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Potent orally Active Inhibitors of Angiotensin-Converting Enzyme (ACE)

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1-[3-Mercaptoalkanoyl]pyroglutamic acids and 1-[3-mercaptoalkanoyl]-4-hetero(oxa, thia and aza)pyroglutamic acids were prepared and their inhibitory activities against angiotensin-converting enzyme (ACE) were examined.

(2*S*)-1-[(2*S*)-3-Mercapto-2-methylpropanoyl]pyroglutamic acid **2** was found to be the most potent orally active inhibitor of ACE of rabbit lung among the derivatives synthesized in the present study and appeared to have great potential for use as an oral antihypertensive agent.

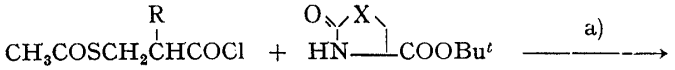
Keywords——angiotensin-converting enzyme; angiotensin-converting enzyme inhibitor; antihypertensive agent; L-pyroglutamic acid; 1-[3-mercapto-2-methylpropanoyl]pyroglutamic acid

Recently (2*S*)-1-[(2*S*)-3-mercapto-2-methylpropanoyl]proline (SQ 14225) was found to be a potent inhibitor of angiotensin-converting enzyme (ACE), and was considered to be potentially useful as a therapeutic agent for renal hypertension.[1]

ACE converts the inactive decapeptide angiotensin I to the potent vasopressor octapeptide angiotensin II and the vasodepressor nonapeptide bradykinin to the inactive heptapeptide.[2]

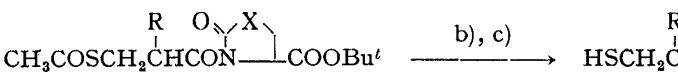
In our search for new inhibitors of ACE, we synthesized 1-[3-mercaptoalkanoyl]pyroglutamic acids **1**, **2**, **3** and **4** and 1-[3-mercaptoalkanoyl]-4-hetero(oxa, thia and aza)pyroglutamic acids **5**, **6** and **7** (Table I), and examined their inhibitory activities against ACE *in vitro* and *in vivo*.

(2*S*)-1-[(2*S*)-3-Mercapto-2-methylpropanoyl]pyroglutamic acid **2** was found to be the most potent orally active inhibitor of ACE and was considered to have great potential for use as an oral antihypertensive agent.



8a: R=H
8b: R=CH_3(*S*)
8c: R=CH_3(*R*)
8d: R=CH_3(*R*, *S*)

9a: X=CH_2, COOBu: (*S*)
9b: X=CH_2, COOBu: (*R*)
9c: X=O, COOBu: (*S*)
9d: X=S, COOBu: (*R*)
9e: X=N-THP, COOBu: (*S*)



10a: R=H, X=CH_2, COOBu: (*S*)
10b: R=CH_3(*S*), X=CH_2, COOBu: (*S*)
10c: R=CH_3(*R*), X=CH_2, COOBu: (*S*)
10d: R=CH_3(*S*), X=CH_2, COOBu: (*R*)
10e: R=CH_3(*S*), X=O, COOBu: (*S*)
10f: R=CH_3(*S*), X=S, COOBu: (*R*)
10g: R=CH_3(*S*), X=N-THP, COOH: (*R*, *S*)

1: R=H, X=CH_2, COOH: (*S*)
2: R=CH_3(*S*), X=CH_2, COOH: (*S*)
3: R=CH_3(*R*), X=CH_2, COOH: (*S*)
4: R=CH_3(*S*), X=CH_2, COOH: (*R*)
5: R=CH_3(*S*), X=O, COOH: (*S*)
6: R=CH_3(*S*), X=S, COOH: (*R*)
7: R=CH_3(*S*), X=NH, COOH: (*R*, *S*)

a) NaH, THF, b) CF_3COOH, anisole, c) $H_2NCH_2CH_2SH$, CH_3CN

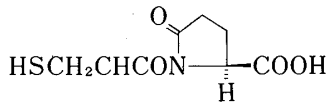
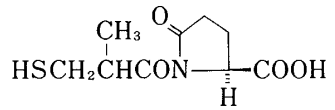
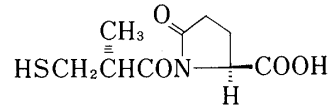
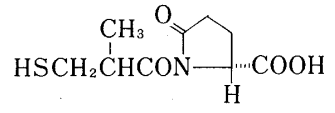
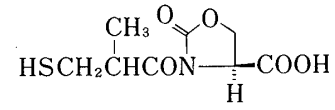
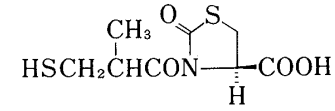
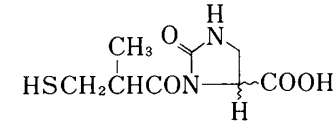
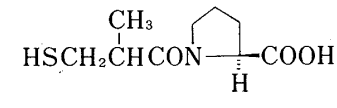
Chart 1

Chemistry

1-[3-Mercaptoalkanoyl]pyroglutamic acids **1**, **2**, **3** and **4** were synthesized from *tert*-butyl pyroglutamates **9a** and **9b** according to Chart 1.

Sodium salts of *tert*-butyl pyroglutamates **9a** and **9b** were treated with 3-acetylthiopropanoyl chlorides **8a**, **8b** and **8c**[3] in anhydrous THF at 0° C to afford *tert*-butyl 1-[3-acetylthiopropanoyl]pyroglutamates **10a**, **10b**, **10c** and **10d** in 60—80% yields. Deprotection of the acetyl group and *tert*-butyl group was achieved by treatment with trifluoroacetic acid and anisole at 0 °C followed by 2-mercaptoethylamine (1 eq) in acetonitrile at room temperature to afford 1-[3-mercaptopropanoyl]pyroglutamic acids **1**, **2**, **3** and **4** in about 90% yields.

Table I. Physical Constants and Inhibitory Activities

Compound		mp, °C (uncorr.)	$[\alpha]_D$, ° (MeOH)	IC_{50}, nM against ACE[a]
1		Oil	−58.2(*c*, 0.95)	60
2		109—110	−113.6(*c*, 0.55)	3.6
3		108—109	−21.0(*c*, 1.00)	45
4		110—111	+7.1(*c*, 1.28)	300
5		101—102	−154.4(*c*, 0.58)	6.4
6		Oil	−191.8(*c*, 1.00)	20
7[b]		Oil	−45.7(*c*, 0.51)	42
SQ 14225		103—105	−131.0(*c*, 2.00)	23

a) Inhibitory activities against ACE were determined according to the reported procedure: D.W. Cushman, H.S. Cheund, E.F. Sabo and M.A. Ondetti, *Biochemistry*, **16**, 5484 (1977).
b) A roughly 1: 1 mixture of diastereomers.

An improved method for large scale preparation of optically pure **2** was developed as follows. 1) L-Pyroglutamic acid was treated with racemic 3-acetylthio-2-methylpropanoyl chloride **8d** in acetonitrile in the presence of triethylamine (2 eq) at 0 °C for 2 h to afford (2*S*)-1-[(2*RS*)-3-acetylthio-2-methylpropanoyl]pyroglutamic acid **12**. 2) The dicyclohexylammonium salt of **12** was recrystallized once from acetonitrile, followed by treatment with dil. HCl to afford optically pure (2*S*)-1-[(2*S*)-3-acetylthio-2-methylpropanoyl]pyroglutamic acid **13** 3) Treatment of **13** with 2-mercaptoethylamine (2 eq) in acetonitrile at room temperature for 1 h afforded optically pure (2*S*)-1-[(2*S*)-3-mercapto-2-methylpropanoyl]pyroglutamic acid **2**.

(4*S*)-3-[(2*S*)-3-Mercapto-2-methylpropanoyl]-2-oxazolidone-4-carboxylic acid **5**, (4*R*)-3-[(2*S*)-3-mercapto-2-methylpropanoyl]-2-thiazolidone-4-carboxylic acid **6** and 3-[(2*S*)-3-mercapto-2-methylpropanoyl]-2-imidazolidone-4-carboxylic acid **7**[4] were also prepared from *tert*-butyl (4*S*)-2-oxazolidone-4-carboxylate **9c**, *tert*-butyl (4*R*)-2-thiazolidone-4-carboxylate **9d** and *tert*-butyl (4*S*)-3-tetrahydropyranyl-2-imidazolidone-4-carboxylate **9e**, respectively, according to Chart 1.

Table II

Compound	Formula	Analysis (%) Calcd C	H	N	S	Found C	H	N	S
1	$C_8H_{11}NO_4S$	44.23	5.10	6.45	14.76	44.08	5.01	6.31	14.88
2	$C_9H_{13}NO_4S$	46.74	5.67	6.06	13.86	46.67	5.62	6.38	13.71
3	$C_9H_{13}NO_4S$	46.74	5.67	6.06	13.86	46.59	5.66	6.43	13.79
4	$C_9H_{13}NO_4S$	46.74	5.67	6.06	13.86	46.63	5.59	6.30	13.81
5	$C_8H_{11}NO_5S$	41.19	4.75	6.01	13.75	41.17	4.73	6.10	13.80
6	$C_8H_{11}NO_4S_2$	38.54	4.45	5.62	25.72	38.59	4.48	5.57	25.85
7	$C_8H_{12}N_2O_4S$	41.37	5.21	12.06	13.80	40.39	5.23	12.11	13.68

Biological and Pharmaceutical Studies

As shown in Table I, we have tested the inhibitory activity of the above compounds against ACE of rabbit lung.

(2*S*)-1-[3-Mercaptopropanoyl]pyroglutamic acid **1** has an IC_{50} value of 60 nM, being 2.5 times less active than SQ 14225. However, the introduction of a 2-methyl substituent with *S* configuration enhanced the inhibitory potency, whereas a 2-methyl substituent with *R* configuration had no effect on the potency. (2*S*)-1-[(2*S*)-3-Mercapto-2-methylpropanoyl]pyroglutamic acid **2** was 20 times more active as an inhibitor of ACE than the unsubstituted analog **1**, but (2*S*)-1-[(2*R*)-3-mercapto-2-methylpropanoyl]pyroglutamic acid **3** has almost the same activity as the unsubstituted analog **1**.[5]

Insertion of a hetero atom (oxygen, nitrogen and sulfur) into the ring of (2*S*)-1-[(2*S*)-3-mercapto-2-methylpropanoyl]pyroglutamic acid **2** weakened the inhibitory potencies against ACE by 2—12 times. The 4-oxa, 4-thia and 4-aza analogs **5**, **6** and **7** of **2** were 2, 6 and 12 times, respectively, less active than the parent compound. Their increasing polarities probably cause their affinities to the enzyme to be weakened.

The most potent compound **2** among the derivatives has a K_i value of 6.16 nM (K_i value of SQ 14225=38.9 nM) and showed reversible competitive inhibition of ACE of rabbit lung. Compound **2** also had an inhibitory effect (IC_{50}=15 nM) against Kininase II of rabbit lung (IC_{50} of SQ 14225=17 nM) and inhibited the vasopressor response of angiotensin I (0.3 ug/kg, *i.v.*) in the normotensive anesthetized rat after oral administration (0.5 mg/kg).

Moreover, compound **2** showed a significant vasodepressor effect on 2-kidney Goldblatt RHR (acute phase) at 3 mg/kg, *p.o.* and on 2-kidney Goldblatt RHR (chronic phase) at 100 mg/kg, *p.o.*[6]

Experimental

Melting points are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-100 machine and signals are given in δ units downfield from TMS as an internal standard. Infrared (IR) spectra were measured on a Hitachi 269-30 spectrometer. Mass spectrum (MS) and specific rotations (25°C) were taken on JMS-01SG and JASCO DIP-4 machines, respectively.

Preparation of 2. Method A: *tert*-Butyl (2*S*)-1-[(2*S*)-3-acetylthio-2-methylpropanoyl]pyroglutamate 10b——A solution of 914 mg of *tert*-butyl L-pyroglutamate in 16 ml of anhydrous THF was treated with 180 mg of 63% NaH under cooling in an ice bath. The mixture was stirred for 20 min, then 891 mg of (2*S*)-3-acetylthio-2-methyl-propanoyl chloride (prepared from (2*S*)-3-acetylthio-2-methylpropionic acid with thionyl chloride in anhydrous benzene at room temperature for 20 h) in 8 ml of anhydrous THF was added at 0°C, and then the reaction mixture was stirred for 1.5 h at 0°C and for 2 h at room temperature. The contents were diluted with EtOAc, washed with water, dried and concentrated *in vacuo*.

The residue was separated by column chromatography on silica gel (EtOAc: cyclohexane/4: 1) to give 1.13 g (54% yield) of the title compound. $[\alpha]_D$ −97.8° (*c*, 1.61, MeOH). NMR ($CDCl_3$) ppm: 1.26 (3H, d, J=6.9 Hz), 1.47 (9H, s), 2.31 (3H, s), 2.0—2.7 (4H, m), 3.08—3.22 (2H, m), 3.70—4.05 (1H, m), 4.62 (1H, dd, J=3.0, 8.5 Hz). MS *m/e*: 329 (M^+), 286, 273, 256, 230, 213, 198. IR (film) cm^{-1}: 1745, 1695.

(2*S*)-1-[(2*S*)-3-Acetylthio-2-methylpropanoyl]pyroglutamic Acid 13——A mixture of a solution of 990 mg of *tert*-butyl (2*S*)-1-[(2*S*)-3-acetylthio-2-methylpropanoyl]pyroglutamate in 10 ml of trifluoroacetic acid and 5 ml of anisole was stirred for 1 h at room temperature then concentrated *in vacuo* to afford 830 mg (100% yield) of the title compound. $[\alpha]_D$ −100° (*c*, 1.65, MeOH). NMR ($CDCl_3$) ppm: 1.24 (3H, d, J=7.0 Hz), 2.30 (3H, s), 2.10—2.90 (4H, m), 3.05 (1H, dd, J=7.0, 14.0 Hz), 3.21 (1H, dd, J=5.5, 14.5 Hz), 3.70—4.05 (1H, m), 4.76 (1H, dd, J=4.0, 8.0 Hz). MS *m/e*: 273 (M^+), 256, 231, 213, 198. IR (film) cm^{-1}: 1750, 1695.

Method B: (2*S*)-1-[(2*RS*)-3-Acetylthio-2-methylpropanoyl]pyroglutamic Acid 12——A solution of 12.9 g of L-pyroglutamic acid in 300 ml of acetonitrile and 20.2 g of NEt_3 at 0°C was treated dropwise with 18 g of (2*RS*)-3-acetylthio-2-methylpropanoyl chloride. The mixture was stirred for 1 h at room temperature, then concentrated *in vacuo*. The residue was dissolved in 200 ml of H_2O and washed with EtOAc. The aqueous layer was acidified with 1 N HCl and extracted with EtOAc. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*.

The residue was purified by column chromatography on silica gel ($CHCl_3$: THF: AcOH/100: 10: 1) to afford 16.3 g (60% yield) of the title compound. MS *m/e*: 273 (M^+), 256, 231, 213, 198. IR (KBr) cm^{-1}: 1754, 1695.

(2*S*)-1-[(2*S*)-3-Acetylthio-2-methylpropanoyl]pyroglutamic Acid 13——A solution of 103 mg of (2*S*)-1-[(2*RS*)-3-acetylthio-2-methylpropanoyl]pyroglutamic acid in 0.7 ml of acetonitrile was treated with 0.075 ml of dicyclohexylamine. The crystalline precipitates were collected and recrystallized from acetonitrile to give 70 mg of (2*S*)-1-[(2*S*)-3-acetylthio-2-methylpropanoyl]pyroglutamic acid-dicyclohexylamine salt, mp 185—187°C, $[\alpha]_D$ −72.6° (*c*, 0.46, MeOH).

A solution of 347 mg of the salt in H_2O was acidified with 0.5 N HCl and extracted with EtOAc. The extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$: THF: AcOH/10: 2: 1) to afford 217 mg (42% yield) of the title compound.

(2*S*)-1-[(2*S*)-3-Mercapto-2-methylpropanoyl]pyroglutamic Acid 2——2-Mercaptoethylamine (338 mg) was added to a solution of 546 mg of (2*S*)-1-[(2*S*)-3-acetylthio-2-methylpropanoyl]pyroglutamic acid in 10 ml of acetonitrile under argon. The contents were stirred for 1 h at room temperature and concentrated *in vacuo*.

The residue was treated with 10 ml of 1% HCl and the resulting aqueous mixture was extracted 3 times with EtOAc. The extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was separated by column chromatography on silica gel ($CHCl_3$: THF: AcOH/20: 2: 1) to afford 415 mg (85% yield) of the title compound. mp 109—110°C. $[\alpha]_D$ −113.6° (*c*, 0.55, MeOH). NMR ($CDCl_3$) ppm: 1.24 (3H, d, J= 6.5 Hz), 1.52 (1H, t, J=9.0 Hz), 2.0—3.0 (6H, m), 3.70—4.10 (1H, m), 4.83 (1H, dd, J=4.0, 8.5 Hz), 8.78 (1H, br s). MS *m/e*: 231 (M^+), 198, 130. Calcd for $C_9H_{13}NO_4S$: 231.05652. Obsd 231.05744. IR (film) cm^{-1}: 1750, 1700.

(2*S*)-1-[3-Mercaptopropanoyl]pyroglutamic Acid 1——The title compound was prepared from *tert*-butyl L-pyroglutamate and 3-acetylthiopropionic acid according to the procedure described for **2**. $[\alpha]_D$ −58.2° (*c*, 0.97, MeOH). NMR ($CDCl_3$) ppm: 1.71 (1H, t, J=8.0 Hz), 2.0—3.0 (6H, m), 3.10—3.40 (2H, m), 4.78 (1H, dd, J=3.5, 8.5 Hz), 8.95 (1H, m). MS *m/e*: 217 (M^+), 199, 184, 130. Calcd for $C_8H_{11}NO_4S$: 217.04087. Obsd 217.03951. IR (film) cm^{-1}: 1740, 1690.

(2*S*)-1-[(2*R*)-3-Mercapto-2-methylpropanoyl]pyroglutamic Acid 3——The title compound was prepared from *tert*-butyl L-pyroglutamate and (2*R*)-3-acetylthio-2-methylpropionic acid according to the procedure described for **2**. mp 108—109°C. $[\alpha]_D$ −21.0° (*c*, 1.00, MeOH). NMR ($CDCl_3$) ppm: 1.24 (3H, d, J=7.0 Hz), 1.54 (1H, t, J=9.0 Hz), 2.0—3.1 (6H, m), 3.90 (1H, m), 4.80 (1H, dd, J=5.0, 8.5 Hz), 9.55 (1H, br s). MS *m/e*: 231 (M^+), 198. Calcd for $C_9H_{13}NO_4S$: 231.05652. Obsd 231.05498. IR (KBr) cm^{-1}: 1750, 1680.

(2*R*)-1-[(2*S*)-3-Mercapto-2-methylpropanoyl]pyroglutamic Acid 4——The title compound was prepared from *tert*-butyl D-pyroglutamate and (2*S*)-3-acetylthio-2-methylpropionic acid according to the procedure described for **2**. mp 110—111°C. $[\alpha]_D$ +7.1° (*c*, 1.28, MeOH). NMR ($CDCl_3$) ppm: 1.24 (3H, d, J=7.0 Hz),

1.55 (1H, t, J=8.0 Hz), 2.0—3.1 (6H, m), 3.90 (1H, m), 4.80 (1H, dd, J=5.0, 8.5 Hz), 9.5 (1H, br s). MS *m/e*: 231 (M^+), 198. Calcd for $C_9H_{13}NO_4S$: 231.05652. Obsd 231.05553. IR (KBr) cm^{-1}: 1740, 1690.

***tert*-Butyl (4*S*)-3-[(2*S*)-3-Acetylthio-2-methylpropanoyl]-2-oxazolidone-4-carboxylate 10e**——A solution of 374 mg of *tert*-butyl (4*S*)-2-oxazolidone-4-carboxylate[7] in 3 ml of anhydrous THF was added to a mixture of 76 mg of 63% NaH in 5 ml of anhydrous THF at 0°C. The whole was stirred for 20 min at 0°C, then 360 mg of (2*S*)-3-acetylthio-2-methylpropanoyl chloride was added at 0°C. The reaction mixture was stirred for 1 h at room temperature, diluted with EtOAc, washed with H_2O and dried over Na_2SO_4.

The organic solution was concentrated *in vacuo* to afford the residue, which was purified by column chromatography on silica gel (cyclohexane: EtOAc/5: 1) to give 419 mg (63% yield) of the title compound. NMR ($CDCl_3$) ppm: 1.30 (3H, d, J=7.0 Hz), 1.50 (9H, s), 2.27 (3H, s), 3.0—3.2 (2H, m), 3.6—4.1 (1H, m), 4.1—4.9 (3H, m). MS *m/e*: 331 (M^+), 275.

The following compounds were also prepared according to the procedure described above.

***tert*-Butyl (4*R*)-3-[(2*S*)-3-Acetylthio-2-methylpropanoyl]-2-thiazolidone-4-carboxylate 10f**——NMR ($CDCl_3$) ppm: 1.20 (3H, d, J=8.0 Hz), 1.40 (9H, s), 2.30 (3H, s), 3.0—4.0 (5H, m), 4.95 (1H, dd, J=9.2 Hz). MS *m/e*: 347 (M^+), 291.

***tert*-Butyl 3-[(2*S*)-3-Acetylthio-2-methylpropanoyl]-1-tetrahydropyranyl-2-imidazolidone-4-carboxylate 10g**——Epimerization of the carboxyl group occurred during the course of the reaction and the product was a roughly 1: 1 mixture of diastereomers as judged from the NMR spectrum.

(4*S*)-3-[(2*S*)-3-Mercapto-2-methylpropanoyl]-2-oxazolidone-4-carboxylic Acid 5——A solution of 903 mg of *tert*-butyl (4*S*)-3-[(2*S*)-3-acetylthio-2-methylpropanoyl]-2-oxazolidone-4-carboxylate in 5.0 ml of trifluoroacetic acid and 2.5 ml of anisole was stirred at room temperature for 40 min, then concentrated *in vacuo* to afford the residue, which was used for the next reaction without purification.

2-Mercaptoethylamine (460 mg) was added to a solution of the above residue in 12 ml of acetonitrile at room temperature. The reaction mixture was stirred for 2.5 h at room temperature then concentrated *in vacuo*. The residue was dissolved in EtOAc, and the solution was washed with dil. HCl, dried over Na_2SO_4 and concentrated *in vacuo* to afford the crude product, which was purified by column chromatography on silica gel ($CHCl_3$: THF: AcOH/100: 10: 1) to give 483 mg (76% yield) of the title compound. NMR ($CDCl_3$) ppm: 1.27 (3H, d, J=6.5 Hz), 2.4—3.0 (3H, m), 3.7—4.1 (1H, m), 4.39 (1H, dd, J=4.0, 9.0 Hz), 4.64 (1H, t, J=9.0 Hz), 4.97 (1H, dd, J=4.0, 9.0 Hz). MS *m/e*: 233 (M^+), 200, 132. Calcd for $C_8H_{11}NO_5S$: 233.03579. Obsd 233.03353. IR (KBr) cm^{-1}: 1800, 1740, 1690.

The following compounds were also prepared according to the procedure described above.

(4*R*)-3-[(2*S*)-3-Mercapto-2-methylpropanoyl]-2-thiazolidone-4-carboxylic Acid 6——NMR ($CDCl_3$) ppm: 1.25 (3H, d, J=8.0 Hz), 1.53 (1H, t, J=9.0 Hz), 2.40—3.05 (2H, m), 3.44 (1H, dd, J=2.0, 12.0 Hz), 3.75 (1H, dd, J=8.5, 12.0 Hz), 3.6—4.0 (1H, m), 5.00 (1H, dd, J=2.0, 8.5 Hz), 9.57 (1H, br s). MS *m/e*: 249 (M^+), 216. Calcd for $C_8H_{11}NO_4S_2$: 249.01294. Obsd 249.01420. IR (film) cm^{-1}: 1740, 1680.

3-[(2*S*)-3-Mercapto-2-methylpropanoyl]-2-imidazolidone-4-carboxylic Acid 7——The NMR spectrum showed the presence of a roughly 1: 1 mixture of diastereomers. MS *m/e*: 232 (M^+), 217, 214, 199, 186. Calcd for $C_8H_{12}N_2O_4S$: 232.05177. Obsd 232.05221. IR (film) cm^{-1}: 1740, 1690.

References and Notes

1) M.A. Ondetti, B. Rubin, and D.W. Cushman, *Science*, **196**, 441 (1977); R.K. Ferguson, G. Turini, H.R. Brunner, H. Gavras, and D.N. Mckinistry, *The Lancet*, **1977**, 775; J. Iwao, T. Iso, and M. Oya, *Yuki Gosei Kagaku Kyokai Shi*, **38**, 1100 (1980).
2) L.T. Skeggs, J.P. Kahn, and N.P. Shummary, *J. Exp. Med.*, **103**, 295 (1956); H.Y.T. Yang, E.G. Erdös, and Y. Levin, *J. Pharmacol. Exp. Ther.*, **197**, 291 (1971).
3) The optical resolution of racemic 3-acetylthio-2-methylpropionic acid was achieved by recrystallization of its cinchonidine salt to afford the (2*S*)-isomer, $[\alpha]_D$ −33.3° (*c*, 1.00, EtOH), according to J. Iwao, M. Oya, E. Kato, and T. Watanabe, Japan Patent 151912 (1979).
4) Epimerization of the carboxyl group of *tert*-butyl (4*S*)-3-tetrahydropyranyl-2-imidazolidone-4-carboxylate **9e** occurred during the course of the reaction. **7** was a roughly 1: 1 mixture of diastereomers as judged from the NMR spectrum.
5) (6*S*)-1-[(2*S*)-3-Mercapto-2-methylpropanoyl]-2-piperidone-6-carboxylic acid **14** and (2*S*)-1-[(2*S*)-3-acetylthio-2-methylpropanoyl]pyroglutamic acid **13** were also prepared. **14** showed an IC_{50} value of 130 nM. **13** had almost none of the activity of **2** *in vitro*, but showed the same effect on pressor response induced by angiotensin I in conscious rats (administered orally).
6) The results of detailed pharmacological evaluations will be published elsewhere.
7) This compound was prepared from (4*S*)-2-oxazolidone-4-carboxylic acid [T. Kaneko and T. Inui, *Nippon Kagaku Zasshi*, **82**, 1075 (1961)] and isobutene in the presence of conc. H_2SO_4 in dioxane.