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Studies on Angiotensin-converting Enzyme Inhibitors. I. Syntheses and Angiotensin-converting Enzyme Inhibitory Activity of 2-(3-Mercaptopropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid Derivatives[1)]

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(3*S*)-2-[(2*S*)-3-Mercapto-2-methylpropionyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid [(3*S*), (2*S*)-**6a**] was prepared by the reaction of (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid *tert*-butyl ester [(3*S*)-**2a**] or benzyl ester [(3*S*)-**2b**] with 3-benzoylthio-2-methylpropionyl chloride (**3a**), followed by fractional crystallization and removal of the protective group. The absolute configuration of (3*S*), (2*S*)-**6a** was confirmed by X-ray diffraction analysis of the thiazepino[4,3-*b*]isoquinoline compound (**7**) derived from **6a**. Resolution of 3-benzoylthio-2-methylpropionic acid (**8**) was completed by using optically active phenylalanine amide as a resolving agent. The other optical isomers of (3*S*),(2*S*)-**6a** were prepared by the reaction of (3*S*)- or (3*R*)-**2b** with optically active **3a**.

The *in vitro* ACE inhibitory activity of each isomer of **6a** was evaluated. Among them, (3*S*),(2*S*)-**6a** was found to be the most potent inhibitor with an IC_{50} value of 8.6×10^{-9}M. Compound (3*S*),(2*S*)-**6a** induced a dose-dependent inhibition of the pressor response to angiotensin I after oral administration to normotensive anesthetized rats. Moreover, (3*S*),(2*S*)-**6a** markedly reduced the systolic blood pressure in renal hypertensive rats (RHR) and spontaneously hypertensive rats (SHR). The *in vivo* ACE inhibitory activity and the hypotensive effects of (3*S*),(2*S*)-**6a** were comparable to those of captopril.

Keywords——angiotensin-converting enzyme (ACE); ACE inhibitor; inhibitory activity; antihypertensive activity; 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; (3*S*)-2-[(2*S*)-3-mercapto-2-methylpropionyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; optical isomer; 3-benzoylthio-2-methylpropionic acid; optical resolution

Interest in a new class of antihypertensive drugs which work by inhibiting angiotensin-converting enzyme (ACE) has been growing since Ondetti *et al.* developed captopril in 1977.[2)] Captopril, D-3-mercapto-2-methylpropionyl-L-proline is a potent orally active ACE inhibitor which is designed for optimal interaction with the active center of the enzyme.

Reports[3a-b)] on the structural variation of the amino acid moiety show that the inhibitory activity is greatly enhanced by the use of either cyclic imino acids such as proline and thiazolidine-4-carboxylic acid, or amino acids having a hydrophobic aromatic ring such as tryptophan and phenylalanine. Thus, our interest was drawn to 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; this fused ring has an imino acid structure with a hydrophobic aromatic ring.

In the present paper, we describe the syntheses of 2-(3-mercaptopropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives and their biological activities.

Synthesis

The starting materials, (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid *tert*-butyl ester [(3*S*)-**2a**] and benzyl ester [(3*S*)-**2b**] were prepared from (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid [(3*S*)-**1**], which was obtained by Pictet–Spengler reaction of L-phenylalanine and formalin.[4a-b)] The corresponding (3*R*)-**2b** was obtained from D-

phenylalanine in the same way.

The target compounds, 2-(3-mercaptopropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids,were prepared by the procedure shown in Chart 1.

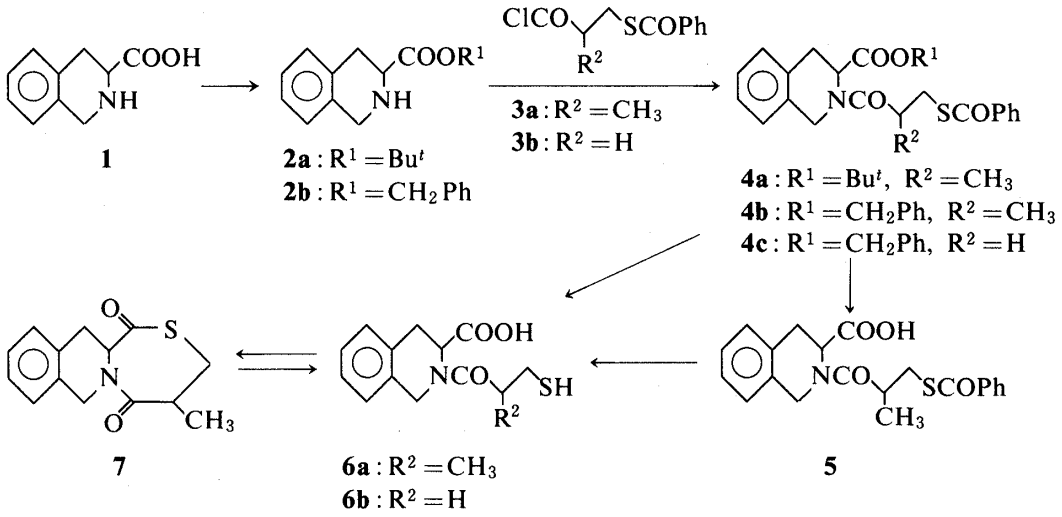


Chart 1

(3*S*)-2-[(2*S*)-3-Mercapto-2-methylpropionyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid [(3*S*),(2*S*)-6a]

Acylation of the *tert*-butyl ester [(3*S*)-**2a**] or the benzyl ester [(3*S*)-**2b**] with 3-benzoylthio-2-methylpropionyl chloride (**3a**) in the presence of triethylamine gave the corresponding (3*S*)-2-(3-benzoylthio-2-methylpropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate [(3*S*)-**4a** or (3*S*)-**4b**] as a mixture of diastereomers.

In the case of (3*S*)-**4a**, cleavage of the *tert*-butyl group was carried out by treatment with trifluoroacetic acid (TFA) to afford (3*S*)-2-(3-benzoylthio-2-methylpropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid [(3*S*)-**5**] as a mixture of diastereomers in approximately equal ratio asindicatedby thin-layer chromatography (TLC). Fractional crystallization of (3*S*)-**5** dicyclohexylamine (DCHA) salt from methanol–ether gave a single isomer [α-isomer of (3*S*)-**5**: (3*S*)-**5α**] in 35% yield, while isolation of the other isomer from the mother liquor was unsuccessful. After deprotection of the *S*-benzoyl group of (3*S*)-**5α** with aqueous ammonia, a single isomer of (3*S*)-2-(3-mercapto-2-methylpropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxlic acid [(3*S*)-**6aα**], mp 133—135°C, was obtained.

On the other hand, (3*S*)-**4b** was subjected to alcoholysis with methanolic potassium hydroxide to remove the *S*-benzoyl group and hydrolyzed subsequently with aqueous potassium hydroxide to give a mixture of diastereomers of (3*S*)-**6a**. Fractional crystallization of the DCHA salt of (3*S*)-**6a** from ethanol gave a single isomer [(3*S*)-**6aα**] in 35.4% yield; this product was identical with the specimen prepared from (3*S*)-**5α**.

X-Ray diffraction analysis was used to confirm the absolute configuration of the methyl group at position 2 in the side chain of (3*S*)-**6aα**. In order to obtain a more suitable crystalline form for X-ray diffraction analysis, an attempt was made to convert (3*S*)-**6aα** to a thiazepino[4,3-*b*]isoquinoline compound (**7**) having a three-membered rigid ring system. Treatment with *N*-hydroxybenzotriazole (HOBt) and dicyclohexylcarbodiimide (DCC) gave, as expected, colorless prisms of **7**, mp 163—165°C, which was confirmed to have (12a *S*),(4*S*)-configuration by X-ray diffraction analysis. Since the compound **7** was easily reconverted to (3*S*)-**6aα** by alkaline hydrolysis, the structure of (3*S*)-**6aα** was determincd to be (3*S*)-2-[(2*S*)-3-mercapto-2-methylpropionyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

Other Optical Isomers of (3*S*),(2*S*)-6a

In order to synthesize the other optical isomers of (3*S*), (2*S*)-**6a**, optical resolution of racemic 3-benzoylthio-2-methylpropionic acid (**8**) was investigated.

Several methods for the resolution of 3-acylthio-2-methylpropionic acid have been reported. In these methods, optically active amines such as (−)-2-tolyl-1-phenethylamine[5a] (−)-2-(4-chlorophenyl)-1-phenethylamine,[5b] D-(+)-*N*-benzyl-1-phenethylamine,[5c] and cinchonidine[5d] were used as a resolving agent.

We found that optically active phenylalanine amide was well suited for resolution of the acid **8**. As shown in Chart 2, resolution of racemic **8** in ethyl acetate with L-phenylalanine amide gave a crystalline salt composed of L-phenylalanine amide and (2*S*)-**8** in 27.8% yield. Subsequently, treatment of the salt with dilute hydrochloric acid gave optically pure (2*S*)-**8**. Similarly, the enantiomeric (2*R*)-**8** was obtained by resolution of racemic **8** using D-phenylalanine amide in the same manner.

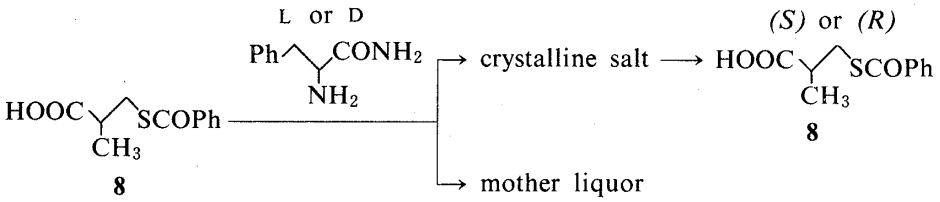
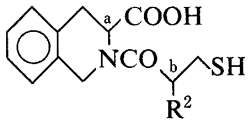


Chart 2

The optical isomers of (3*S*),(2*S*)-**6a** were prepared by the reaction of (3*S*)- or (3*R*)-**2b** with (2*S*)- or (2*R*)-**3a**, which was derived from the corresponding optically active **8**, followed by cleavage of the protective group in the same manner as for (3*S*), (2*S*)-**6a**. The desmethyl analog, (3*S*)-2-(3-mercaptopropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid [(3*S*)-**6b**] was also prepared from (3*S*)-**2b** and 3-benzoylthiopropionyl chloride (**3b**).

The physical constants and analytical data for four optical isomers of **6a** and **6b** are shown in Table I.

TABLE I. Physical Constants and Analytical Data for 2-(3-Mercaptopropionyl)-1, 2, 3, 4-tetrahydroisoquinoline-3-carboxylic Acids



Compd. No.	Config. (a, b)	R_2	mp (°C)	$[\alpha]_D^{25}$,° (*c*=1, MeOH)	Formula	Analysis (%) Calcd (Found) C H N
6a	(*S*, *S*)	CH_3	133—135	−23.5	$C_{14}H_{17}NO_3S$	60.19 6.13 5.01 (59.84 6.11 4.97)
6a	(*S*, *R*)	CH_3	110—112	+42.3	$C_{14}H_{17}NO_3S$	60.19 6.13 5.01 (59.85 6.27 4.86)
6a	(*R*, *S*)	CH_3	109—111	−43.9	$C_{14}H_{17}NO_3S$	60.19 6.13 5.01 (60.00 6.03 4.80)
6a	(*R*, *R*)	CH_3	131—133	+23.0	$C_{14}H_{17}NO_3S$	60.19 6.13 5.01 (59.91 6.08 5.01)
6b	(*S*)	H	151—152	+28.5	$C_{13}H_{15}NO_3S$	58.85 5.70 5.28 (58.67 5.74 5.16)

Biological Results

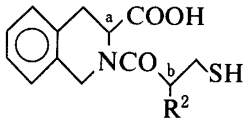
In Vitro ACE Inhibitory Activity

The *in vitro* ACE inhibitory activities of **6a** and **6b** are shown in Table II.

The activities were different depending on the configuration of the carboxylic acid at the 3-position of tetrahydroisoquinoline and the methyl group at the 2-position of the mercaptopropionyl moiety. The isomer of **6a** having (3*S*),(2*S*)-configuration [(3*S*),(2*S*)-**6a**] was the most potent inhibitor with an IC_{50} value of 8.6×10^{-9} M (compared to 2.3×10^{-8}M for captopril). Compound (3*S*),(2*R*)-**6a** showed almost the same activity as the desmethyl analog (3*S*)-**6b**. Compounds (3*R*),(2*S*)-**6a** and (3*R*),(2*S*)-**6a** were weaker inhibitors.

The most active compound, (3*S*),(2*S*)-**6a** was a competitive inhibitor of ACE with a Ki value of 1.1×10^{-9}M, which is comparable to that of captopril (1.4×10^{-9}M).

TABLE II. *In Vitro* ACE Inhibitory Activity of the Synthetic Compounds



Compd. No.	Config. (a, b)	R^2	IC_{50} (M)
6a	(*S*, *S*)	CH_3	8.6×10^{-9}
6a	(*S*, *R*)	CH_3	2.5×10^{-8}
6a	(*R*, *S*)	CH_3	7.2×10^{-8}
6a	(*R*, *R*)	CH_3	5.0×10^{-8}
6b	(*S*)	H	2.8×10^{-8}
Captopril[a)]			2.3×10^{-8}

a) This compound was prepared in our laboratory, mp 88—89°C, $[\alpha]_D^{20}$: −131.8° (*c*=1, EtOH).

In Vivo ACE Inhibitory Activity

As shown in Fig 1, (3*S*),(2*S*)-**6a** induced a dose-dependent inhibition of the pressor response to angiotensin I, like captopril, at oral doses of 0.1, 0.3 and 1.0 mg/kg in anesthetized rats. The activities of both compounds reached the maximum at 15 to 30 min after administration and lasted for about 2 h.

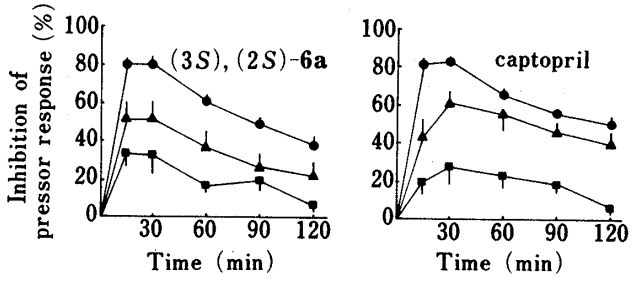


Fig. 1. Effects of (3*S*), (2*S*)-**6a** and Captopril on Pressor Responses to Angiotensin I in Anesthetized Rats

Dose: ●, 1 mg/kg (*p.o.*); ▲, 0.3 mg/kg (*p.o.*); ■, 0.1 mg/kg (*p.o.*).
Angiotensin I was given at a dose of 300 ng/kg, *i.v.*
Each point represents the mean±S.E. of 4 to 7 animals.

Antihypertensive Activity in Conscious Rats

Compound (3*S*),(2*S*)-**6a** markedly reduced the systolic blood pressure in renal hypertensive rats (RHR) and spontaneously hypertensive rats (SHR), but did so only slightly in normotensive rats (NR). The hypotensive effect of (3*S*),(2*S*)-**6a** in RHR was more potent than that in SHR. Compound (3*S*),(2*S*)-**6a** showed almost the same activities as captopril in SHR and NR (Table III).

TABLE III. Antihypertensive Activities of (3*S*), (2*S*)-**6a** and Captopril in SHR, RHR and NR

Compd.	Dose (mg/kg, *p. o.*)	Fall in blood pressure (mmHg)		
		SHR	RHR	NR
Control		−5±3	−5±2	−6±2
(3*S*), (2*S*)-**6a**	10		−29±3	
	30	−24±3	−38±5	−12±4
	100	−37±5		−16±3
Captopril	10		−28±2	
	30	−24±3	−42±7	−15±4
	100	−35±5		−11±3

Six to 8 rats were used for each dose.
Initial blood pressure were 178±3, 170±7 and 121±4 mmHg in SHR, RHR and NR, respectively.

Experimental

Melting points are uncorrected. Infrared (IR) spectra were obtained on a Shimadzu IR-27G spectrophotometer. Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Hitachi R-20A instrument, using tetramethylsilane as an internal standard. Mass spectra (MS) were taken on a Hitachi M-60 mass spectrometer. Specific rotations were measured with a Perkin-Elmer 243 polarimeter.

***tert*-Butyl (3*S*)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylate [(3*S*)-2a]**———A mixture of (3*S*)-**1** hydrochloride (10 g, 0.468 mol), isobutene (80 ml), conc. sulfuric acid (8 ml) and dioxane (80 ml) was shaken at room temperature for 24 h. The resulting clear solution was poured into a mixture of AcOEt, saturated aqueous $NaHCO_3$ and crushed ice. The separated organic layer was washed with water and dried over $MgSO_4$. The solvent was removed *in vacuo* to afford (3*S*)-**2a** (8.0 g, 73.3%) as a viscous oil. IR ν_{max}^{Nujol} cm^{-1}: 3320, 1730. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (9H, s, *tert*-Bu), 2.45 (1H, s, NH), 2.80—3.05 (2H, m, $ArC\underline{H}_2CH$), 3.55—3.75 (1H, m, CHN), 3.82—4.25 (2H, m, $ArCH_2N$), 7.04 (4H, s, aromatic H). MS *m/e*: 233 (M^+).

***tert*-Butyl (3*S*)-2-(3-Benzoylthio-2-methylpropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate [(3*S*)-4a]**———A solution of **3a** [prepared from **8** (6.25 g, 0.028 mol) and thionyl chloride (10 g) by heating at 50°C for 3 h] in tetrahydrofuran (THF) (10 ml) was added dropwise to a solution of (3*S*)-**2a** (6.5 g, 0.028 mol) and triethylamine (3.33 g, 0.033 mol) in THF (100 ml) with stirring at 0—5°C. After being stirred at room temperature for 4 h, the mixture was concentrated *in vacuo* and the residue was extracted with AcOEt. The extract was washed successively with 3% citric acid solution, water, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with toluene–AcOEt (10:1) as an eluent to give a mixture of diastereomers of (3*S*)-**4a** as a syrup (10.0 g, 81.6%). IR ν_{max}^{film} cm^{-1}: 1740, 1660, 1640. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (9H, s, *tert*-Bu), 1.15—1.45 (3H, m, CH_3), 3.0—3.45 (5H, m, $ArC\underline{H}_2CH$, $CHCH_2S$), 4.65—4.82 (2H, m, $ArCH_2N$), 5.25—5.42 (1H, m, CHN), 7.13 (4H, s, aromatic H), 7.2—7.6 (3H, m, aromatic H), 7.78—8.06 (2H, m, aromatic H). MS *m/e*: 439 (M^+).

(3*S*)-2-[(2*S*)-3-Benzoylthio-2-methylpropionyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid [(3*S*),(2*S*)-5]———A mixture of (3*S*)-**4a** (10 g, 0.023 mol) and TFA (50 ml) was allowed to stand for 1 h at room temperature and then concentrated to dryness *in vacuo*. The residue was dissolved in ether and the solution was treated with DCHA (4.1 g). The crystalline precipitate was collected and recrystallized from methanol–ether to give the DCHA salt of (3*S*)-**5α** (4.5 g, 35%) as colorless needles, mp 182—183°C, $[\alpha]_D^{25}$ −50.7° (*c*=1, MeOH). The DCHA salt was shaken with AcOEt and 5% aqueous potassium hydrogen sulfate. The organic layer was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was crystallized from AcOEt–*n*-hexane to give (3*S*),(2*S*)-**5**, mp 122—124°C, $[\alpha]_D^{26}$ −67.7° (*c*=1, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1725, 1650. ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.42 (3H, m, CH_3), 2.92—3.40 (5H, m, $ArCH_2C\underline{H}$, $CHCH_2S$), 4.6—4.9 (2H, m,

$ArCH_2N$), 5.38 (1H, t, J=5 Hz, CHN), 7.12 (s, 4H, aromatic H), 7.32—7.55 (3H, m, aromatic H), 7.78—8.05 (2H, m, aromatic H), 8.75 (1H, s, COOH). MS m/e: 383 (M^+). *Anal.* Calcd for $C_{21}H_{21}NO_4S \cdot 1/8CH_3COOC_2H_5$: C, 65.46; H, 5.62; N, 3.55; S, 8.13. Found: C, 65.16; H, 5.44; N, 3.68; S, 8.33.

Benzyl (3*S*)-2-(3-Benzoylthio-2-methylpropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate [(3*S*)-4b]——A solution of **3a** [prepared from **8** (4.48 g, 0.02 mol) and thionyl chloride (8 g)] in THF (10 ml) was added dropwise to a solution of (3*S*)- **2b** *p*-toluenesulfonate[6] (8.79 g, 0.02 mol) and triethylamine (4.55 g, 0.045 mol) in THF (100 ml) with stirring at 0—5°C. After being stirred at 5—10°C for 4 h, the reaction mixture was worked up as described for the preparation of (3*S*)-**4a** to afford a colorless syrup of (3*S*)-**4b** (9.1 g, 96.2%) as a mixture of diastereomers. IR ν_{max}^{Nujol} cm^{-1}: 1740, 1650 (broad). ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.42 (3H, m, CH_3), 2.90—3.55 (5H, m, $ArCH_2CH$, $CHCH_2S$), 4.55—4.90 (2H, m, $ArCH_2N$), 5.02 (2H, s, CH_2Ph), 5.38—5.62 (1H, m, CHN), 7.02—8.05 (14H, m, aromatic H). MS m/e: 473 (M^+).

(3*S*)-2-[(2*S*)-3-Mercapto-2-methylpropionyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid [(3*S*),(2*S*)-6a]——a) A mixture of (3*S*),(2*S*)-**5** (3.83 g, 0.01 mol) and 10% aqueous ammonia (50 ml) was stirred for 2 h at room temperature under a nitrogen atmosphere. The reaction mixture was washed with AcOEt to remove precipitated benzamide. The excess ammonia was removed *in vacuo* and the aqueous solution was acidified to pH 2 with 10% hydrochloric acid. The acidic solution was extracted with AcOEt and the extract was washed with water, dried over $MgSO_4$, and concentrated to dryness *in vacuo*. The residue was dissolved in ether,and DCHA (1.8 g) was added to the ethereal solution to yield the DCHA salt. Recrystallization of the salt from ethanol gave the DCHA salt of (3*S*)-**6a*α*** (4.37 g, 95%) as colorless needles, mp 191—192°C (dec., sintering 172°C), $[\alpha]_D^{19}$ −21.0° (c=1, MeOH). Treatment of the DCHA salt according to the procedure described for (3*S*),(2*S*)-**5** gave (3*S*),(2*S*)-**6a** as colorless needles, mp 134—136°C, $[\alpha]_D^{26}$ −22.8° (c=1, MeOH). *Anal.* Calcd for $C_{14}H_{17}NO_3S \cdot 1/8CH_3COOC_2H_5$: C, 59.98; H, 6.25; N, 4.82; S, 11.04. Found: C, 59.84; H, 6.20; N, 4.79; S, 11.12. The above sample was recrystallized from aqueous ethanol to give colorless crystals, mp 133—135°C, $[\alpha]_D^{25}$ −23.5° (c=1, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 2600, 1730, 1600. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, d, J=7 Hz, CH_3), 1.51 (1H, t, J=9 Hz, D_2O-exchangeable SH), 2.35—3.40 (5H, m, $ArCH_2CH$, $CHCH_2S$), 4.55—4.94 (2H, m, $ArCH_2N$), 5.42 (1H, t, J=5 Hz, CHN), 7.20 (4H, s, aromatic H), 9.80 (1H, s, COOH). MS m/e: 279 (M^+).

b) A solution of KOH (1.3 g) in MeOH (20 ml) was added dropwise to a solution of (3*S*)-**4b** (9.1 g, 0.019 mol) in MeOH (150 ml) with stirring at 0—5°C under a nitrogen atmosphere. After being stirred at 5°C for 30 min, ether (40 ml), *n*-hexane (40 ml) and water (80 ml) were added to the reaction mixture. A solution of KOH (1.63 g) in water (5 ml) was added to the separate aqueous layer and the mixture was stirred at room temperature for 2 h under a nitrogen atmosphere. The mixture was washed with AcOEt, acidified with 10% hydrochloric acid, and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo* to give a syrup of (3*S*)-**6a** as a mixture of diastereomers. The product thus obtained was dissolved in isopropyl ether (150 ml) and treated with DCHA (3.5 g). The crystalline precipitate was collected by filtration and recrystallized from ethanol to give the DCHA salt of (3*S*),(2*S*)-**6a** (3.13 g, 35.4%). The physical constants of this product were identical with those of the sample obtained from (3*S*),(2*S*)-**5**.

12a(*S*),4(*S*)-4-Methyl-1,5-dioxo-1,4,5,7,12,12a-hexahydro-3*H*-1,4-thiazepino[4,3-*b*]isoquinoline (7)——Dicyclohexylcarbodiimide (3.1 g, 0.015 mol) was added portionwise to a solution of (3*S*),(2*S*)-**6a** (4.5 g, 0.015 mol) and HOBt (2.03 g, 0.015 mol) in THF (50 ml) at −5—0°C with stirring. The stirring was continued for 40 min at the same temperature and then at room temperature overnight. After the precipitate had been removed by filtration, the filtrate was concentrated *in vacuo* and the residue was dissolved in AcOEt. The solution was washed with saturated aqueous $NaHCO_3$ and water, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was recrystallzed from AcOEt to afford colorless prisms of **7** (1.52 g, 38.8%), mp 163—165°C, $[\alpha]_D^{27}$ +32.6° (c=1, THF). IR ν_{max}^{Nujol} cm^{-1}: 1675, 1660. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, d, J=7 Hz, CH_3), 2.70—3.80 (5H, m, $ArCH_2CH$, $CHCH_2S$), 4.43, 4.84 (2H, ABq, J=16 Hz, $ArCH_2N$), 5.09 (1H, t, J=5 Hz, CHN), 7.22 (4H, s, aromatic H). *Anal.* Calcd for $C_{14}H_{15}NO_2S$: C, 64.34; H, 5.79; N, 5.36; S, 12.27. Found: C, 64.16; H, 6.16; N, 5.60; S, 12.13.

Hydrolysis of 7 to (3*S*),(2*S*)-6a——A solution of NaOH (0.44 g) in water (20 ml) was added dropwise to a solution of **7** (2.62 g, 0.01 mol) in MeOH (10 ml) with stirring at 0—5°C under a nitrogen atmosphere. After being stirred at 5°C for 1 h, the reaction mixture was acidified with dilute hydrochloric acid and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized from *n*-hexane to afford (3*S*),(2*S*)-**6a** (2.54 g, 91%).

Optical Resolution of 3-Benzoylthio-2-methylpropionic Acid (8)——Racemic acid **8** (61.5 g, 0.27 mol) and L-phenylalanine amide (45 g, 0.27 mol) were dissolved in AcOEt (1200 ml) by heating on a water bath and the solution was allowed to stand overnight at room temperature. The crystalline precipitate was collected by filtration and recrystallized from AcOEt (660 ml) to afford the L-phenylalanine amide salt of (2*S*)-**8** (29 g, 27.8%) as colorless needles, mp 124—125°C, $[\alpha]_D^{21}$ −59.4° (c=0.5, $CHCl_3$). The salt (28 g) thus obtained was shaken with ether and 5% hydrochloric acid. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to dryness. The resultant syrup was crystallized from *n*-hexane to give colorless crystals of (2*S*)-**8** (15.0 g, 92.8%), mp 69—71°C, $[\alpha]_D^{21}$ −41.7° (c=1, MeOH).[5c]

The (2*R*)-isomer of **8** was prepared from racemic acid **8** and D-phenylalanine amide according to the

procedure described for (2*S*)-**8**. mp 69—71°C, $[\alpha]_D^{21}$ +41.5° (*c*=1, MeOH).

Preparation of Optical Isomers of (3*S*),(2*S*)-6a: (3*S*)-2-[(2*R*)-3-Mercapto-2-methylpropionyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid [(3*S*),(2*R*)-6a]———The reaction of (3*S*)-**2b** with (2*R*)-**3a** [prepared from (2*R*)-**8** and thionyl chloride] was carried out according to the procedure described for the preparation of (3*S*)-**4b** to give (3*S*),(2*R*)-**4b** as a colorless syrup in 90% yield. The compound (3*S*),(2*R*)-**4b** was treated with a methanolic KOH solution as described for the preparation of (3*S*),(2*S*)-**6a** to afford (3*S*),(2*R*)-**6a** as colorless crystals in 81% yield. IR ν_{max}^{Nujol} cm^{-1}: 2600, 1730, 1600. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, d, *J*=7 Hz, CH_3), 1.51 (1H, t, *J*=9 Hz, D_2O-exchangeable SH), 2.40—3.40 (5H, m, $ArCH_2CH$, $CHCH_2S$), 4.55—4.94 (2H, m, $ArCH_2N$), 5.42 (1H, t, *J*=5 Hz, CHN), 7.20 (4H, s, aromatic H), 8.61 (1H, s, COOH). MS *m/e*: 279 (M^+).

(3*R*),(2*S*)-**6a** and (3*R*),(2*R*)-**6a** were prepared by a similar method in 79 and 85% yields, respectively.

(3*S*)-2-(3-Mercaptopropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid [(3*S*)-6b]———The title compound was prepared from (3*S*)-**2b** (2.67 g, 0.01 mol) and **3b** (2.23 g, 0.01 mol) according to the procedure described for the preparation of (3*S*),(2*R*)-**6a** in 80% yield. IR ν_{max}^{Nujol} cm^{-1}: 2580, 1740, 1615. ^{1}H-NMR ($CDCl_3$) δ: 2.65—3.35 (6H, m, $ArCH_2CH$, CH_2CH_2S), 4.60—4.90 (2H, m, $ArCH_2N$),5.44 (1H, t, *J*=5 Hz, CHN), 7.20 (4H, s, aromatic H), 8.3—8.9 (1H, broad, COOH). MS *m/e*: 265 (M^+).

Biological Methods: *In Vitro* ACE Inhibitory Activity———ACE was prepared from swine renal cortex by the method of Oshima *et al.*[7] ACE inhibitory activity was investigated by the following methods. The reaction mixture contained: Tris-hydrochloride (pH 7.4), 60 μmol; sodium chloride, 60 μmol; hippuryl-histidyl-leucine, 0.5 μmol, testing inhibitor and converting enzyme (6 μg of protein) in a final volume of 0.5 ml. Incubation was carried out for 20 min at 37°C. The reaction was stopped by placing the tubes in ice water. Histidyl-leucine formed was measured microbiologically by the use of *Leuconostoc mesenteroides* p-60. Each sample was run in duplicate and the average of the two readings obtained was calculated. A standard curve of histidyl-leucine was always prepared with each assay.

Activity was designated in terms of the IC_{50}, which was the molar concentration of test inhibitor causing 50% inhibition of the control converting-enzyme activity.

***In Vivo* ACE Inhibitory Activity**———Groups of 4 to 7 male Wistar rats weighing 300 to 400 g were used. The rats were anesthetized subcutaneously with urethane at a dose of 1.5 g/kg. The carotid artery blood pressure was measured with a pressure transducer (MPU-0.5A, Nihonkohden) and recorded on a polygraph pen-recorder (RM-45, Nihonkohden). The pressor response to intravenous injection of 300 ng/kg of angiotensin I was obtained at intervals of 15 to 30 min.

Antihypertensive Activity in Conscious Rats———As hypertensive rats, male spontaneously hypertensive rats (SHR, Charles River Japan Inc.) weighing 300 to 350 g and male renal hypertensive rats (RHR) prepared by clipping the left renal artery of Wistar rats were used. The effect on normotension was also examined in male Wistar rats (NR) weighing 250 to 300 g.

The systolic blood pressure was measured by a tail cuff method.[8] Groups of 6 to 8 rats were used for each dose. In all experiments, the test drugs were suspended in 0.5% CMC solution and orally administered to rats which had been fasted overnight.

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