[Chem. Pharm. Bull. 29(1) 63—70 (1981)]

Thiol Compounds. I.[1] Synthesis and Antihypertensive Activity of Mercaptoacylamino Acids

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(Received June 26, 1980)

A series of mercaptoacylamino acids was synthesized and screened as angiotensin I-converting enzyme inhibitors. The inhibitory activities of these compounds were compared with that of (2*S*)-1-[(2*S*)-3-mercapto-2-methylpropanoyl]proline. The structure-activity relationship of the compounds is discussed.

Keywords——thiol; amino acid; mercaptoacylamino acid; angiotensin I-converting enzyme inhibitor; antihypertensive agent; structure–activity relationship

We have been seeking novel compounds related to tiopronin[3] (**1**), which is a liver protective agent, and have synthesized various derivatives thereof.

Recently Cushman and Ondetti[4a-c] found that (2*S*)-1-[(2*S*)-3-mercapto-2-methylpropanoyl]proline (SQ 14225) has a strong inhibitory activity against angiotensin I-converting enzyme *in vitro*. They also stated in their patent application that tiopronin may have a weak hypotensive activity.[4d]

This paper describes the synthesis of various mercaptoacylamino acids and their structure–activity relationship for inhibitory activity against angiotensin I-converting enzyme. The length of the methylene chain between thiol and carbonyl groups in the mercaptoacyl moiety, and the length and bulkiness of the methylene chain between amino and carboxyl groups in the amino acid moiety varied in these compounds.

Syntheses

The compounds described in Table I—III were synthesized by the procedures (methods A—D) shown in Chart 1.

The four methods are described in "Experimental" for a typical compound in each case.

In method A, S-benzoylmercaptoacylamino acids or amides prepared by the reaction of the corresponding amino acids or amides with S-benzoylmercaptoacyl halide in the presence of a base such as sodium hydroxide were treated with aqueous ammonia to give the mercaptoacylamino acids.[6]

In method B, the haloacylamino acids or amides obtained by the reaction of amino acids or amides with haloacyl halides in the presence of sodium hydroxide were treated with thiobenzoic acid to give the S-benzoylmercaptoacylamino acids.

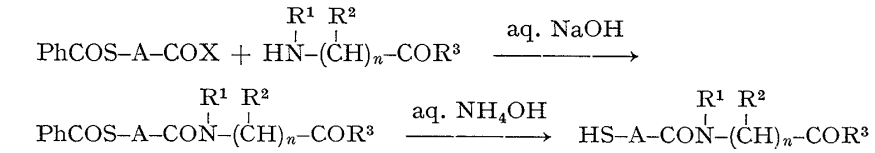
In method C, S-benzylmercaptoacylamino acids or amides prepared by the reaction of the corresponding amino acids or amides with S-benzylmercaptoacyl halide in the same manner as in method A were treated with metallic sodium in liquid ammonia to give the mercaptoacylamino acids.

In method D, mercaptoalkanoic acids provided the corresponding polythioesters by reaction with N,N′-dicyclohexylcarbodiimide (DDC).[7] The resulting polythioesters were converted to mercaptoacylamino acids by coupling with amino acids.[8]

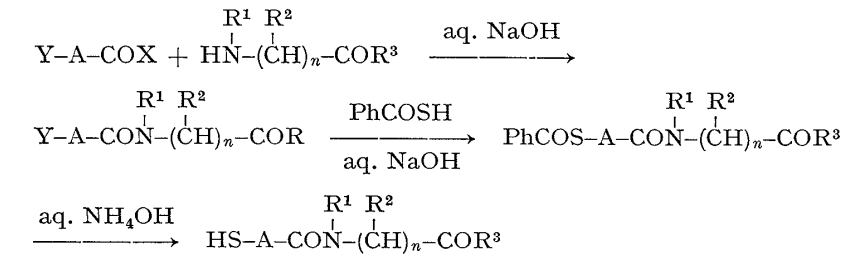
When an S-benzoylmercaptoacylamino acid was diastereomeric, the two forms were separated by fractional recrystallization or chromatography on silica gel.

2-Bromophenylacetic acid, the starting material for N-(2-mercaptophenylacetyl)glycine

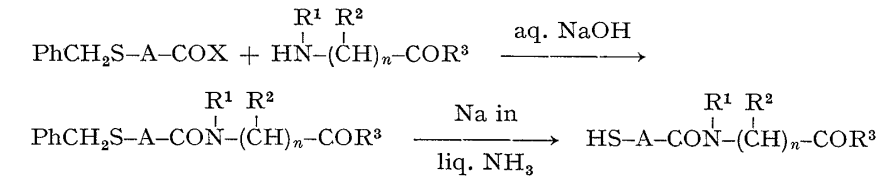
Method A



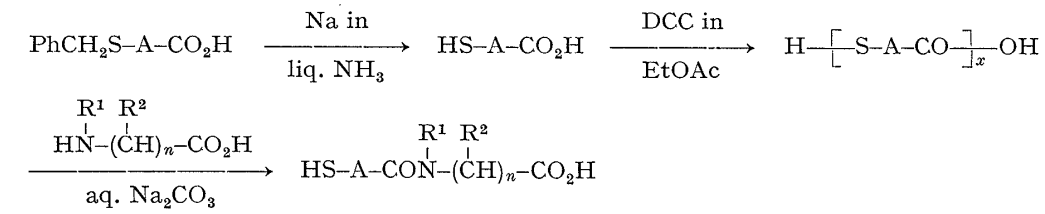
Method B



Method C[5]



Method D



A: (un) substituted alkylene or (un) substituted aralkylene group.
R^1: H or CH_3 group.
R^2: H, alkyl, (un) substituted aryl or aralkyl group.
R^3: OH or (un) substituted amino group.
X, Y: halogen atom.
n: 1—5.
x: polymer.

Chart 1

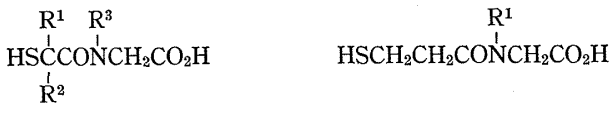
(**5**), was obtained in good yield from mandelic acid by treatment with 48% hydrobromic acid, and 2-bromo-*p*-chlorophenylacetic acid was also prepared in the same manner.[10] To prepare S-benzyl-2-mercapto-2-methylpropanoic acid, the starting material for N-(2-mercapto-2-methylpropanoyl)glycine (**8**), benzylmercaptan was condensed with acetone and chloroform in the presence of alkali.[11]

Compounds **1**—**7**, **9**, **17**—**26**, **30** and **31** were racemic compounds and **12**—**14** and **27** were mixtures of two racemic diastereoisomers. Compounds **15b**, **16b**, **28b** and **29b** were racemic diastereoisomers of **15a**, **16a**, **28a** and **29a**, respectively.

Structure–Activity Relationships

In this series of mercaptoacylamino acids, there was some correlation between the structure and activity *in vitro* (Table IV). The activity of **1** was about 100 times less than that of (2*S*)-1-[(2*S*)-3-mercapto-2-methylpropanoyl]proline. Cysteine and glutathione showed no activity, despite possessing a thiol group or mercaptopropanoyl moiety. Alkylation of

TABLE I. N-(2- and 3-Mercaptoacyl)glycines



No. 1—9 No. 10, 11

Compd. No.	Confign.	R^1	R^2	R^3	mp (°C)[a]	Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	Method of prepn.[b]
1[c]	(±)	CH_3	H	H	97.5—98.5	EtOAc	$C_5H_9NO_3S$	36.80	5.56	8.58	A, B, C
					(159.5—160.5	EtOAc–MeOH)[f]		(37.04	5.65	8.66)	
					(133—134	EtOAc–MeOH)[g]					
2[c]	(±)	$CH(CH_3)_2$	H	H	118—119	EtOAc–benzene	$C_7H_{13}NO_3S$	43.96	6.85	7.32	B
					(136—137	EtOAc)[f]		(43.71	6.79	7.54)	
3[c]	(±)	$(CH_2)_3CH_3$	H	H	83.5—84.5	EtOAc–benzene	$C_8H_{15}NO_3S$	46.81	7.37	6.82	B
					(87—91	EtOAc–benzene)[f]		(46.78	7.44	6.71)	
4[c,d]	(±)	CH_2SH	H	H	87—89	EtOAc–$CHCl_3$	$C_5H_9NO_3S_2$	30.76	4.65	7.17	B
					(155—157	EtOAc)[f]		(30.75	4.56	6.92)	
5[c]	(±)	C_6H_5	H	H	91—93	EtOAc–benzene	$C_{10}H_{11}NO_3S$	53.32	4.92	6.22	B
					(133—138	benzene)[f]		(53.35	5.01	6.27)	
6[c]	(±)	C_6H_4-*p*-Cl	H	H	148—149	EtOAc–benzene	$C_{10}H_{10}ClNO_3S$	46.25	3.88	5.39	B
					(158—159	EtOAc–benzene)[f]		(46.39	3.99	5.45)	
7[c]	(±)	$CH_2C_6H_5$	H	H	126—127	EtOAc	$C_{11}H_{13}NO_3S$	55.21	5.48	5.85	B
					(181—182	EtOAc–EtOH)[f]		(55.16	5.51	5.86)	
8		CH_3	CH_3	H	117—118	EtOAc	$C_6H_{11}NO_3S$	40.67	6.26	7.90	C, D
								(40.79	6.30	7.79)	
9[c]	(±)	CH_3	H	CH_3	Oil[e]		$C_6H_{11}NO_3S$				A
					(oil)[f,h]						
10		H			100—103	EtOAc	$C_5H_9NO_3S$	36.80	5.56	8.58	A, B, C
					(128—129	EtOAc)[f]		(37.01	5.46	8.64)	
					(115—117	EtOAc–MeOH)[g]					
11		CH_3			oil[e]		$C_6H_{21}NO_3S$				A
					(141—142.5	EtOAc–EtOH)[f]					

a) Melting points are uncorrected.
b) Methods are those described in "Experimental."
c) Mixtures of enantiomers.
d) Lit.[9] The S-acetyl derivative is yellow oil.
e) Purified by chromatography.
f) Melting point of the S-benzoyl derivative.
g) Melting point of the S-benzyl derivative.
h) Starting materials were S-benzoyl-2-mercaptopropanoyl chloride and ethyl N-methylglycinate. Glycine moiety is ethyl ester.

TABLE II. N-(2-Mercaptopropanoyl)amino Acids

No. **12**—**18**

Compd. No.	Confign.	R	*n*	mp (°C)[a]	Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	Method of Prepn.[b]
12[c]	Mixt. of (±)-a and (±)-b	CH_3	1	131—132 (138—152	EtOAc EtOAc)[f]	$C_6H_{11}NO_3S$	40.67 (40.77	6.26 6.35	7.90 7.81)	B
13[c]	Mixt. of (±)-a and (±)-b	$CH_2CH(CH_3)_2$	1	122—125 (oil)[f]	EtOAc-pet. ether	$C_9H_{17}NO_3S$	49.29 (49.54	7.81 7.94	6.39 6.68)	B
14[c]	Mixt. of (±)-a and (±)-b	C_6H_5	1	135—136 (149—151	EtOAc EtOAc)[f]	$C_{11}H_{13}NO_3S$	55.21 (55.17	5.48 5.43	5.85 5.62)	B
15a[e]	(±)-a	C_6H_4-*p*-Cl	1	170 (188—189	EtOAc EtOAc)[f]	$C_{11}H_{12}ClNO_3S$	48.27 (48.50	4.42 4.47	5.12 4.99)	B
15b[e]	(±)-b	C_6H_4-*p*-Cl	1	174—175 (148—156	EtOAc benzene)[f]	$C_{11}H_{12}ClNO_3S$	48.27 (48.46	4.42 4.43	5.12 4.97)	B
16a[e]	(±)-a	$CH_2C_6H_5$	1	131—132 (173—175	EtOAc EtOAc)[f]	$C_{12}H_{15}NO_3S$	56.90 (57.00	5.97 6.01	5.53 5.54)	A, B
16b[e]	(±)-b	$CH_2C_6H_5$	1	105—108 (98—103	EtOAc–benzene EtOAc–benzene)[f]	$C_{12}H_{15}NO_3S$	56.90 (57.17	5.97 6.06	5.53 5.54)	A, B
17[d]	(±)	H	2	102.5—105 (94—97	EtOAc benzene)[f]	$C_6H_{11}NO_3S$	40.67 (40.50	6.26 6.29	7.90 7.96)	B
18[d]	(±)	H	5	78—79 (oil)[f,g]	EtOAc–pet. ether	$C_9H_{17}NO_3S$	49.29 (49.26	7.81 7.78	6.39 6.42)	B

a,b) See the corresponding footnotes in Table I.
c) Mixtures of racemic diastereoisomers.
d) Mixtures of enantiomers.
e) Compounds **15b** and **16b** are diastereoisomers of **15a** and **16a**, respectively.
f) Melting point of the S-benzoyl derivative.
g) Purified by chromatography.

TABLE III. N-(2- and 3-Mercaptoacyl)amino Acid Amides and N-(S-Alkyl-2-mercaptopropanoyl)glycines

No. **19**—**29**

No. **30**, **31**

Compd. No.	Confign.	R^1	R^2	R^3	mp (°C)[a]	Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	Method of Prepn.[b]
19[c]	(±)	CH_3	H	H	139—141 (149.5—150 (88—90	EtOAc–H_2O MeOH)[f] EtOAc)[g]	$C_5H_{10}N_2O_2S$	37.02 (36.88	6.21 6.25	17.27 17.39)	B
20[c]	(±)	CH_3	H	C_6H_5	132—133 (134—135	EtOAc EtOAc)[f]	$C_{11}H_{14}N_2O_2S$	55.44 (55.63	5.92 5.91	11.76 11.77)	B
21[c]	(±)	CH_3	H	C_6H_4-*o*-CH_3	154—155 (138—139	EtOAc EtOH)[f]	$C_{12}H_{16}N_2O_2S$	57.12 (56.98	6.39 6.30	11.10 11.12)	B
22[c]	(±)	CH_3	H	C_6H_3-2,6-$(CH_3)_2$	179—180 (156—157	EtOH–EtOAc EtOH)[f]	$C_{13}H_{18}N_2O_2S$	58.62 (58.53	6.81 6.79	10.52 10.27)	B
23[c]	(±)	CH_3	H	C_6H_3-2,6-Cl_2	186—187 (164	EtOH MeOH)[f]	$C_{11}H_{12}Cl_2N_2O_2S$	43.01 (42.99	3.94 3.80	9.12 8.93)	B
24[c]	(±)	C_6H_5	H	H	128—129 (165.5—166	EtOH–EtOAc EtOH)[f]	$C_{10}H_{12}N_2O_2S$	53.57 (53.56	5.39 5.49	12.50 12.26)	B
25[c]	(±)	C_6H_4-*p*-Cl	H	H	141—142 (157—158	EtOH EtOH)[f]	$C_{10}H_{11}ClN_2O_2S$	48.44 (48.33	4.80 4.77	10.27 10.43)	B
26[c]	(±)	$CH_2C_6H_5$	H	H	172—173 (134	MeOH EtOH–EtOAc)[f]	$C_{11}H_{14}N_2O_2S$	55.45 (55.35	5.92 6.08	11.76 11.53)	B
27[d]	Mixt. of (±)-a and (±)-b	CH_3	C_6H_5	H	187—189 (176—181	MeOH MeOH)[f]	$C_{11}H_{14}N_2O_2S$	55.44 (55.27	5.92 5.88	11.75 11.77)	B
28a[e]	(±)-a	CH_3	C_6H_4-*p*-Cl	H	219—220 (228—229	MeOH MeOH)[f]	$C_{11}H_{13}ClN_2O_2S$	48.44 (48.56	4.80 4.76	10.27 10.18)	B
28b[e]	(±)-b	CH_3	C_6H_4-*p*-Cl	H	215—216 (170—173	MeOH MeOH)[f]	$C_{11}H_{13}ClN_2O_2S$	48.44 (48.47	4.80 4.77	10.27 10.29)	B
29a[e]	(±)-a	CH_3	$CH_2C_6H_5$	H	186—188 (121—123	MeOH benzene)[f]	$C_{12}H_{16}N_2O_2S$	57.12 (57.07	6.39 6.39	11.10 11.14)	B
29b[e]	(±)-b	CH_3	$CH_2C_6H_5$	H	148—150 (199—200	MeOH–EtOAc MeOH)[f]	$C_{12}H_{16}N_2O_2S$	57.12 (57.04	6.39 6.35	11.10 11.13)	B
30[c]	(±)	CH_3			112—113	EtOAc	$C_6H_{11}NO_3S$	40.67 (40.64	6.26 6.14	7.90 7.70)	*h*)
31[c]	(±)	C_2H_5			88.5—89.5	EtOAc	$C_7H_{13}NO_3S$	43.96 (44.02	6.85 6.85	7.32 7.26)	*h*)
32		$HS(CH_2)_2CONHCH_2CONH_2$			142—143.5 (132—134	EtOH MeOH)[f]	$C_5H_{10}N_2O_2S$	37.02 (36.78	6.15 6.30	11.27 11.04)	A, B, C

a,b) See the corresponding footnotes in Table I. *b*) Amino acid amides reacted instead of amino acids. *c*) Mixtures of enantiomers. *d*) Mixtures of racemic diastereoisomers. *e*) Compounds **28b** and **29b** are diastereoisomers of **28a** and **29a**, respectively. *f*) Melting point of the S-benzoyl derivative. *g*) Melting point of the S-benzyl derivative. *h*) Prepared by alkylation of **1** with alkyl iodide.

Table IV. Inhibitory Activities against ACE of Mercaptoacylamino Acids[a]

Compd. No.	AI pI_{50}	ACE pI_{50}	BK pA_{50}
SQ 14225[b]	6.68	8.44	7.09
Cysteine	<3.5	3.00	<3
Glutathione	<3.5	<3	<4
1	4.70	4.82	6.17
2		4.22	
3	4.15	4.17	
4	5.34	3.18	
5		3.00	
6		2.40	
7	4.89	2.89	6.60
8	2.77	2.74	
9	4.53	4.42	
10	4.28	4.35	5.60
11		4.35	
12	4.70	5.02	
13		4.68	
14	4.00	4.36	
15a	3.28	<3	
15b	4.10	<4	
16a	<4	3.27	
16b	5.00	4.70	6.62
17	2.92	<3	3.46
18	3.52	<3	4.68
19—32	<4	<4	<4

a) Inhibitory activities of the compounds against ACE were determined according to the procedures in ref. 4 (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.

b) Physical constants were as follows: mp 104—106°, $[\alpha]_D^{25}$ −131.0° (c=2.0, EtOH). The compound was synthesized by Santen Pharmaceutical Co., Ltd.

the thiol and amidation of the carboxylic acid moiety, as seen in compounds **19—32**, reduced the activity. The activity varied depending on the steric bulkiness of the substituent at the α-position in the mercaptoacyl moiety. That is, the increases in steric bulkiness in going from a methyl group to isopropyl, butyl, phenyl, and benzyl groups reduced the potency in that order (*e.g.*, compounds **1**, **2**, **3**, **5**, **6**, and **7**). Moreover, N-(2-mercapto-2-methylpropanoyl)-glycine (**8**), which is disubstituted, had little potency. A similar reduction in potency was seen on increasing the length of the methylene chain and removing the methyl group at the α-position (compound **10**). Alkylation of the nitrogen atom did not affect the activity (compound **9**).

Conversion of the substituent from methyl (**12**) to isobutyl (**13**) or phenyl (**14**) reduced the inhibitory activity, and substitution by chlorine (**15a** and **15b**) in the aromatic ring of (±)-N-(2-mercaptopropanoyl)phenylglycine-a and -b (**14**) further reduced it, whereas on conversion to benzyl (**16b**) the activity became stronger than that of **1**. Further, one of the diastereoisomers had higher activity (*e.g.*, **15a** and **15b**, **16a** and **16b**). Modification of **1** by changing the length of the methylene chain in the amino acid moiety (*e.g.*, compounds **17** and **18**) decreased the potency *in vitro*.

Thus, it appears that mercaptoacylamino acids, consisting of thiol, amide and carboxyl groups, have activity as angiotensin I-converting enzyme inhibitors. In order to attain more potent activity, the amino acid should be an α-amino acid substituted with a small group at the α-position. Exceptionally, the use of phenylalanine did not lead to a decrease in activity, despite its large substituent. It is presumed that the phenyl skeleton in phenylalanine could rotate freely and adopt a favorable conformation.

It appears that the absolute configuration of the mercaptoacyl or amino acid moiety affects the inhibitory potency, and we intend to examine the activity of the various mercaptoacylamino acids synthesized from optically active amino acids in order to determine whether (*R*)- or (*S*)-configuration results in stronger activity.

Experimental

Melting points were determined in open capillary tubes with a Yamato melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO IR-E spectrometer. Specific rotations were measured with a JASCO DIP-4 polarimeter.

Method A. (±)-N-(2-Mercaptopropanoyl)phenylalanine-a and -b (16a and 16b)——S-Benzoyl-2-mercaptopropanoyl chloride (45.7 g, 0.20 mol) [prepared by the reaction of S-benzoyl-2-mercaptopropanoic acid (42.0 g, 0.20 mol) with thionyl chloride (26.2 g, 0.22 mol)] and 2 N sodium hydroxide (110 ml) were added dropwise with ice-cooling to a mixture of DL-phenylalanine (33.0 g, 0.20 mol) and 2 N sodium hydroxide (100 ml). After the addition, the mixture was stirred for 2 hr at room temperature, acidified, and extracted with ethyl acetate. The extract was dried (Na_2SO_4) and concentrated. Benzene was added to the residue to yield colorless crystals of (±)-N-(S-benzoyl-2-mercaptopropanoyl)phenylalanine-a (25.0 g, 35.0%): mp 173—175°; IR ν_{max}^{Nujol} cm^{-1}: 3240 (NH), 1710 (COOH), 1630 (CONH), 1540 (CONH).

The filtrate was evaporated to dryness and the residue was recrystallized from ethyl acetate–benzene to yield (±)-N-(S-benzoyl-2-mercaptopropanoyl)phenylalanine-b (28.6 g, 40.0%): mp 99—103°; IR ν_{max}^{Nujol} cm^{-1}: 3380 (NH), 1720 (COOH), 1650 (CONH), 1510 (CONH).

(±)-N-(S-Benzoyl-2-mercaptopropanoyl)phenylalanine-a or -b (7.1 g, 0.02 mol) was added to aqueous ammonia (70 ml) and the mixture was stirred for 1 hr at room temperature. The resulting solution was extracted with chloroform to remove benzamide. The aqueous layer was concentrated under reduced pressure, washed with ethyl acetate, and acidified with hydrochloric acid. The precipitates were collected, and recrystallized from ethyl acetate or ethyl acetate–benzene. (±)-Acid-a (**16a**) was obtained (3.6 g, 71.1%): mp 131—132°; IR ν_{max}^{Nujol} cm^{-1}: 3320 (NH), 1715 (COOH), 1620 (CONH), 1525 (CONH). *Anal.* Calcd for $C_{12}H_{15}NO_3S$: C, 56.90; H, 5.97; N, 5.53. Found: C, 57.00; H, 6.01; N, 5.54. (±)-Acid-b (**16b**) was obtained (3.7 g, 73.0%): mp 105—108°; IR ν_{max}^{Nujol} cm^{-1}: 3300 (NH), 1705 (COOH), 1625 (CONH), 1525 (CONH). *Anal.* Calcd for $C_{12}H_{15}NO_3S$: C, 56.90; H, 5.97; N, 5.53. Found: C, 57.17; H, 6.06; N, 5.54.

Method B. N-(2-Mercaptophenylacetyl)glycine (5)——2-Bromophenylacetic acid (62.9 g, 0.29 mol) was refluxed with thionyl chloride (200 ml, 2.76 mol) for 2 hr. Removal of the excess thionyl chloride by evaporation, followed by distillation of the residue gave oily 2-bromophenylacetyl chloride (62.8 g, 91.9%): bp 105° (5 mmHg). 2-Bromophenylacetyl chloride (20.0 g, 0.085 mol) and 2 N sodium hydroxide (43 ml) were then added dropwise to a mixture of glycine (7.5 g, 0.10 mol) and 2 N sodium hydroxide (50 ml) with ice-cooling over a period of 1 hr. The mixture was stirred for 4 hr at room temperature, then sodium thiobenzoate solution [prepared from thiobenzoic acid (13.8 g, 0.10 mol) and 2 N sodium hydroxide (50 ml)] was added with stirring at room temperature. After standing overnight, the mixture was acidified with hydrochloric acid. The precipitates were collected and recrystallized from benzene to yield N-(S-benzoyl-2-mercaptophenylacetyl)glycine (23.9 g, 72.6%): mp 133—138°.

N-(S-Benzoyl-2-mercaptophenylacetyl)glycine (9.9 g, 0.03 mol) was added to aqueous ammonia and the mixture was stirred for 1 hr at room temperature. The resulting solution was extracted with ether to remove benzamide. The aqueous layer was concentrated under reduced pressure, treated with hydrochloric acid, and extracted with ethyl acetate. The extract was dried over Na_2SO_4, filtered and concentrated. The residue was crystallized from benzene to yield the acid **5** (5.6 g, 82.8%): mp 91—93°; IR ν_{max}^{Nujol} cm^{-1}: 3260 (NH), 1760 (COOH), 1610 (CONH), 1550 (CONH). *Anal.* Calcd for $C_{10}H_{11}NO_3S$: C, 53.32; H, 4.92; N, 6.22. Found: C, 53.35; H, 5.01; N, 6.27.

Method C. N-(3-Mercaptopropanoyl)glycine (10)——Glycine (16.0 g, 0.21 mol) was dissolved in 1 N sodium hydroxide (220 ml). The solution was stirred and heated at 50°, then S-benzyl-3-mercaptopropanoyl chloride (46.0 g, 0.21 mol) [obtained by the reaction of S-benzyl-3-mercaptopropanoic acid (50.5 g, 0.26 mol) with thionyl chloride (40.0 g, 0.34 mol)] and 1 N sodium hydroxide (330 ml) were added dropwise with stirring at this temperature. The mixture was then allowed to cool to room temperature with continuous stirring, further stirred for 3 to 4 hr, and extracted with ether. The aqueous layer was acidified with hydrochloric acid. The precipitates were washed with water and then 60% methanol to yield N-(S-benzyl-3-mercaptopropanoyl)glycine (43.6 g, 80.7%): mp 115—117°.

N-(S-Benzyl-3-mercaptopropanoyl)glycine (40.0 g, 0.185 mol) was dissolved in liquid ammonia (260 ml), and metallic sodium (8.0 g, 0.348 g atom) was added in small portions with stirring. After completion of the reaction, ammonium chloride was added and ammonia was removed by distillation. Water was added to dissolve the residue. The aqueous layer was separated, washed with ether, and acidified with hydrochloric acid with cooling. The aqueous solution was concentrated under reduced pressure. The resulting crystalline deposit was collected, washed with cold water, and dried *in vacuo*. Recrystallization from ethyl acetate yielded the acid **10** (16.0 g, 62.0%): mp 100—103°. *Anal.* Calcd for $C_5H_9NO_3S$: C, 36.80; H, 5.56; N, 8.58.

Found: C, 37.01; H, 5.46; N, 8.64.

Method D. N-(2-Mercapto-2-methylpropanoyl)glycine (8)——A solution of N,N′-dicyclohexylcarbodiimide (61.9 g, 0.30 mol) in ethyl acetate (100 ml) was added dropwise to a solution of 2-mercapto-2-methylpropanoic acid (36.0 g, 0.30 mol) in ethyl acetate (150 ml). After the addition, the mixture was stirred overnight. The precipitates of N,N′-dicyclohexylurea were collected and washed with ethyl acetate. The filtrate was combined with the washings and concentrated to 200 ml. Glycine (22.5 g, 0.30 mol) and potassium carbonate (20.7 g, 0.15 mol) were dissolved in methanol (150 ml) and water (150 ml), and the concentrated ethyl acetate solution obtained above was added dropwise with stirring. The resulting mixture was further stirred overnight, mixed with water (150 ml), and washed with ethyl acetate. The aqueous layer was acidified with hydrochloric acid. Sodium chloride was added, and the solution was extracted with ethyl acetate. The organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on silica gel to yield an oil (34.9 g). The oil was dissolved in benzene (200 ml) and extracted with water. The aqueous layer was washed with benzene, and sodium chloride was added. The solution was extracted with ethyl acetate. The extract was dried (Na_2SO_4) and concentrated to give a solid. Recrystallization from ethyl acetate yielded the acid **8** (18.7 g, 35%): mp 117—118°. *Anal.* Calcd for $C_6H_{11}NO_3S$: C, 40.67; H, 6.26; N, 7.90. Found: C, 40.79; H, 6.30; N, 7.79.

Acknowledgement The authors are most grateful to Prof. M. Suzuki of Meijo University for valuable suggestions. Thanks are also due to Mr. S. Mita, the president, and Dr. I. Mita, the executive vice-president, of Santen Pharmaceutical Co., Ltd. for their encouragement throughout this work, and to Dr. T. Iso and Dr. T. Chiba for pharmacological assay.

References and Notes

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