{25}$ +34.7° (*c* = 0.5, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1720 and 1685 (CO_2H), 1605 (CON). ^{1}H-NMR (DMSO-d_6) δ: 1.43—2.83 (9H, m, C_3-H_2, C_4-H_2, $COCH_2CH_2SH$), 4.27 (1H, dd, *J* = 8.0, 6.0 Hz, C_5-H), 5.22 (1H, br d, *J* = 6.0 Hz, C_2-H), 6.43—7.23 (3H, m, aromatic H), 7.45 (br d, *J* = 6.0 Hz) and 7.81 (d, *J* = 6.0 Hz) (1H, aromatic H), 9.20 (br s) and 9.45 (br s) (1H, OH), 10.30—13.00 (1H, br, CO_2H). *Anal.* Calcd for $C_{14}H_{17}NO_4S$: C, 56.93; H, 5.80; N, 4.74. Found: C, 56.82; H, 5.81; N, 4.64.

(2*R*,5*S*)-(−)-5-(2-Hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic Acid (4b)——This compound was obtained by using **15b** (9.4 g, 0.02 mol) instead of **15a** in the above procedure, in 77% yield (4.5 g) as colorless prisms of mp 198—199 °C (dec.), $[\alpha]_D^{25}$ −35.3° (*c* = 0.5, MeOH). *Anal.* Calcd for $C_{14}H_{17}NO_4S$: C, 56.93; H, 5.80; N, 4.74. Found: C, 56.79; H, 5.87; N, 4.65. The IR and ^{1}H-NMR spectra of **4b** were identical with those of **4a**.

(±)-5-(2-Hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic Acid (4)——This compound was obtained in a manner similar to that used for **15a** from **15** (4.0 g, 0.01 mol) in 85% yield (2.5 g), mp 213—214 °C (AcOEt–benzene). IR ν_{max}^{Nujol} cm^{-1}: 1720 (CO_2H), 1618 (CON). *Anal.* Calcd for $C_{14}H_{17}NO_4S$: C, 56.93; H, 5.80; N, 4.74. Found: C, 56.85; H, 5.81; N, 4.77. The ^{1}H-NMR spectrum of **4** was identical with that of **4a**.

(2*S*,5*R*)-(+)-5-(2-Acetoxyphenyl)-1-[3-(acetylthio)propionyl]-2-pyrrolidinecarboxylic Acid (16a)——Acetic an-

hydride (2.3 ml, 24 mmol) was added to a stirred solution of **4a** (2.91 g, 9.8 mmol) in 1 N NaOH (35 ml) with ice-cooling. The resulting solution was stirred at room temperature for 1 h, acidified with conc. HCl, and extracted with AcOEt (100 ml). The organic layer was washed with water and satd. NaCl, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was recrystallized from AcOEt to give **16a** (3.13 g, 83%) as colorless needles of mp 175.5—176.5 °C, $[\alpha]_D^{25}$ +33.2° (c=1.0, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1759 (CO_2), 1683 (CO_2H), 1603 (COS, CON). *Anal.* Calcd for $C_{18}H_{21}NO_6S$: C, 56.98; H, 5.58; N, 3.69. Found: C, 56.92; H, 5.62; N, 3.68. The ^{1}H-NMR spectral data of **16a** are shown in Table I.

(2*R*,5*S*)-(−)-5-(2-Acetoxyphenyl)-1-[3-(acetylthio)propionyl]-2-pyrrolidinecarboxylic Acid (16b)——The above treatment of **4b** (4.6 g, 0.016 mol) yielded **16b** (5.38 g, 91%) as colorless needles of mp 173—174 °C, $[\alpha]_D^{25}$ −32.9° (c=1.0, MeOH). *Anal.* Calcd for $C_{18}H_{21}NO_6S$: C, 56.98; H, 5.58; N, 3.69. Found: C, 56.82; H, 5.57; N, 3.71. The IR and ^{1}H-NMR spectra of **16b** were identical with those of **16a**.

(2*R*,5*R*)-(+)-5-(2-Acetoxyphenyl)-1-[3-(acetylthio)propionyl]-2-pyrrolidinecarboxylic Acid (17a)——A solution of **16a** (2.38 g, 6.27 mmol) in acetic acid (15 ml) and acetic anhydride (8 ml) was refluxed for 30 min, then concentrated *in vacuo*. The residue was separated by column chromatography on silica gel (benzene–AcOEt–AcOH system) to give recovered (+)-**16a** (1.1 g, 46%) and **17a** (0.35 g, 15%). Recrystallization of **17a** from AcOEt gave colorless needles of mp 123—124 °C, $[\alpha]_D^{25}$ +101.4° (c=0.9, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1743 (CO_2), 1684 (CO_2H), 1599 (COS, CON). *Anal.* Calcd for $C_{18}H_{21}NO_6S$: C, 56.98; H, 5.58; N, 3.69. Found: C, 56.94; H, 5.58; N, 3.67. The ^{1}H-NMR spectrum of **17a** was identical with that of **17b**.

In thin-layer chromatography (TLC: SiO_2; benzene–AcOEt–AcOH=25:25:1), **16a** and **17a** gave *Rf* values of 0.28 and 0.19, respectively.

(2*S*,5*S*)-(−)-5-(2-Acetoxyphenyl)-1-[3-(acetylthio)propionyl]-2-pyrrolidinecarboxylic Acid (17b)——Application of the above procedure to **16b** (1.8 g, 4.7 mmol) gave recovered **16b** (0.67 g, 37%) and **17b** (0.33 g, 18%) as colorless needles of mp 125—126 °C, $[\alpha]_D^{25}$ −99.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{18}H_{21}NO_6S$: C, 56.98; H, 5.58; N, 3.69. Found: C, 56.89; H, 5.60; N, 3.71. The IR spectrum of **17b** was identical with that of **17a**. The ^{1}H-NMR spectral data of **17b** are shown in Table I.

(2*R*,5*R*)-(+)-5-(2-Hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic Acid(18a)——The (+)-*O*,*S*-diacetate (**17a**) (100 mg, 0.26 mmol) was added to a mixture of conc. ammonia water (0.5 ml) and water (3 ml), and the whole was stirred for 0.5 h, acidified with 2 N HCl and extracted with AcOEt (20 ml). The organic layer was washed with water and satd. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (benzene–AcOEt system) and solidified with benzene and cyclohexane to give **18a** (69 mg, 89%). $[\alpha]_D^{25}$ +132.0° (c=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1707 (CO_2H), 1617 (CON). ^{1}H-NMR (DMSO-d_6) δ: 1.43—2.93 (9H, m, C_3-H_2, C_4-H_2, $COCH_2CH_2SH$), 4.55 (1H, br d, J=5.5 Hz, C_5-H), 5.32 (1H, br d, J=6.0 Hz, C_2-H), 6.43—7.20 (4H, m, aromatic H), 9.37 (br s) and 9.60 (br s) (1H, OH), 10.00—14.00 (1H, br, CO_2H).

(2*S*,5*S*)-(−)-5-(2-Hydroxyphenyl)-1-(3-mercaptopropionyl)-2-pyrrolidinecarboxylic Acid (18b)——Ammonolysis of **17b** (260 mg, 0.69 mmol) according to the above procedure yielded **18b** (191 mg, 94%) as an amorphous solid of $[\alpha]_D^{25}$ −136.0° (c=0.5, MeOH). The IR and ^{1}H-NMR spectra of **18b** were identical with those of **18a**.

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References and Notes

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