Chem. Pharm. Bull. 29(5)1203—1213(1981)

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

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(Received August 29, 1980)

A series of mercaptoacylamino acids containing tyrosine, dopa and α-methyldopa moieties is reported, and the structure-activity relationships are discussed. These compounds were tested for antihypertensive activity, and some of the compounds exhibited inhibitory activity against angiotensin I-converting enzyme. N-(2-Mercaptopropanoyl)-L-tyrosine-b (**6b**) was found to be 5 times more potent than N-(2-mercaptopropanoyl)-L-phenylalanine-a (Ia).

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

As part of a research program aimed at the development of new antihypertensive agents, we have investigated compounds showing inhibitory activity against angiotensin I-converting enzyme (ACE) and reported recently the synthesis and pharmacological effects of a series of mercaptoacylamino acids (*e.g.*, N-(mercaptoacyl)glycines, -alanines, -leucines, -phenylglycines, -phenylalanines, -glutamic acids, -cysteines, -histidines, and -tryptophans).[1,2] In prior studies on structure-activity relationships of active compounds against ACE, N-(mercaptoacyl)-phenylalanines were found to show potent activity.

On the other hand, tyrosine (*p*-hydroxyphenylalanine) is biologically capable of participating in interactions with the structural components of its acceptor because the phenolic hydroxyl group of tyrosine forms a hydrogen bond as a proton donor.[3] Thus, N-(mercaptoacyl) tyrosine, -dopa (-3,4-dihydroxyphenylalanine) and -α-methyldopa (-α-methyl-3,4-dihydroxyphenylalanine) were synthesized and tested for inhibitory activity against ACE on the basis that they retained the structural features of phenylalanine but were substituted on the benzene ring by hydroxyl groups. Further, the optically active forms (Ia and Ib) of (±)-N-(2-mercaptopropanoyl)phenylalanine-b[2] were synthesized as standard compounds, and their inhibitory activities were compared with those of the above compounds.

Syntheses

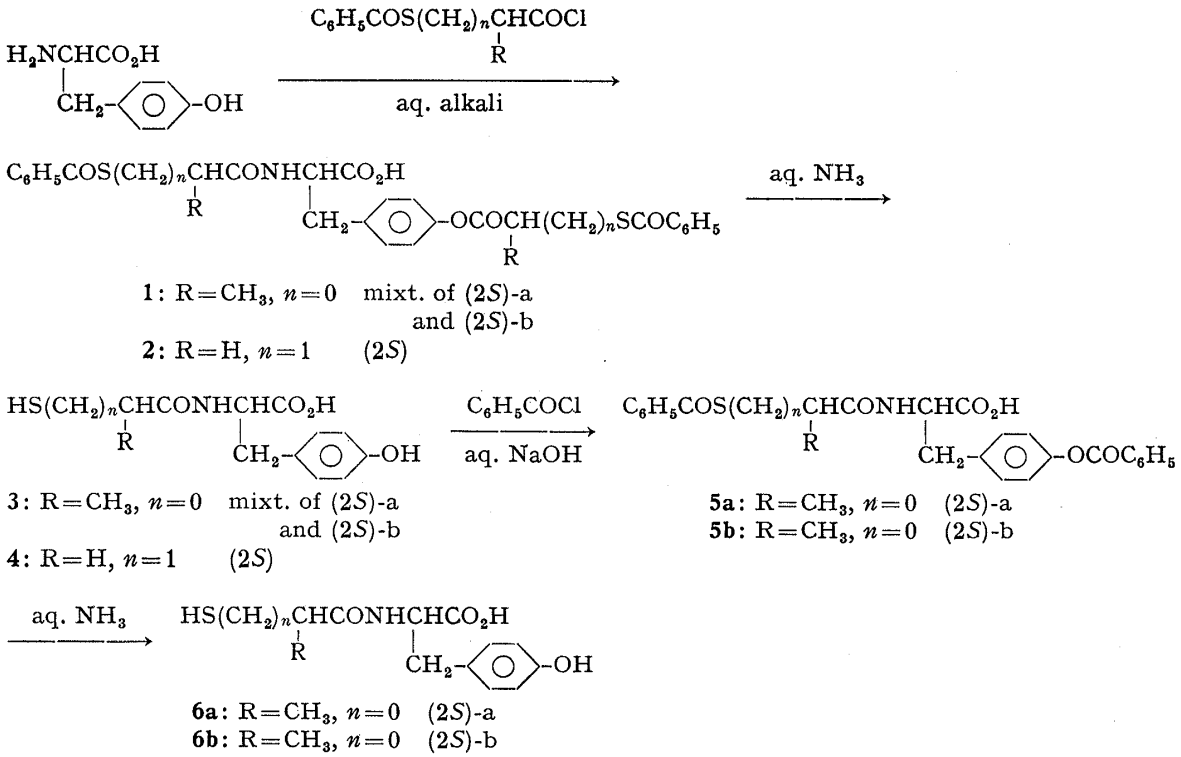
N-(Mercaptoacyl)tyrosines were synthesized by two methods (represented in Chart 1), as follows.

Method A: Tyrosine was reacted with acid halide without protecting the hydroxyl group, and ammonolysis of the resulting N,O-(diacyl)tyrosines was performed.

Method B: Tyrosine protected at the hydroxyl group with a benzyloxycarbonyl group[4] was reacted with acid halide by means of the Schotten–Baumann reaction, and the N-acyl derivatives obtained were hydrolyzed under alkaline conditions.

L-Tyrosine was reacted with DL-S-benzoyl-2-mercaptopropanoyl chloride to give N,O-(diacyl)tyrosine (**1**) in 68% yield. Because compound **1** is a mixture of diastereoisomers and the ratio of isomers is not clear, the reaction mixture was ammonolyzed without isolation to give a mixture of N-(2-mercaptopropanoyl)-L-tyrosine-a and -b (**3**) diastereoisomers, in 44% yield. The *Rf* values were 0.34 and 0.37.[5] As it was difficult to separate the diastereoisomers, **3** was converted to O,S-dibenzoyl derivatives. It was recrystallized from ethyl acetate–

Method A



Method B

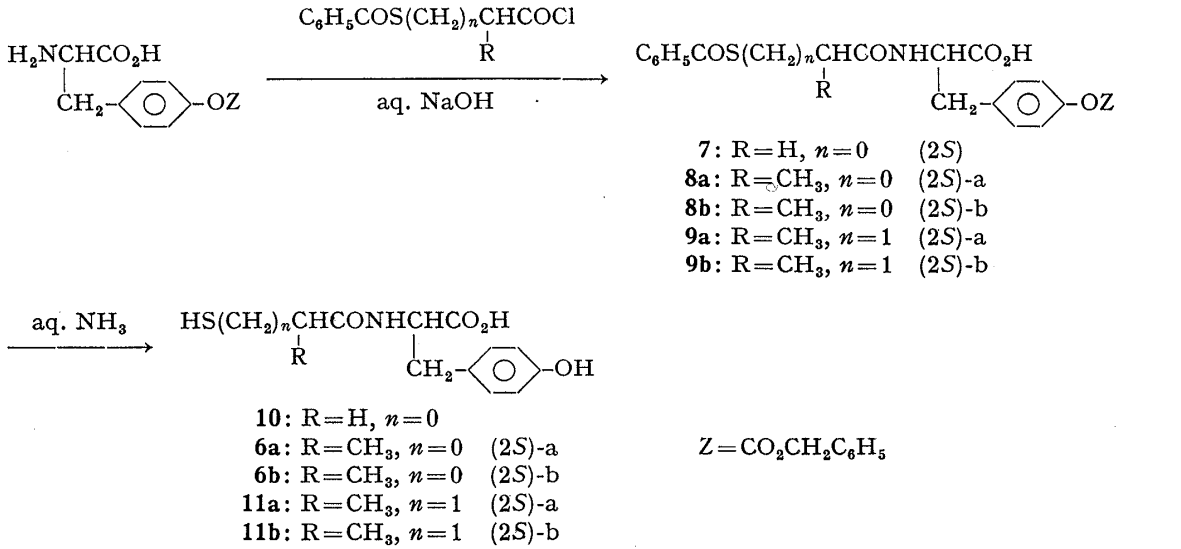


Chart 1

benzene, ethyl acetate and ethyl acetate–methanol successively to give O-benzoyl-N-(S-benzoyl-2-mercaptopropanoyl)-L-tyrosine-a (**5a**): *Rf* value 0.31.[6] The filtrate of **5a** was purified by column chromatography to give O-benzoyl-N-(S-benzoyl-2-mercaptopropanoyl)-L-tyrosine-b (**5b**): *Rf* value 0.20.[6] Ammonolysis of compounds **5a** and **5b** gave the corresponding thiols, N-(2-mercaptopropanoyl)-L-tyrosine-a and -b (**6a** and **6b**), respectively.

Similarly, L-tyrosine was reacted with S-benzoyl-3-mercaptopropanoyl chloride, and the resulting N,O-diacyl derivative (**2**) was treated with aqueous ammonia. The crude product was purified by column chromatography to give N-(3-mercaptopropanoyl)-L-tyrosine (**4**).

Method C

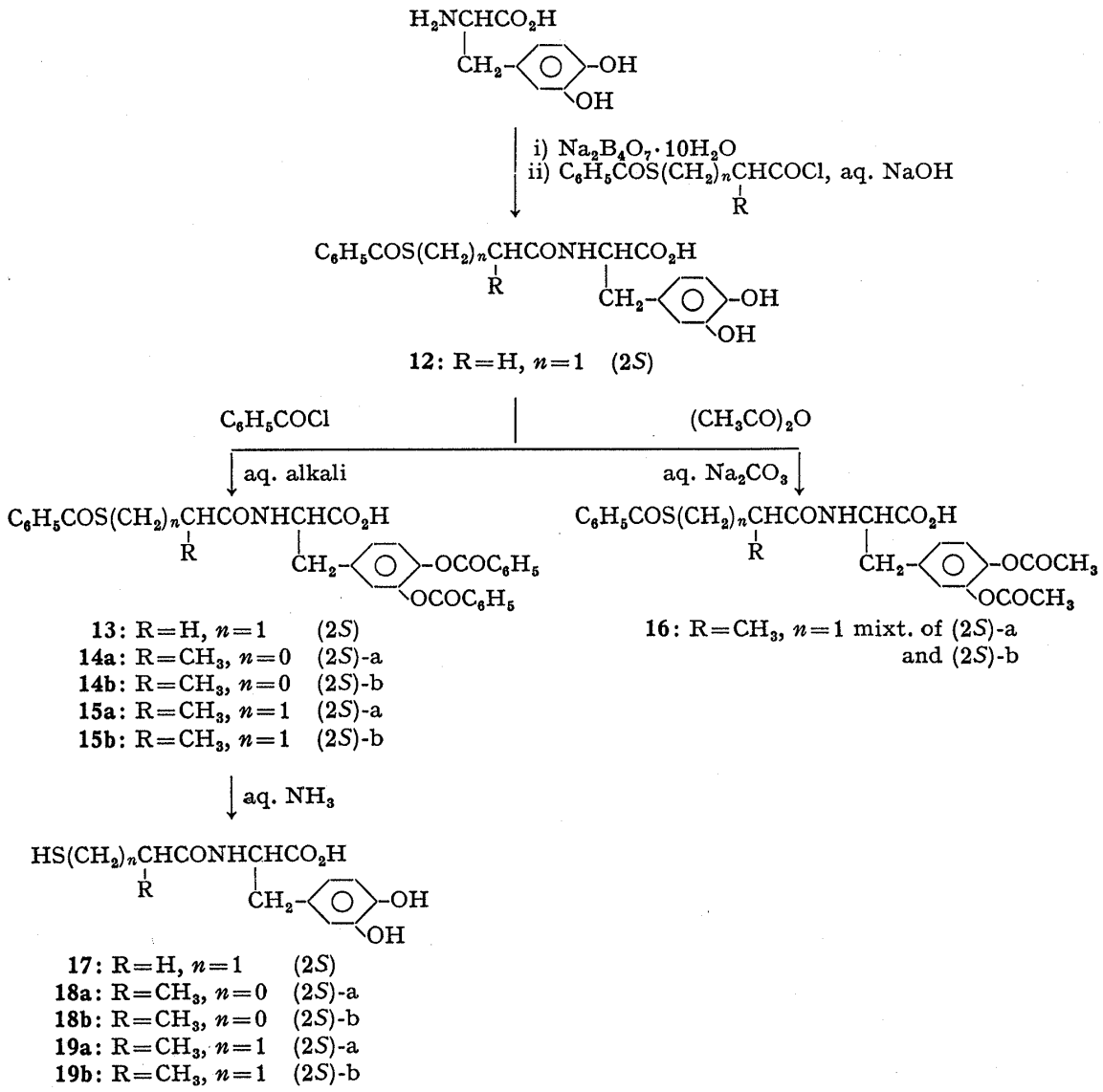


Chart 2

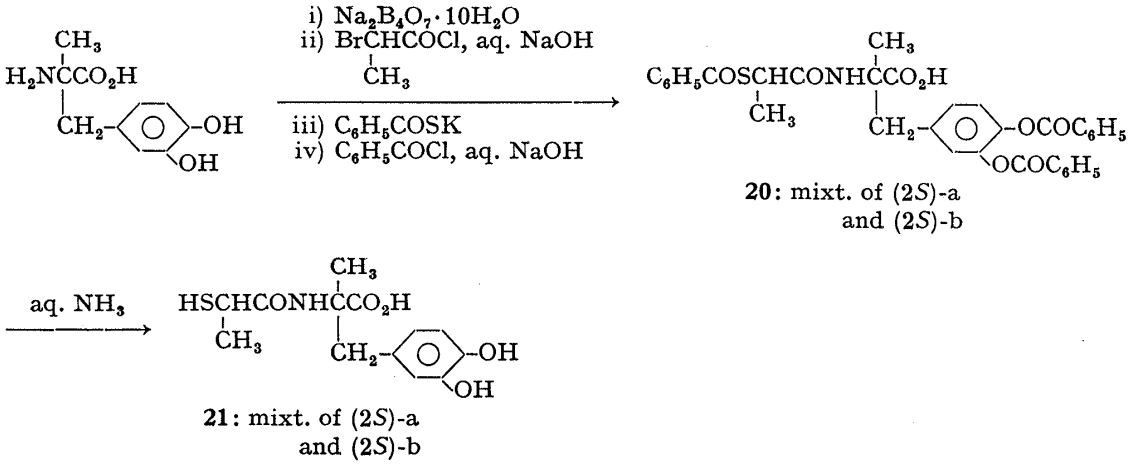
O-Benzyloxycarbonyl-L-tyrosine was reacted with S-benzoylmercaptoacetyl chloride, and the resulting oil was purified by column chromatography to give N-(S-benzoylmercaptoacetyl)-O-benzyloxycarbonyl-L-tyrosine (**7**). Then **7** was hydrolyzed and purified by column chromatography to give N-(mercaptoacetyl)-L-tyrosine (**10**). When the acid halide was DL-S-benzoyl-2-mercaptopropanoyl chloride in the above reaction, the mixture of diastereoisomers was separated by column chromatography to give oily N-(S-benzoyl-2-mercaptopropanoyl)-O-benzyloxycarbonyl-L-tyrosine-a and -b (**8a**: *Rf* value 0.31 and **8b**: *Rf* value 0.21).[6] Hydrolysis of **8a** and **8b** gave N-(2-mercaptopropanoyl)-L-tyrosine-a and -b (**6a** and **6b**), respectively.

DL-S-Benzoyl-3-mercapto-2-methylpropanoyl chloride gave N-(S-benzoyl-3-mercapto-2-methylpropanoyl)-O-benzyloxycarbonyl-L-tyrosine-a and -b (**9a**: *Rf* value 0.17 and **9b**: *Rf* value 0.27)[6] when reacted in the manner described for the synthesis of compounds **8a** and **8b**. Hydrolysis of **9a** and **9b** gave N-(3-mercapto-2-methylpropanoyl)-L-tyrosine-a and -b (**11a**: *Rf* value 0.48 and **11b**: *Rf* value 0.57).[5]

Dopa derivatives were synthesized as follows. The phenolic 3,4-diol groups of dopa were protected by the formation of a complex with boric acid[7] and the product was reacted

with acid halide by means of the Schotten–Baumann reaction. The structure of oily N-(S-benzoyl-3-mercaptopropanoyl)-L-dopa (**12**) was identified as the dicyclohexylamine salt. Moreover, benzoylation of hydroxyl groups of **12** gave crystalline N-(S-benzoyl-3-mercaptopropanoyl)-O,O′-dibenzoyl-L-dopa (**13**). The diastereomeric compounds **14** and **15** were separated to **14a** (*Rf* value 0.29) and **14b** (*Rf* value 0.20), and **15a** (*Rf* value 0.47) and **15b** (*Rf* value 0.59),[6] respectively. Although the acetyl derivative (**16**) was crystallized, separation of the diastereoisomers was difficult. Ammonolysis of the tribenzoyl derivatives gave the corresponding thiols, **17**, **18a** (*Rf* value 0.45), **18b** (*Rf* value 0.41), **19a** (*Rf* value 0.21) and **19b** (*Rf* value 0.28).[5]

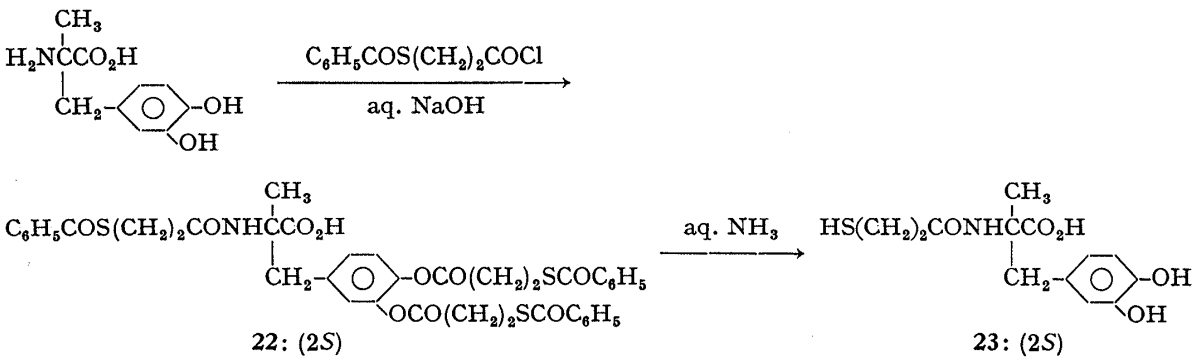
Method D



20: mixt. of (2*S*)-a and (2*S*)-b

21: mixt. of (2*S*)-a and (2*S*)-b

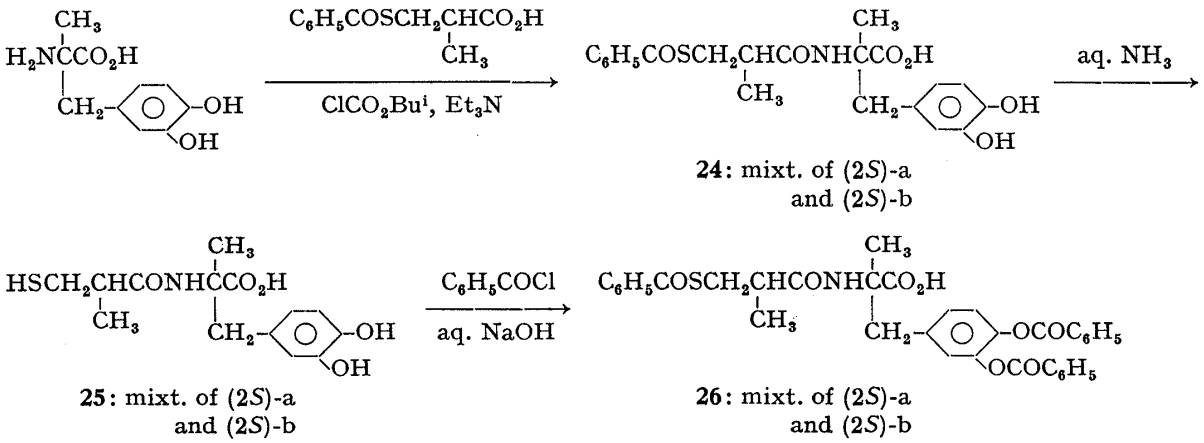
Method E



22: (2*S*)

23: (2*S*)

Method F



24: mixt. of (2*S*)-a and (2*S*)-b

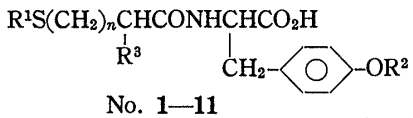
25: mixt. of (2*S*)-a and (2*S*)-b

26: mixt. of (2*S*)-a and (2*S*)-b

Chart 3

In the reaction of L-α-methyldopa with acid halide,[8] when the acid halide was DL-S-benzoyl-2-mercaptopropanoyl chloride or DL-S-benzoyl-3-mercapto-2-methylpropanoyl chloride, the desired compound could not be obtained by means of the Schotten–Baumann reaction, and the starting material was mostly recovered. Accordingly, as shown in method D, the hydroxyl groups of L-α-methyldopa were protected with boric acid as described for dopa, and the product was reacted with DL-2-bromopropanoyl chloride by means of the Schotten–Baumann reaction. Next, the S-benzoylmercapto group was introduced into the reaction product by treatment with potassium thiobenzoate. Benzoylation of the hydroxyl groups gave a mixture of diastereoisomers (**20**: *Rf* value 0.36[6]). Ammonolysis of **20** gave **21**: *Rf* value 0.55.[5]

Table I. N-(S-Unsubstituted and -substituted mercaptoacyl)-L-tyrosines

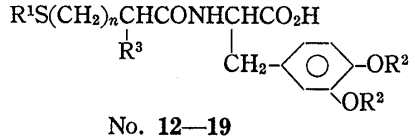


No. **1**—**11**

Compd. No.	mp (°C)[a]	Recrystn. solvent	$[\alpha]_D$ deg. (*c*, solv., °C)	Yield (%)	IR $\nu_{C=O}^{Nujol}$ cm^{-1} COOC COOH COSC CONH					
1[b]	Amorph.[d]		−8.3 (1.0, MeOH, 25)	68		1720	1650			(KBr)
2	132—134	iso-PrOH–benzene	−4.0 (2.0, MeOH, 25)	88	1750	1710	1660	1625	1550	
3[b]	140—142	EtOAc–benzene	+46.2 (1.0, MeOH, 25)	44[e]		1703		1637	1542	
4	136—138	EtOAc–benzene	+30.6 (1.0, MeOH, 25)	70		1701		1632	1540	
5a[c]	202—203	EtOAc–MeOH	+25.7 (1.0, MeOH, 25)	37		1728	1661	1646	1530	
5b[c]	150—152	Benzene	−55.8 (1.0, MeOH, 25)	32		1726		1649	1529	
6a[c]	148—150	EtOAc–benzene	+54.4 (1.0, MeOH, 25)	62[f] 73[g]		1706		1642	1543	
6b[c]	146—148	EtOAc–benzene	+32.3 (1.0, MeOH, 25)	71[f] 67[g]		1706		1637	1546	
7	118—119	Benzene–cyclohexane	+12.4 (1.0, MeOH, 25)	59	1751	1730	1670	1621	1541	
8a[c]	Oil[d]		+27.3 (1.0, MeOH, 25)	20		1759		1670		($CHCl_3$)
8b[c]	Oil[d]		−42.3 (1.1, MeOH, 25)	22		1759		1652		($CHCl_3$)
9a[c]	165—165.5	EtOAc	−4.4 (0.9, MeOH, 25)	33	1753	1720	1653	1643	1540	
9b[c]	133—134	EtOAc	+2.8 (1.0, MeOH, 25)	31	1762	1700	1656	1645	1523	
10	149—150	EtOAc	+50.1 (1.0, MeOH, 25)	71		1704		1637	1549	
11a[c]	144.5—145	EtOAc–benzene	+0.6 (1.1, MeOH, 25)	88		1720		1632	1545	
11b[c]	123.5—124	EtOAc–benzene	+0.8 (1.1, MeOH, 25)	74		1714		1645	1535	

a) Melting points are uncorrected.
b) Mixture of diastereoisomers.
c) Compounds **5b**, **6b**, **8b**, **9b**, and **11b** are diastereoisomers of **5a**, **6a**, **8a**, **9a**, and **11a**, respectively.
d) Purified by chromatography.
e) From tyrosine.
f) Method A.
g) Method B.

Table II. N-(S-Unsubstituted and -substituted mercaptoacyl)-L-dopas



No. **12**—**19**

Compd. No.	mp (°C)[a]	Recrystn. solvent	$[\alpha]_D$ deg (c, solv., °C)	Yield (%)	IR $\nu_{C=O}^{Nujol}$ cm^{-1} COOC	COOH	COSC	CONH		
12	Oil[e]		−0.6 (1.1, MeOH, 25)	66						
12·DCHA[b]	116 (dec.)	EtOAc–$(iso\text{-}Pr)_2O$	+30.7 (1.0, MeOH, 25)			1578		1653		
13	198—200	EtOH	+5.9 (1.0, MeOH, 25)	62	1736	1721	1652	1638	1530	
14a[c]	186—186.5	EtOAc	+10.8 (1.0, MeOH, 25)	25	1735	1730	1655	1640	1530	
14b[c]	140.5—141	EtOAc	−1.3 (1.0, MeOH, 25)	23	1735	1707	1650	1640	1538	
15a[c]	179—180	EtOAc	−19.3 (1.0, MeOH, 25)	36	1740		1660	1640	1530	
15b[c]	170—171	EtOAc	+0.7 (1.0, MeOH, 25)	33	1740		1660	1640	1530	
16[d]	122—128	EtOAc–benzene	+4.5 (1.0, MeOH, 25)	46	1760	1710	1655	1635	1540	
17	114—116	EtOAc–benzene	+28.0 (1.0, MeOH, 25)	81		1706		1652	1541	
18a[c]	Amorph.[e]		+35.6 (1.1, MeOH, 25)	88		1720		1650		(Film)
18b[c]	Amorph.[e]		+17.2 (1.1, MeOH, 25)	84		1720		1650		(Film)
19a[c]	Amorph.[e]		+6.6 (1.0, MeOH, 25)	85		1720		1635	1516	(Film)
19b[c]	155—156	EtOAc	−0.5 (1.0, MeOH, 25)	85		1720		1635	1516	(Film)

a) See footnote in Table I.
b) DCHA is dicyclohexylamine.
c) Compounds **14b**, **15b**, **18b**, and **19b** are diastereoisomers of **14a**, **15a**, **18a**, and **19a**, respectively.
d) Mixture of diastereoisomers.
e) Purified by chromatography.

In method E, N,O,O′-triacyl derivative (**22**) obtained by means of the Schotten–Baumann reaction without protecting the hydroxyl groups of α-methyldopa was treated with ammonia to give the thiol **23**. In method F, the acyl derivative **24** obtained by the mixed anhydride method was treated with ammonia to give the oily thiol **25**. Compounds **24** and **25** (*Rf* values: **24**, 0.66: **25**, 0.52)[5] were the mixture of diastereoisomers. Compound **25** could not be separated by benzoylation because it gave an oily tribenzoyl derivative (**26**: *Rf* value 0.43).[6]

Compounds Ia and Ib described above were derivatives of L-phenylalanine, and were synthesized by method A. L-Phenylalanine was reacted with DL-S-benzoyl-2-mercaptopropanoyl chloride by means of the Schotten–Baumann reaction, and the resulting mixture of diastereoisomers was separated by column chromatography to give N-(S-benzoyl-2-mercaptopropanoyl)-L-phenylalanine-a and -b (IIa: *Rf* value 0.40 and IIb: *Rf* value 0.48).[5] Ammonolysis of compounds IIa and IIb gave N-(2-mercaptopropanoyl)-L-phenylalanine-a and -b (Ia: *Rf* value 0.34 and Ib: *Rf* value 0.38),[5] respectively.

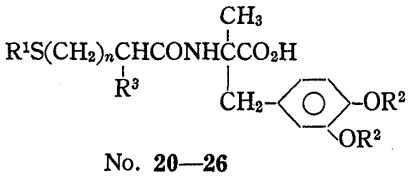
Structure-Activity Relationships

In the first report,[2] the inhibitory activity of (±)-N-(2-mercaptopropanoyl)phenylalanine-b against angiotensin I-converting enzyme (ACE) was shown to be pI_{50}: 5.00 (angiotensin I).

In the present study, N-(2-mercaptopropanoyl)-L-phenylalanine-a and -b (Ia and Ib) which are optically active in both the mercaptoacyl and amino acid moieties, were synthesized and tested for activity. The activity of Ia was pI_{50}: 5.34 (angiotensin I); Ia was about twice as potent as the racemic form.

On the other hand, the inhibitory activity of **6b** (having a *para* hydroxyl group on the benzene ring of Ia) was pI_{50}: 6.00. A comparison of the activities of Ia and 6b indicates that the introduction of a phenolic hydroxyl group augmented the activity by 5 times, which is mpiortant from the standpoint of drug design.

TABLE III. N-(S-Unsubstituted and -substituted mercaptoacyl)-L-α-methyldopas

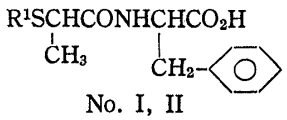


No. **20—26**

Compd. No.	mp (°C)[a]	Recrystn. solvent	$[\alpha]_D$ deg (c, solv., °C)	Yield (%)	IR $\nu_{C=O}^{Nujol}$ cm^{-1} COOC	COOH	COSC	CONH		
20[b]	Amorph.[c]		−47.6 (1.1, MeOH, 25)	31	1740	1715	1650	1630	1520	
21[b]	Amorph.[c]		−24.5 (1.3, MeOH, 23)	87		1720		1655	1520	
22	88—91	$(iso\text{-}Pr)_2O$	−46.4 (1.0, MeOH, 25)	66	1765	1705	1650	1625	1559	
23	Amorph.[c]		−53.2 (1.0, MeOH, 25)	64		1720		1640		
24[b]	Amorph.[c]		−33.5 (1.8, MeOH, 25)	13		1713		1652		($CHCl_3$)
25[b]	Oil[c]		−29.4 (0.8, MeOH, 25)	80		1710		1635	1514	(Film)
26[b]	Oil[c]		−54.2 (1.0, MeOH, 25)	86		1740		1660		

a) See footnote in Table I.
b) Mixture of diastereoisomers.
c) Purified by chromatography.

TABLE IV. N-(2-Mercaptopropanoyl)-L-phenylalanines and Their S-Benzoyl Derivatives



No. I, II

Compd. No.[a]	R^1	mp (°C)[b]	Recrystn. solvent	$[\alpha]_D$ deg (c, solv., °C)	Yield (%)	IR $\nu_{C=O}^{Nujol}$ cm^{-1} COOH	COSC	CONH		
Ia[c]	H	106—107	Benzene–hexane	+27.3 (1.0, MeOH, 25)	73	1720 1710		1658 1650	1498 1540	($CHCl_3$)
Ib[c]	H	Oil		+34.0 (1.1, MeOH, 25)	66	1722		1660	1498	($CHCl_3$)
IIa	COC_6H_5	122—122.5	EtOAc	−91.6 (0.9, MeOH, 25)	22	1725	1651	1640	1554	
IIb	COC_6H_5	149.5—150	EtOAc	+65.5 (1.0, MeOH, 25)	22	1762	1653	1636	1524	

a) Compounds Ib and IIb are diastereoisomers of Ia and IIa, respectively.
b) See footnote in Table I.
c) Purified by chromatography.

Table V. Elemental Analyses

Compd. No.	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
2	$C_{29}H_{27}NO_7S_2$	61.58	4.81	2.48	61.52	4.92	2.48
3	$C_{12}H_{15}NO_4S$	53.52	5.61	5.20	53.61	5.66	5.20
4	$C_{12}H_{15}NO_4S$	53.52	5.61	5.20	53.58	5.66	5.13
5a	$C_{26}H_{23}NO_6S$	65.40	4.85	2.93	65.49	4.91	2.91
5b	$C_{26}H_{23}NO_6S$	65.40	4.85	2.93	65.38	4.83	2.95
6a	$C_{12}H_{15}NO_4S$	53.52	5.61	5.20	53.54	5.66	5.08
6b	$C_{12}H_{15}NO_4S$	53.52	5.61	5.20	53.43	5.64	5.17
7	$C_{26}H_{23}NO_7S$	63.28	4.70	2.84	63.41	4.78	2.75
9a	$C_{28}H_{27}NO_7S$	64.48	5.22	2.69	64.53	5.26	2.76
9b	$C_{28}H_{27}NO_7S$	64.48	5.22	2.69	64.54	5.24	2.71
10	$C_{11}H_{13}NO_4S$	51.75	5.13	5.49	51.78	5.05	5.27
11a	$C_{13}H_{17}NO_4S$	55.11	6.05	4.94	55.25	6.06	4.88
11b	$C_{13}H_{17}NO_4S$	55.11	6.05	4.94	55.20	6.10	4.85
12·DCHA[a]	$C_{19}H_{19}NO_6S \cdot C_{12}H_{23}N$	65.24	7.42	4.91	65.31	7.58	4.63
13	$C_{33}H_{27}NO_8S$	66.32	4.55	2.34	66.29	4.66	2.33
14a	$C_{33}H_{27}NO_8S$	66.32	4.55	2.34	66.25	4.57	2.38
14b	$C_{33}H_{27}NO_8S$	66.32	4.55	2.34	66.30	4.53	2.36
15a	$C_{34}H_{29}NO_8S$	66.76	4.78	2.29	67.00	4.63	2.28
15b	$C_{34}H_{29}NO_8S$	66.76	4.78	2.29	66.93	4.70	2.25
16	$C_{24}H_{25}NO_8S$	59.13	5.17	2.87	59.40	5.08	2.73
17	$C_{12}H_{15}NO_5S$	50.52	5.30	4.91	50.88	5.34	4.77
22	$C_{40}H_{37}NO_{10}S_3$	60.98	4.73	1.78	61.04	4.81	1.75

a) DCHA is dicyclohexylamine.

Table VI. Inhibitory Activities of N-(Mercaptoacyl)amino Acids against ACE[a]

Compd. No.	AI pI_{50}	ACE pI_{50}	BK pA_{50}
SQ 14225[b]	6.68	7.09	8.44
3	5.62	5.44	7.70
4	5.46	3.97	7.42
6a	4.85	2.99	6.44
6b	6.00	5.42	7.57
10	5.40	5.70	7.59
11a	5.77	6.39	5.97
11b	5.07	5.30	6.47
17	5.46	5.43	7.04
18a	4.36	5.82	6.03
18b	5.21	6.08	7.10
19a	5.77	6.60	7.59
19b	5.28	5.96	6.90
21	<4	5.41	<4
23	3.96	4.09	5.96
25	4.36	5.92	6.83
Ia	5.34	—	7.00
Ib	—	—	5.34

a) The inhibitory activities of compounds against ACE were determined according to the reported procedures.[9] (AI; angiotensin I, BK; bradykinin). pI_{50}; $-\log$ of the molar concentration of compound which inhibits the enzyme activity or agonist effect by 50%. pA_{50}; $-\log$ of the molar concentration of compound which enhances the agonist effect by 50%.

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

α-Methyldopa derivatives showed the lowest activity among the synthesized compounds, possibly because of steric hindrance by the methyl group at the α-position with respect to the amino acid moiety. Thus, it seems to be essential for activity that the α-carbon of the amino acid is a methine group.

Experimental

Melting points were determined in open capillary tubes on 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,O-Bis(S-benzoyl-3-mercaptopropanoyl)-L-tyrosine (2)——A solution of L-tyrosine (9.1 g, 0.05 mol) in 1 N sodium hydroxide (100 ml) were treated dropwise with S-benzoyl-3-mercaptopropanoyl chloride (25.8 g, 0.11 mol) and 2 M potassium carbonate (25 ml), with ice-cooling and stirring. After the addition, the solution was stirred for 1 hr and acidified with 6 N hydrochloric acid. The crystals were collected, washed with water, and recrystallized from isopropyl alcohol–benzene to give 25.0 g (88%) of **2**.

N-(3-Mercaptopropanoyl)-L-tyrosine (4)——N,O-Bis(S-benzoyl-3-mercaptopropanoyl)-L-tyrosine (**2**) (5.7 g, 0.01 mol) was dissolved in methanol (20 ml), and 28% aqueous ammonia (20 ml) was added. The reaction mixture was stirred for 1.5 hr at room temperature, then concentrated *in vacuo*, and water (10 ml) was added. The resulting solution was washed with ethyl acetate. The aqueous layer was acidified with 6 N hydrochloric acid, and extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was placed on an SiO_2 column packed with benzene, and eluted with benzene–ethyl acetate (10: 3). The eluate was evaporated to dryness *in vacuo* to give solid, which was recrystallized from benzene–ethyl acetate to give 1.9 g (70%) of **4**.

N-(S-Benzoyl-3-mercapto-2-methylpropanoyl)-O-benzyloxycarbonyl-L-tyrosine-a and -b (9a and 9b)——Ether (30 ml) was added to a solution of O-benzyloxycarbonyl-L-tyrosine (15.7 g, 0.05 mol) in 1 N sodium hydroxide (90 ml). To this solution, S-benzoyl-3-mercapto-2-methylpropanoyl chloride (12.2 g, 0.05 mol) and 1 N sodium hydroxide (10 ml) were added dropwise with ice-cooling and stirring. After the addition, the solution was stirred for 1 hr. The resulting precipitate was collected by decantation, and dissolved in water (150 ml). The solution was acidified with 6 N hydrochloric acid and extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried over $MgSO_4$, and concentrated *in vacuo*. This mixture of two diastereoisomers (19.3 g, 74%) was separated by recrystallization to give 8.6 g (33%) of **9a**.

The combined filtrate was applied to an SiO_2 column packed with benzene, and eluted with benzene–ethyl acetate (4: 1). The eluate was evaporated to dryness *in vacuo* to give a solid, which was recrystallized from ethyl acetate to give 8.1 g (31%) of **9b**.

N-(3-Mercapto-2-methylpropanoyl)-L-tyrosine-a and -b (11a and 11b)——N-(S-Benzoyl-3-mercapto-2-methylpropanoyl)-O-benzyloxycarbonyl-L-tyrosine-a or -b (**9a** or **9b**) (5.2 g, 0.01 mol) was dissolved in methanol (130 ml), and aqueous ammonia (70 ml) was added. The mixture was treated in the same manner as compound **4** to give 2.5 g (88%) of **11a** or 2.1 g (74%) of **11b**, respectively.

N-(S-Benzoyl-2-mercaptopropanoyl)-O,O′-dibenzoyl-L-dopa-a and -b (14a and 14b)——L-Dopa (9.9 g, 0.05 mol) and sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) (9.5 g, 0.025 mol) were dissolved in water (100 ml), and ether (30 ml) was added. S-Benzoyl-2-mercaptopropanoyl chloride (12.6 g, 0.055 mol) and 1 N sodium hydroxide (90 ml) were then added dropwise to the solution with ice-cooling and stirring. After the addition, the solution was stirred for 1 hr. The aqueous layer was acidified with 6 N hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried over $MgSO_4$, and concentrated *in vacuo*. The residual oil was purified by column chromatography to give a mixture of two diastereoisomers (13.6 g). The mixture was dissolved in 1 N sodium hydroxide (120 ml), and ether (30 ml) was added. Benzoyl chloride (11.2 g, 0.08 mol) was added dropwise to the solution with ice-cooling and stirring. After the addition, the solution was stirred for 1 hr. The resulting precipitate was collected by decantation, and dissolved in water (150 ml). The solution was acidified with 6 N hydrochloric acid, and extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried over $MgSO_4$, and concentrated *in vacuo*. This mixture of two diastereoisomers (18.5 g, 62%) was separated by recrystallization from ethyl acetate to give 7.6 g (25%) of **14a**.

The combined filtrate was purified by column chromatography to give 6.8 g (23%) of **14b**.

N-(2-Mercaptopropanoyl)-L-dopa-a and -b (18a and 18b)——N-(S-Benzoyl-2-mercaptopropanoyl)-O,O′-dibenzoyl-L-dopa-a or -b (**14a** or **14b**) (6.0 g, 0.01 mol) was dissolved in methanol (160 ml), and 28% aqueous ammonia (80 ml) was added. The mixture was treated in the same manner as compound **4** to give 2.5 g (88%) of **18a** or 2.4 g (84%) of **18b**, respectively.

Mixture of N-(S-benzoyl-2-mercaptopropanoyl)-O,O′-dibenzoyl-L-α-methyldopa-a and -b (20)——L-α-Methyldopa (10.6 g, 0.05 mol) and sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) (9.5 g, 0.025 mol) was dissolved in water (100 ml), and ether (30 ml) was added. 2-Bromopropanoyl chloride (9.5 g, 0.055 mol) and 1 N sodium hydroxide (120 ml) were then added dropwise to the solution with ice-cooling and stirring. After the addi-

tion, the solution was stirred for 1 hr at the same temperature and for a further 1 hr at room temperature. Potassium thiobenzoate (10.6 g, 0.06 mol) in water (60 ml) was added to the reaction mixture, and stirred at room temperature. After standing overnight, the mixture was washed with ethyl acetate, acidified with 6 N hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried over $MgSO_4$, and concentrated *in vacuo*. The residual oil was washed with hot cyclohexane to give 8.4 g of crude N-(S-benzoyl-2-mercaptopropanoyl)-L-α-methyldopa. Benzoyl chloride (6.5 g, 0.046 mol) was added dropwise to the crude product in 1 N sodium hydroxide (67 ml) with ice-cooling and stirring. After the addition, the solution was stirred for 1 hr. The resulting precipitate was collected by decantation, and dissolved in water (70 ml). The solution was acidified with 6 N hydrochloric acid, and extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography to give 9.6 g (31%) of **20**.

Mixture of N-(2-mercaptopropanoyl)-L-α-methyldopa-a and -b (21)——A mixture of N-(S-benzoyl-2-mercaptopropanoyl)-O,O′-dibenzoyl-L-α-methyldopa-a and -b (**20**) (6.1 g, 0.01 mol) was dissolved in methanol (100 ml), and 28% aqueous ammonia (80 ml) was added. The mixture was treated in the same manner as compound **4** to give 2.6 g (87%) of **21**.

N,O,O′-Tris(S-benzoyl-3-mercaptopropanoyl)-L-α-methyldopa (22)——A suspension of L-α-methyldopa (10.6 g, 0.05 mol) in water (50 ml) was treated with 1 N sodium hydroxide (100 ml), with ice-cooling and stirring. S-Benzoyl-3-mercaptopropanoyl chloride (34.3 g, 0.15 mol) and 1 N sodium hydroxide (130 ml) were added dropwise to the clear solution. After the addition, the reaction mixture was stirred for 2 hr, then washed with ether, acidified with 6 N hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried over $MgSO_4$, and concentrated *in vacuo*. The residual oil (38 g) was purified by column chromatography to give a solid, which was recrystallized from isopropyl ether to give 26 g (66%) of **22**.

N-(3-Mercaptopropanoyl)-L-α-methyldopa (23)——N,O,O′-Tris(S-benzoyl-3-mercaptopropanoyl)-L-α-methyldopa (**22**) (7.9 g, 0.01 mol) was dissolved in methanol (45 ml), and 28% aqueous ammonia (70 ml) was added. The mixture was treated in the same manner as compound **4** to give 1.9 g (64%) of **23**.

Mixture of N-(S-Benzoyl-3-mercapto-2-methylpropanoyl)-L-α-methyldopa-a and -b (24)——S-Benzoyl-3-mercapto-2-methylpropanoic acid (4.5 g, 0.02 mol) was dissolved in anhyd. tetrahydrofuran (50 ml), and triethylamine (2.8 ml, 0.02 mol) was added. Isobutyl chloroformate (2.6 ml, 0.02 mol) was then added dropwise to the solution, with stirring at −10°. After 10 min a solution of L-α-methyldopa (4.2 g, 0.02 mol), sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) (3.8 g, 0.01 mol), and triethylamine (2.8 ml, 0.02 mol) in water (50 ml) was added at once to the above solution, and the reaction mixture was stirred for 1 hr at room temperature. Water (50 ml) was added, and the solution was washed with ethyl acetate, acidified with 6 N hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was washed with hot cyclohexane 5 times by decantation, and purified by column chromatography to give 1.1 g (13%) of **24**.

Mixture of N-(3-Mercapto-2-methylpropanoyl-L-α-methyldopa-a and -b (25)——A mixture of N-(S-benzoyl-3-mercapto-2-methylpropanoyl)-L-α-methyldopa-a and -b (**24**) (1.0 g, 0.0023 mol) was dissolved in methanol (10 ml), and 28% aqueous ammonia (8 ml) was added. The mixture was treated in the same manner as compound **4** to give 0.6 g (80%) of **25**.

Mixture of N-(S-Benzoyl-3-mercapto-2-methylpropanoyl)-O,O′-dibenzoyl-L-α-methyldopa-a and -b (26)——Under a nitrogen atmosphere, the mixture of N-(3-mercapto-2-methylpropanoyl)-L-α-methyldopa-a and -b (**25**) (0.4 g, 0.0013 mol) was dissolved in 1 N sodium hydroxide (6 ml), and ether (4 ml) was added. Benzoyl chloride (0.6 g, 0.0043 mol) was added dropwise to the solution with ice-cooling and stirring. After the addition, the reaction mixture was stirred for 1 hr at the same temperature and for a further 1 hr at room temperature. Water (10 ml) and ethyl acetate (20 ml) were added, and the organic layer was washed with 2 N hydrochloric acid and then with saturated sodium chloride solution, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography to give 0.7 g (86%) of **26**.

Acknowledgement The authors are very grateful to Prof. M. Suzuki of Meijo University for his valuable suggestions. They are also grateful 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.

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