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Thiol Compounds. II.¹⁾ Synthesis and Antihypertensive Activity of Mercaptoacylamino Acids

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The synthesis and structure-activity relationship of a series of mercaptoacylamino acids are described. These compounds were tested for antihypertensive activity. When the compounds were screened as angiotensin I-converting enzyme inhibitors *in vitro*, N-(2-mercaptopropanoyl)-L-cysteine-a (**7a**) and N²-(2-mercaptopropanoyl)-L-tryptophan-a (**20a**) were found to be 10 and 20 times more active than tiopronin, respectively.

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

Previously, we reported that mercaptoacylamino acids, which are tiopronin analogs, had an antihypertensive effect.^{1b)} The amino acids used were as follows: neutral ones such as glycine, and DL-alanine, DL-leucine and DL-phenylalanine (having a lower alkyl or benzyl group at the α -position of glycine). Diastereomeric products were separated into two racemic diastereoisomers, and assayed for efficacy. Phenylalanine derivatives showed the highest activity.

In this study, amino acids having a functional group or heterocyclic ring in the side chain were selected, and optically active ones were mainly used. We synthesized N-(mercaptoacyl)-glutamic acids, N-(mercaptoacyl)cysteines, N²-(mercaptoacyl)histidines and N²-(mercaptoacyl)-tryptophans. When the products had diastereoisomers, they were separated. We found that N-(2-mercaptopropanoyl)-L-cysteine-a (**7a**) and N²-(2-mercaptopropanoyl)-L-tryptophan-a (**20a**) had high inhibitory activities against angiotensin I-converting enzyme (ACE) among the compounds tested. The activities of **7a** and **20a** were about 10 and 20 times higher than that of tiopronin and about 10 and 5 times lower than that of (2S)-1-[(2S)-3-mercapto-2-methylpropanoyl]proline, SQ 14225.²⁾

This paper describes the synthesis and the structure-activity relationship of this series of analogs.

Syntheses

N-(S-Benzoyl-2-mercapto-2-methylpropanoyl)-L-glutamic acid (**1**) was obtained in 14% yield by method A and in 8% yield by method D (Chart 1). N-(S-Benzoyl-3-mercapto-2-methylpropanoyl)-L-glutamic acid (**3**) was obtained by method B (Chart 1).

N-(Mercaptoacyl)-L-cysteines (**5**, **9** and **10**) were prepared by method D. The molecular weight distributions of the mercaptoalkanoic acid polythioesters³⁾ are given in Table I.

In method D, a by-product shown in Chart 2, (5R)-3-cyclohexyl-2-cyclohexylimino-5-mercaptomethyl-4-imidazolidinone hydrochloride, was produced regardless of the kind of alkylene (*e.g.*, $-\text{CH}_2-$, $-(\text{CH}_2)_2-$ and $-\text{C}(\text{CH}_3)_2-$).⁴⁾ Cysteine derivatives⁵⁾ (**6a**, **6b** and **8**) were prepared by means of the Schotten-Baumann reaction (method C) while the thiol group was protected with a benzyl group.⁶⁾ Compounds **6b** and **7b** were diastereoisomers of **6a** and **7a**, respectively.

Histidine derivatives (**11** and **13**) could be prepared by either method A or B, but high yields of **11** and **13** were obtained by method B and method A, respectively. Sodium hydroxide

Method A

Method B

Method C

Method D

A: (un) substituted alkylene

X: halogen

x: polymer

Chart 1

was not suitable as a base in the Schotten-Baumann reaction, and sodium carbonate was used for the reaction.⁷⁾

Method B gave N²-(chloroacetyl)-DL-tryptophan,⁸⁾ an intermediate of **15**, mp 149–152°; N²-(chloroacetyl)-L-tryptophan,⁸⁾ an intermediate of **17**, mp 160–161°; and N²-(3-bromopropanoyl)-DL-tryptophan, an intermediate of **23**, mp 156–159°. The compounds **15** and **17** were also prepared by method A. S-Benzoyl derivatives (**19a** and **19b**) were derived from N²-(haloacyl)-L-tryptophan without isolation by method B, resolved, and purified by column chromatography and recrystallization. The intermediates, N²-(2-bromopropanoyl)-L-

TABLE I. Molecular Weight Distributions of Polythioesters
(H-[S-A-CO]-_xOH)

A	Mn ^{a)}	Mw ^{b)}	Mz ^{c)}	Mw/Mn	Mz/Mw
-CH ₂ -	377	483	641	1.28	1.33
-(CH ₂) ₂ -	792	1094	1358	1.38	1.24
-C(CH ₃) ₂ -	401	477	564	1.19	1.13

a) Number-average molecular weight.

b) Weight-average molecular weight.

c) Z-average molecular weight.

Chart 2

TABLE II. N-(Mercaptoacyl)amino Acids and Related Compounds

Compd. No.	Confign.	R ¹	R ²	R ³	R ⁴	n	mp(°C) ^{a)}	Recrystn. solvent	[α] _D deg (c, solv., °C)	Method ^{b)} of prepn.	Yield (%)	Formula	Analysis (%)			
													Calcd (Found)	C	H	N
Glutamic acid derivatives																
1	(2S)	COC ₆ H ₅	CH ₃	CH ₃	CH ₂ CO ₂ H	0	120—122	Benzene-ether	−4.5 (1.0, MeOH, 25)	A	14	C ₁₆ H ₁₉ NO ₆ S	54.38	5.42	3.96	
2	(2S)	H	CH ₃	CH ₃	CH ₂ CO ₂ H	0	Oil ^{c)}		−5.4 (4.2, MeOH, 25)	A	80		54.62	5.43	3.76)	
3	(2S)	COC ₆ H ₅	H	H	CH ₂ CO ₂ H	1	138—139	EtOAc	−18.1 (3.2, MeOH, 25)	B	37	C ₁₅ H ₁₇ NO ₆ S	53.09	5.05	4.13	
4	(2S)	H	H	H	CH ₂ CO ₂ H	1	Oil ^{c)}		−12.8 (3.9, MeOH, 25)	B	63		53.44	5.07	3.88)	
Cysteine derivatives																
5	(2R)	H	H	H	SH	0	113 (dec.)	EtOAc	+24.9 (1.0, EtOH, 25)	D	52	C ₆ H ₉ NO ₃ S ₂	30.76	4.65	7.17	
6a ^{c)}	(2R)–a	CH ₂ C ₆ H ₅	CH ₃	H	SCH ₂ C ₆ H ₅	0	111.5—112.5	EtOAc	−135.9 (1.4, EtOH, 25)	C	37	C ₂₀ H ₂₃ NO ₃ S ₂	30.80	4.71	7.24	
6b ^{c)}	(2R)–b	CH ₂ C ₆ H ₅	CH ₃	H	SCH ₂ C ₆ H ₅	0	Oil ^{c)}		+46.2 (5.2, EtOH, 25)	C	37		61.69	5.95	3.60	
7a ^{c)}	(2R)–a	H	CH ₃	H	SH	0	114—115	Ether	+3.5 (2.0, EtOH, 24)	C	72	C ₆ H ₁₁ NO ₃ S ₂	34.45	5.30	6.70	
7b ^{c)}	(2R)–b	H	CH ₃	H	SH	0	124—125	EtOAc	+16.8 (1.7, EtOH, 24)	C	75	C ₆ H ₁₁ NO ₃ S ₂	34.45	5.30	6.70	
8	(2R)	CH ₂ C ₆ H ₅	CH ₃	CH ₃	SCH ₂ C ₆ H ₅	0	Oil ^{c)}		−23.4 (4.1, MeOH, 25)	C	74		34.67	5.28	6.77)	
9	(2R)	H	CH ₃	CH ₃	SH	0	139—140	EtOAc	+32.3 (1.0, EtOH, 25)	C	81	C ₇ H ₁₃ NO ₃ S ₂	37.65	5.87	6.27	
10	(2R)	H	H	H	SH	1	97	EtOAc	+4.9 (1.0, EtOH, 25)	D	65	C ₆ H ₁₁ NO ₃ S ₂	37.78	5.86	6.16)	
Histidine derivatives																
11	(2S)	COC ₆ H ₅	H	H	4-Imidazolyl	0	176—177	H ₂ O	−6.1 (1.0, H ₂ O, 24)	B	30	C ₁₈ H ₁₅ N ₃ O ₄ S	54.05	4.54	12.60	
12	(2S)	H	H	H	4-Imidazolyl	0	198—200 (dec.)	EtOH–H ₂ O	+29.9 (1.0, H ₂ O, 24)	B	80	C ₈ H ₁₁ N ₃ O ₃ S	54.18	4.41	12.70)	
13	(2S)	COC ₆ H ₅	H	H	4-Imidazolyl	1	170—171	H ₂ O	−7.0 (1.0, H ₂ O, 24)	A	39	C ₁₆ H ₁₇ N ₃ O ₄ S	41.91	4.84	18.33	
14	(2S)	H	H	H	4-Imidazolyl	1	219 (dec.)	EtOH–H ₂ O	+14.1 (1.0, H ₂ O, 24)	A	60	C ₉ H ₁₃ N ₃ O ₃ S	55.32	4.93	12.10	
Tryptophan derivatives																
15 ^{d)}	(±)	COC ₆ H ₅	H	H	3-Indolyl	0	140—141	EtOAc	—	B	66	C ₂₀ H ₁₈ N ₂ O ₄ S	55.28	4.96	11.99)	
16 ^{d)}	(±)	H	H	H	3-Indolyl	0	138—139	EtOAc	—	B	50	C ₁₈ H ₁₄ N ₂ O ₃ S	44.43	5.39	17.27)	
17	(2S)	COC ₆ H ₅	H	H	3-Indolyl	0	151—152	EtOAc	+31.0 (1.8, EtOH, 25)	B	63	C ₂₀ H ₁₈ N ₂ O ₄ S	62.81	4.74	7.32	
18	(2S)	H	H	H	3-Indolyl	0	158—159	EtOAc	+28.7 (1.5, EtOH, 25)	B	70	C ₁₉ H ₁₄ N ₂ O ₃ S	62.75	4.71	7.32)	
19a ^{c)}	(2S)–a	COC ₆ H ₅	CH ₃	H	3-Indolyl	0	178—179	EtOAc	−43.3 (1.0, EtOH, 25)	B	18	C ₂₁ H ₂₀ N ₂ O ₄ S	56.10	5.07	10.06	
19b ^{c)}	(2S)–b	COC ₆ H ₅	CH ₃	H	3-Indolyl	0	168—169	EtOAc	+67.1 (1.0, EtOH, 25)	B	21	C ₂₁ H ₂₀ N ₂ O ₄ S	56.08	5.10	10.09)	
20a ^{c)}	(2S)–a	H	CH ₃	H	3-Indolyl	0	103—105	EtOAc–benzene	+31.0 (1.0, EtOH, 25)	B	63	C ₁₄ H ₁₆ N ₂ O ₃ S	63.62	5.08	7.06	
20b ^{c)}	(2S)–b	H	CH ₃	H	3-Indolyl	0	120—122	EtOAc–benzene	+34.6 (1.0, EtOH, 25)	B	35	C ₁₄ H ₁₆ N ₂ O ₃ S	63.60	5.05	6.98)	
21	(2S)	COC ₆ H ₅	CH ₃	CH ₃	3-Indolyl	0	170—171	EtOAc	+21.4 (1.0, EtOH, 25)	A	81	C ₂₂ H ₂₂ N ₂ O ₄ S	63.62	5.40	6.82	
22	(2S)	H	CH ₃	CH ₃	3-Indolyl	0	64—65	Benzene	+15.6 (1.0, EtOH, 25)	A	65	C ₁₃ H ₁₈ N ₂ O ₃ S	63.62	5.08	7.06	
23	(±)	COC ₆ H ₅	H	H	3-Indolyl	1	115—118	EtOAc–benzene	—	B	11	C ₂₁ H ₂₀ N ₂ O ₄ S	63.60	5.07	7.06	
24	(±)	H	H	H	3-Indolyl	1	131—133	EtOAc	—	B	40	C ₁₄ H ₁₆ N ₂ O ₃ S	57.52	5.52	9.58	
25	(2S)	COC ₆ H ₅	H	H	3-Indolyl	1	123—125	EtOAc–benzene	+17.5 (0.7, EtOH, 25)	A	72	C ₂₁ H ₂₀ N ₂ O ₄ S	57.49	5.60	9.58)	
26	(2R)	COC ₆ H ₅	H	H	3-Indolyl	1	118—120	EtOAc–benzene	+27.1 (1.0, EtOH, 25)	A	93	C ₂₁ H ₂₀ N ₂ O ₄ S	68.60	7.50	7.27	
27	(2S)	H	H	H	3-Indolyl	1	126—128	EtOAc	−18.3 (1.5, EtOH, 25)	A	62	C ₁₄ H ₁₆ N ₂ O ₃ S	68.88	7.56	7.14)	
28	(2R)	H	H	H	3-Indolyl	1	131—133	EtOAc	−25.3 (1.0, EtOH, 25)	A	82	C ₁₄ H ₁₆ N ₂ O ₃ S	57.52	5.50	9.57)	
29a ^{c)}	(2S)–a	COC ₆ H ₅	CH ₃	H	3-Indolyl	1	166—167	EtOAc	+20.0 (1.1, EtOH, 25)	A	31	C ₂₂ H ₂₂ N ₂ O ₄ S	57.65	5.47	9.59)	
29b ^{c)}	(2S)–b	COC ₆ H ₅	CH ₃	H	3-Indolyl	1	169—170	EtOH	−19.0 (1.0, EtOH, 25)	A	26	C ₂₂ H ₂₂ N ₂ O ₄ S	64.37	5.40	6.82	
30a ^{c)}	(2S)–a	H	CH ₃	H	3-Indolyl	1	Oil ^{c)}		−39.7 (1.1, EtOH, 25)	A	80	C ₁₅ H ₁₈ N ₂ O ₃ S	64.37	5.50	6.81)	
30b ^{c)}	(2S)–b	H	CH ₃	H	3-Indolyl	1	Oil ^{c)}		−5.5 (1.0, EtOH, 25)	A	73	C ₁₂ H ₁₈ N ₂ O ₃ S	69.00	7.66	7.10	
							189—190	EtOH	+27.1 (0.8, EtOH, 25)				69.12	7.79	7.11)	
							188—189	EtOH	+54.5 (1.2, EtOH, 25)				69.06	7.76	7.17)	
									−0.8 (1.1, EtOH, 25)							
									+16.3 (1.2, EtOH, 25)							
									+27.1 (1.2, EtOH, 25)							

a) Melting points are uncorrected. b) Method: see "Experimental". c) The absolute configuration of the mercaptoacyl moiety is not clear, and may be (R) or (S). Compounds 6a, 7a, 19a, 20a, 29a, and 30a are diastereoisomers of 6b, 7b, 19b, 20b, 29b, and 30b, respectively. d) Mixtures of enantiomers. e) Purified by chromatography. f) C₁₂H₁₈N₂ is dicyclohexylamine.

tryptophan-a and -b have the following physical constants: mp 128—129°, $[\alpha]_D^{25} + 27.0^\circ$ ($c=1.3$, EtOH), R_f 0.56 (thin layer chromatography (TLC): SiO₂, benzene-EtOAc-HOAc (7 : 7 : 1)); oil, R_f 0.65 (above conditions), respectively.⁹⁾ Both compounds reacted with thiobenzoic acid to give **19a** (R_f 0.67) and **19b** (R_f 0.60) (TLC under the above conditions), respectively. It was proved that **22** contained benzene as solvent of crystallization by nuclear magnetic resonance (NMR) analysis. (±)- S-Benzoyl-3-mercapto-2-methylpropanoic acid, the starting material for **29a** and **29b**, was prepared by the addition of thiobenzoic acid to methacrylic acid.¹⁰⁾ The R_f values (TLC: SiO₂, benzene-EtOAc-HOAc (10: 10: 1)) of **29a** and **29b** were 0.30 and 0.39, respectively.

Structure-Activity Relationships

Mercaptoacyl Moiety—In the previous paper, the inhibitory activity of tiopronin (α -position thiol, that is, a compound having a thiol group at the α -position on the mercaptoacyl moiety) against ACE was pI_{50} : 4.70 (angiotensin I) and that of N-(3-mercaptopropanoyl)-glycine (β -position thiol, that is, a compound having a thiol group at the β -position on the mercaptoacyl moiety) was pI_{50} : 4.28 (angiotensin I). The relationship of activity to thiol position was not obvious, but the activity of β -position thiols was less than that of α -position thiols. It seems that the activity was influenced by the presence of a methyl group at the α -position.

When the inhibitory activities of α -position and β -position thiols were compared, the activity of N-(3-mercaptopropanoyl)-L-cysteine (**10**, β -position thiol) was 3 times higher than that of N-(mercaptoacetyl)-L-cysteine (**5**, α -position thiol). The activity of N-(2-mercaptopropanoyl)-L-cysteine-a (**7a**), which possesses a methyl group at the α -position, was 20 times higher than that of N-(2-mercaptopropanoyl)-L-cysteine-b (**7b**) and 10 times higher than that of **5**. N-(2-Mercapto-2-methylpropanoyl)-L-cysteine (**9**), with a dimethyl group at the α -position,

TABLE III. Inhibitory Activities against ACE of N-(Mercaptoacyl)amino Acids^{a)}

Compd. No.	AI pI_{50}	ACE pI_{50}	BK pA_{50}
SQ 14225 ^{b)}	6.68	7.09	8.44
tiopronin	4.70	4.82	6.17
2	<4	2.46	
4	4.40	4.11	
5	4.57	4.72	
7	3.30	4.11	
7a	5.52	5.10	6.46
7b	4.19	3.27	
9	<4	2.14	
10	4.85	4.17	
12	3.96	3.59	
14	4.49	3.64	
16	5.07	4.57	6.85
18	5.27	6.12	7.55
20a	5.85	7.19	7.77
20b	4.10	6.44	4.15
22	<4	4.42	5.92
24	4.85	4.57	7.39
27	5.32	5.43	7.60
28	<3	<3	
30a	5.77	7.46	7.92
30b	5.47	5.48	7.20

^{a)} Inhibitory activities of the compounds against ACE were determined according to the published procedures²⁾ (AI, angiotensin I; BK, bradykinin). pI_{50} ; -log of the molar concentration of compound which inhibits the enzyme activity or agonist effect 50%. pA_{50} ; -log of the molar concentration of compound which augments the agonist effect 50%.

^{b)} Physical constants were as follows: mp 104—106°, $[\alpha]_D^{25} - 131.0^\circ$ ($c=2.0$, EtOH).

had little potency. As judged from the results for 5, 7a and 10, a diastereoisomer of the compound with a 3-mercapto-2-methylpropanoyl group in place of the 2-mercaptopropanoyl group in 7a should show high activity. As we did not have this cysteine derivative, the activity of N²-(2-mercaptopropanoyl)-L-tryptophan-a (20a) which possesses a thiol group at α -position and a methyl group at the α -position was compared with that of N²-(3-mercapto-2-methylpropanoyl)-L-tryptophan-a (30a). However, these compounds showed nearly the same activity.

We stated in the previous paper that the activity of N-(2,3-dimercaptopropanoyl)glycine is 4 times higher than that of tiopronin, and it was assumed that a thiol group at the α -position in the mercaptoacyl moiety could replace a methyl group.

It is desirable to determine the absolute configuration of the methyl group at the α -position in the future, since the activity differs between the diastereoisomers.

Amino Acid Moiety—Glutamic acid derivatives (acidic amino acids) and histidine derivatives (basic amino acids) showed lower activity than cysteine derivatives. It is known that ACE is a zinc-containing enzyme, and that the thiol group and imidazole skeleton form a complex with zinc.^{2,11} However, the activity of N²-(3-mercaptopropanoyl)-L-histidine (14), which possesses a thiol group and imidazole skeleton was not high. On the contrary, it is considered to be due to the high chelation potency of cysteine that the activity of cysteine derivatives possessing a dithiol group, particularly 7a, is 10 times higher than that of tiopronin. Among tryptophan derivatives, the D-form (28) had no activity and the DL-form (24) showed half the activity of the L-form (27).

As phenylalanine derivatives (previous paper) and tryptophan derivatives (this study) showed high activity, it is considered that compounds having an aromatic ring at the β -position of the amino acid moiety are likely to show good 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.

N-(S-Benzoyl-3-mercaptopropanoyl)-L-glutamic Acid (3) (in Method B)—L-Glutamic acid (44.1 g, 0.30 mol) was dissolved in 2 N sodium hydroxide (360 ml) with ice-cooling. 3-Bromopropanoyl chloride (34.2 g, 0.2 mol) and 2 N sodium hydroxide (100 ml) were added dropwise with cooling and stirring. The resulting mixture was further stirred at the same temperature for 1 hr and then at room temperature for 1 hr. Dilute hydrochloric acid was added to the mixture, which was adjusted to pH 7–8. Thiobenzoic acid (33.1 g, 0.24 mol) in 2 N potassium hydroxide (120 ml) was added to this solution. The solution was kept overnight, acidified with 6 N hydrochloric acid, and extracted with ethyl acetate. The extract was washed with water, dried over Na₂SO₄, and concentrated. The residue was applied to an SiO₂ column and eluted with benzene–ethyl acetate (2:1). The eluate was evaporated to dryness *in vacuo* to give a solid, which was recrystallized from ethyl acetate to give 25.2 g (37%) of 3; mp 138–139°.

N-(3-Mercaptopropanoyl)-L-glutamic Acid (4)—N-(S-Benzoyl-3-mercaptopropanoyl)-L-glutamic acid (3) (3.4 g, 0.01 mol) was dissolved in 2 N sodium hydroxide (25 ml) at room temperature, and the solution was stirred for 1 hr. The reaction mixture was acidified with 6 N hydrochloric acid. The precipitates were removed by filtration, and the filtrate was washed with benzene. The aqueous layer was saturated with sodium chloride and extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried over Na₂SO₄, and evaporated to dryness *in vacuo* to give 1.5 g (63%) of 4 as an oily material; $[\alpha]_D^{25} - 12.8^\circ$ ($c=3.9$, methanol).

N-(Mercaptoacetyl)-L-cysteine (5) (in Method D)—Thioglycolic acid (27.6 g, 0.30 mol) was dissolved in ethyl acetate (300 ml). N,N'-Dicyclohexylcarbodiimide (61.9 g, 0.3 mol) in ethyl acetate (250 ml) was added dropwise to the above solution with ice-cooling and stirring. The resulting mixture was further stirred at room temperature for 1 hr. The precipitated N,N'-dicyclohexylurea was removed by filtration, and the filtrate was concentrated to 200 ml. A solution of polythioesters of thioglycolic acid was thus obtained. L-Cysteine (36.3 g, 0.3 mol) and potassium carbonate (22.1 g, 0.16 mol) were dissolved in water (170 ml), and methanol (170 ml) was added. The polythioester ethyl acetate solution was added to this solution, and the reaction mixture was stirred at room temperature for 4 hr. After standing overnight, the solution was concentrated *in vacuo*, acidified with hydrochloric acid, and extracted with ethyl acetate. The extract was washed with water, dried over Na₂SO₄, and concentrated. The crystals were recrystallized from ethyl acetate

to give 30.5 g (52%) of **5**: mp 113° (dec.); $[\alpha]_D^{25} + 24.9^\circ$ ($c=1.0$, ethanol). *Anal.* Calcd for $C_5H_9NO_3S_2$: C, 30.76; H, 4.65; N, 7.17. Found: C, 30.80; H, 4.71; N, 7.24.

The materials insoluble in ethyl acetate were recrystallized from ethyl acetate–ethanol to give 0.8 g of (5*R*)-3-cyclohexyl-2-cyclohexylimino-5-mercaptomethyl-4-imidazolidinone hydrochloride: mp 235° (dec.); $[\alpha]_D^{25} - 20.6^\circ$ ($c=1.0$, ethanol). IR $\nu_{\max}^{NaCl}$ cm^{-1} : 1770 (C=N⁺H), 1650 (C=O), 1560 (C=O). *Anal.* Calcd for $C_{16}H_{27}N_3OS \cdot HCl$: C, 55.55; H, 8.16; N, 12.15. Found: C, 55.52; H, 8.15; N, 11.88.

S-Benzyl-N-(S-benzyl-2-mercaptopropanoyl)-L-cysteine-a and -b (6a and 6b) (in Method C)—S-Benzyl-L-cysteine (21.2 g, 0.10 mol) was dissolved in 0.1 N sodium hydroxide (2.2 l), then S-benzyl-2-mercaptopropanoyl chloride (23.6 g, 0.11 mol) was added dropwise with cooling and stirring. The resulting mixture was further stirred for 1 hr, acidified with hydrochloric acid, and extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 , and concentrated to form crystals. The crystals were collected by filtration, washed with a small amount of ethyl acetate, and recrystallized from ethyl acetate to give 15.4 g (40%) of **6a**: mp 111.5–112.5°; $[\alpha]_D^{25} - 135.9^\circ$ ($c=1.4$, ethanol). *Anal.* Calcd for $C_{20}H_{23}NO_3S_2$: C, 61.69; H, 5.95; N, 3.60. Found: C, 61.73; H, 5.92; N, 3.62. After **6a** had been removed, the filtrate was concentrated. The residue was applied to an SiO_2 column, and eluted with ethyl acetate. The eluate was evaporated to dryness *in vacuo* to give 14.4 g (37%) of **6b** as an oily material: $[\alpha]_D^{25} + 46.2^\circ$ ($c=5.2$, ethanol).

N-(2-Mercaptopropanoyl)-L-cysteine-a and -b (7a and 7b)—S-Benzyl-N-(S-benzyl-2-mercaptopropanoyl)-L-cysteine-a (**6a**) (7.8 g, 0.02 mol) was dissolved in liquid ammonia (250 ml). Metallic sodium (1.6 g, 0.07 g atom) was added in small portions with stirring. After the completion of reaction, ammonium chloride (2.5 g, 0.047 mol) was added, and thereafter ammonia was removed by distillation. Water was added to the residue to dissolve the solid. The aqueous layer was concentrated, washed with ethyl acetate, and acidified with hydrochloric acid with cooling. The precipitates were extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 , and evaporated to dryness *in vacuo*. The crystals were recrystallized from ether to give 3.0 g (72%) of **7a**: mp 114–115°; $[\alpha]_D^{25} + 3.5^\circ$ ($c=2.0$, ethanol). *Anal.* Calcd for $C_6H_{11}NO_3S_2$: C, 34.45; H, 5.30; N, 6.70. Found: C, 34.95; H, 5.36; N, 6.61.

According to the above procedure, the reaction of S-benzyl-N-(S-benzyl-2-mercaptopropanoyl)-L-cysteine-b (**6b**) (14.4 g, 0.037 mol), liquid ammonia (200 ml), metallic sodium (2.8 g, 0.12 g atom) and ammonium chloride (3.9 g, 0.069 mol) provided a solid, 7.1 g (92%), which was recrystallized from ethyl acetate to give 5.8 g (75%) of **7b**: mp 124–125°; $[\alpha]_D^{25} + 16.8^\circ$ ($c=1.7$, ethanol). *Anal.* Calcd for $C_6H_{11}NO_3S_2$: C, 34.45; H, 5.30; N, 6.70. Found: C, 34.67; H, 5.28; N, 6.77.

S-Benzyl-N-(S-benzyl-2-mercapto-2-methylpropanoyl)-L-cysteine (8) (in Method C)—S-Benzyl-L-cysteine (73.9 g, 0.35 mol) was dissolved in 1 N sodium hydroxide (700 ml, 0.70 mol). The solution was cooled in an ice bath and stirred. S-Benzyl-2-mercapto-2-methylpropanoyl chloride, which was obtained by reacting S-benzyl-2-mercapto-2-methylpropanoic acid (63.1 g, 0.30 mol) with thionyl chloride (39.3 g, 0.33 mol), was added dropwise to this solution. The resulting mixture was further stirred for 1 hr, acidified with hydrochloric acid, and extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 , and concentrated. The residue was applied to an SiO_2 column, and eluted with benzene–ethyl acetate (1:1). The eluate was evaporated to dryness *in vacuo* to give 89.6 g (74%) of **8** as an oily material: $[\alpha]_D^{25} - 23.4^\circ$ ($c=4.1$, methanol).

N-(2-Mercapto-2-methylpropanoyl)-L-cysteine (9)—The above obtained S-benzyl-N-(S-benzyl-2-mercapto-2-methylpropanoyl)-L-cysteine (**8**) (89.6 g, 0.222 mol) was dissolved in liquid ammonia (500 ml), and metallic sodium (21.2 g, 0.917 g atom) was added in small portions with stirring. After the completion of the reaction, ammonium chloride (59.4 g, 1.11 mol) was added, and thereafter ammonia was removed by distillation. Water was added to the residue to dissolve it. The aqueous layer was separated, washed with ethyl acetate, and acidified with hydrochloric acid with cooling. The precipitates were extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 , and concentrated *in vacuo*. The crystals were recrystallized from ethyl acetate to give 40.1 g (81%) of **9**: mp 139–140°; $[\alpha]_D^{25} + 32.3^\circ$ ($c=1.0$, ethanol). *Anal.* Calcd for $C_7H_{13}NO_3S_2$: C, 37.65; H, 5.87; N, 6.27. Found: C, 37.78; H, 5.86; N, 6.16.

N²-(S-Benzoyl-3-mercaptopropanoyl)-L-histidine (13) (in Method A)—L-Histidine·HCl·H₂O (69.9 g, 0.33 mol) was dissolved in water (500 ml) in an ice bath, with stirring. Sodium hydroxide (13.3 g, 0.33 mol) in water (160 ml) was added dropwise, then potassium carbonate (23.0 g, 0.167 mol) in water (160 ml) was added. S-Benzoyl-3-mercaptopropanoyl chloride, which was obtained by reacting S-benzoyl-3-mercaptopropanoic acid (84.1 g, 0.40 mol) with thionyl chloride (57.1 g, 0.48 mol), and potassium carbonate (87.7 g, 0.633 mol) in water (160 ml) were added dropwise over a period of 2 hr. The resulting mixture was further stirred for 1 hr, acidified with hydrochloric acid, and washed with ethyl acetate. Next, 1 M potassium carbonate was added to the aqueous layer in small portions to pH 4.5, and sodium chloride was added to saturation. The solution was kept overnight. The resulting crystals were collected, washed with water, and recrystallized from water to give 45.3 g (39%) of **13**: mp 170–171°; $[\alpha]_D^{25} - 7.0^\circ$ ($c=1.0$, water). *Anal.* Calcd for $C_{16}H_{17}N_3O_4S$: C, 55.32; H, 4.93; N, 12.10. Found: C, 55.28; H, 4.96; N, 11.99.

N²-(3-Mercaptopropanoyl)-L-histidine (14)—N²-(S-Benzoyl-3-mercaptopropanoyl)-L-histidine (**13**) (30 g, 0.086 mol) was added to aqueous ammonia (300 ml) and the mixture was stirred at room temperature for 1 hr. The precipitated benzamide was removed by filtration, and the filtrate was concentrated *in vacuo*. The oily residue was washed with ethyl acetate by decantation. Ethanol (50 ml) was added to the residue,

and the precipitated crystals were collected, and washed with ethanol to give 12.6 g (60%) of **14**: mp 219° (dec.); $[\alpha]_D^{25} + 14.1^\circ$ ($c=1.0$, water). *Anal.* Calcd for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27. Found: C, 44.22; H, 5.40; N, 17.02.

N²-(S-Benzoyl-2-mercaptopropanoyl)-L-tryptophan-a and -b (19a and 19b) (in Method B)—2-Bromopropanoyl chloride (68.6 g, 0.4 mol) and potassium carbonate (110 g, 0.80 mol) in water (40 ml) were added dropwise to a mixture of L-tryptophan (81.7 g, 0.40 mol) and 1 N sodium hydroxide (400 ml), with ice-cooling and stirring over a period of 1 hr. After the addition, the mixture was stirred at room temperature for 30 min. Sodium thiobenzoate solution, prepared from thiobenzoic acid (55.2 g, 0.40 mol) and 1 N sodium hydroxide (400 ml), was added to the reaction mixture, and the whole was stirred at room temperature. After standing overnight, the mixture was acidified with hydrochloric acid, and extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried over Na_2SO_4 , filtered, and concentrated. The residue was crystallized from benzene, and recrystallized from ethyl acetate to give 28.7 g (18%) of **19a**: mp 178–179°; $[\alpha]_D^{25} - 43.3^\circ$ ($c=1.0$, ethanol). *Anal.* Calcd for $C_{21}H_{20}N_2O_4S$: C, 63.62; H, 5.08; N, 7.06. Found: C, 63.60; H, 5.05; N, 6.98.

After **19a** had been removed, the filtrate was concentrated, and petr. benzin was added to the residue. The precipitates (116.0 g) were collected, applied to an SiO_2 column, and eluted with ethyl acetate–benzene (1:2). The eluate was evaporated to dryness *in vacuo*. The crystals (50.0 g) were recrystallized from ethyl acetate to give 33.1 g (21%) of **19b**: mp 168–169°; $[\alpha]_D^{25} + 67.1^\circ$ ($c=1.0$, ethanol). *Anal.* Calcd for $C_{21}H_{20}N_2O_4S$: C, 63.62; H, 5.08; N, 7.06. Found: C, 63.58; H, 5.05; N, 6.97.

N²-(2-Mercaptopropanoyl)-L-tryptophan-a and -b (20a and 20b)—N²-(S-Benzoyl-2-mercaptopropanoyl)-L-tryptophan-a or -b (**19a** or **19b**) (20.0 g, 0.05 mol) was added to aqueous ammonia (200 ml) and the mixture was stirred at room temperature for 1 hr. The precipitated benzamide was removed by extraction with ethyl acetate. The aqueous layer was concentrated *in vacuo*, and 6 N hydrochloric acid was added to the oily residue to pH 1. The solution was then extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried over Na_2SO_4 , and concentrated *in vacuo*. Ethyl acetate and benzene were added to the residue to crystallize it. The crystals were collected, washed with ethyl acetate–benzene, and recrystallized from the same solvents. Acid-a (**20a**) was obtained (9.3 g, 63%): mp 103–105°; $[\alpha]_D^{25} + 31.0^\circ$ ($c=1.0$, ethanol). *Anal.* Calcd for $C_{14}H_{16}N_2O_3S$: C, 57.52; H, 5.52; N, 9.58. Found: C, 57.65; H, 5.49; N, 9.50. Acid-b (**20b**) was obtained (5.0 g, 35%): mp 120–122°; $[\alpha]_D^{25} + 34.6^\circ$ ($c=1.0$, ethanol). *Anal.* Calcd for $C_{14}H_{16}N_2O_3S$: C, 57.52; H, 5.52; N, 9.58. Found: C, 57.70; H, 5.49; N, 9.55.

N²-(S-Benzoyl-3-mercaptopropanoyl)-L-tryptophan (25) (in Method A)—L-Tryptophan (10.2 g, 0.05 mol) was suspended in potassium carbonate (3.5 g, 0.025 mol) in water (100 ml), then S-benzoyl-3-mercaptopropanoyl chloride (11.4 g, 0.05 mol) and potassium carbonate (13.8 g, 0.10 mol) in water (50 ml) were added dropwise with cooling and stirring. The resulting mixture was further stirred for 15 min, acidified with hydrochloric acid to pH 1, and extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 , and concentrated to form crystals. The crystals were collected by filtration, washed with ethyl acetate, and recrystallized from ethyl acetate–benzene to give 14.3 g (72%) of **25**: mp 123–125°; $[\alpha]_D^{25} + 17.5^\circ$ ($c=0.7$, ethanol). *Anal.* Calcd for $C_{21}H_{20}N_2O_4S$: C, 63.62; H, 5.08; N, 7.06. Found: C, 63.10; H, 5.03; N, 7.11.

N²-(3-Mercaptopropanoyl)-L-tryptophan (27)—N²-(S-Benzoyl-3-mercaptopropanoyl)-L-tryptophan (**25**) (20.0 g, 0.05 mol) was added to aqueous ammonia (200 ml), and the whole was stirred at room temperature for 1 hr. The resulting solution was extracted with ethyl acetate to remove benzamide. The aqueous layer was concentrated under reduced pressure, acidified with hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried over Na_2SO_4 , and evaporated to dryness. The residue was crystallized from ethyl acetate–benzene. Recrystallization from the same solvents yielded 9.1 g (62%) of **27**: mp 126–128°; $[\alpha]_D^{25} + 20.0^\circ$ ($c=1.0$, ethanol). *Anal.* Calcd for $C_{14}H_{16}N_2O_3S$: C, 57.52; H, 5.52; N, 9.58. Found: C, 57.66; H, 5.50; N, 9.57.

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