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Studies on Angiotensin Converting Enzyme Inhibitors. II. Syntheses and Angiotensin Converting Enzyme Inhibitory Activities of Carboxyethylcarbamoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid Derivatives[1)]

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(3*S*)-2-[*N*-Substituted *N*-(2-carboxyethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives (V) were synthesized by condensation of (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylates (III), 3-alkylaminopropionates (II), and phosgene, followed by cleavage of ester groups.

The *in vitro* angiotensin converting enzyme (ACE) inhibitory activities of these dicarboxylic acid derivatives (V) were evaluated. Among them, *N*-ethyl (**13**) and *N*-isopropyl (**14**) derivatives showed high inhibitory activities with IC_{50} values of 1.1×10^{-8} and 7.7×10^{-8} M, respectively.

These compounds showed only weak inhibition of the pressor response to angiotensin I after oral administration in normotensive rats. Thus, in order to derive orally active inhibitors, the ester derivatives (IV, VI, and VII) were prepared as prodrugs of the dicarboxylic acids (V). Of these esters, the monoester compounds (VI) having the ester function at the side chain were found to be orally active. In particular, (3*S*)-2-[*N*-ethyl-*N*-(2-butoxycarbonylethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**20**) inhibited the pressor response to angiotensin I by up to 82% at an oral dose of 1.0 mg/kg.

Keywords——angiotensin converting enzyme (ACE); ACE inhibitor; inhibitory activity; (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; ureido carbonyl; (3*S*)-2-[*N*-substituted *N*-(2-carboxyethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid

Interest in angiotensin converting enzyme (ACE) inhibitors has increased since Ondetti *et al.*[2a,b)] synthesized captopril on the basis of their hypothesis. Clinical studies[3a–c)] show that captopril displays the expected effectiveness as a new type of orally active antihypertensive agent. However, it has recently been suggested that some side effects[4a,b)] of captopril might be caused by the mercapto group at the side chain, and various synthetic studies[5a–d)] aimed at replacing the mercaptoacyl moiety with other possible binding groups have been reported. The carboxylic acid moiety could serve as a ligand to the zinc ion of the active site of ACE, as has been well discussed in many examples, especially in the cases of ω-carboxyalkanoyl-L-proline derivatives.[2b)] Further, the role of the carbonyl oxygen was recently emphasized as a hydrogen bond acceptor able to bind to the donor site of the enzyme.[6a,b)]

As for the acceptor character, it is expected that the ureido carbonyl may be preferable to amide carbonyl. Thus, we chose a ω-carboxyalkylcarbamoyl group and synthesized 1-[*N*-methyl-*N*-(2-carboxyethyl)carbamoyl]-L-proline (**1**). It should be noted that this compound otherwise fills the same structural requirements as D-2-methylglutaryl-L-proline (SQ-14102),[2b)] and this compound can also be regarded as a positional isomer of *N*-carboxymethyl-L-alanyl-L-proline, reported by the Merck group.[5a)]

We previously found that (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid was a

preferred amino acid moiety for an ACE inhibitor.[7] Therefore, we designed tetrahydroisoquinoline analogs possessing the ureido group, expecting that the inhibitory potency could be further increased.

In the present paper, we describe the syntheses of novel (3*S*)-2-[*N*-substituted *N*-(2-carboxyethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives and their ACE inhibitory activities.

Synthesis

Introduction of an *N*-substituted *N*-(2-carboxyethyl)carbamoyl group into the imino acids was carried out *via* two routes as shown in Chart 1.

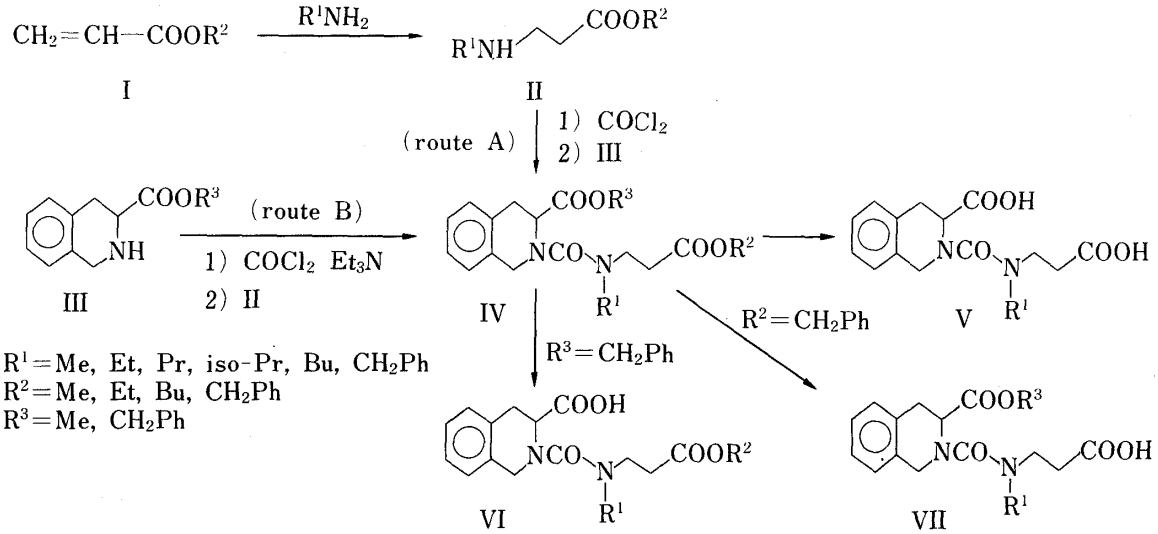


Chart 1

Reaction of 3-(*N*-substituted)aminopropionates (II) with phosgene in the presence of triethylamine gave the corresponding carbamoyl chloride, which was condensed with (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylates (III) to afford (3*S*)-2-[*N*-substituted *N*-(2-alkoxycarbonylethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid esters (IV) in good yields (route A). Alternatively, the diesters (IV) were also obtained by condensation of II with 2-chlorocarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylates, prepared from III and phosgene (route B).

Removal of the ester residues of IV was carried out by alkaline hydrolysis or by catalytic debenzylation to give the corresponding (3*S*)-[*N*-substituted *N*-(2-carboxyethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids (V). Monoesters VI and VII were prepared from IV by catalytic debenzylation of the ester group at the 3-position of tetrahydroisoquinoline (R^3) or at the side chain (R^2), respectively.

Since V, VI, and VII were generally unstable syrups, they were isolated as their calcium salts. The physical constants and analytical data for the synthetic compounds are shown in Table I.

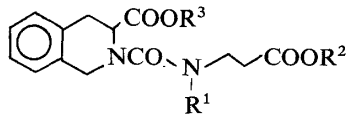
Similarly, compound **1** was prepared by the reaction of benzyl L-prolinate with the carbamoyl chloride derived from benzyl 3-methylamino propionate, followed by deprotection of the benzyl ester groups.

Biological Results and Discussion

In Vitro and *in Vivo* ACE Inhibitory Activities of Dicarboxylic Acid Derivatives (1 and V)

The *in vitro* ACE inhibitory activities of the dicarboxylic acid derivatives were de-

TABLE I. Physical Constants and Analytical Data for the Dicarboxylic Acids (V) and Monoesters (VI and VII)



Compd. No.	R^1	R^2	R^3	mp (°C)	$[\alpha]_D$ deg. (*c*, solv., °C)	Formula	Analysis (%) Calcd (Found)		
							C	H	N
12[a]	Me	H	H	265—268 (dec.)	+7.5 (1, DMSO, 24)	$C_{15}H_{16}CaN_2O_5 \cdot 5/2H_2O$	46.26 (46.18	5.44 5.07	7.19 6.98)
13[a]	Et	H	H	275—280 (dec.)	+17.5 (1, DMSO, 24)	$C_{16}H_{18}CaN_2O_5 \cdot H_2O$	51.05 (50.75	5.36 5.48	7.44 7.23)
14[a]	iso-Pr	H	H	267—269 (dec.)	+10.9 (1, DMSO, 24)	$C_{17}H_{20}CaN_2O_5 \cdot H_2O$	52.59 (52.57	5.68 5.87	7.18 6.91)
15[a]	Pr	H	H	260—263 (dec.)	+29.0 (1, DMSO, 24)	$C_{17}H_{20}CaN_2O_5 \cdot 5/3H_2O$	50.70 (51.01	5.90 5.94	6.96 6.53)
16[a]	Bu	H	H	252—255 (dec.)	+12.2 (1, DMSO, 24)	$C_{18}H_{22}CaN_2O_5 \cdot 3/2H_2O$	52.29 (52.24	6.09 6.27	6.78 6.60)
17[a]	CH_2Ph	H	H	263—265 (dec.)	+30.3 (1, DMSO, 24)	$C_{21}H_{20}CaN_2O_5 \cdot 9/4H_2O$	54.71 (54.78	5.36 5.24	6.08 6.00)
18[b]	iso-Pr	H	Me	Amorphous	−0.4 (1, DMSO, 24)	$C_{36}H_{46}CaN_4O_{10}$	58.84 (58.75	6.31 6.57	7.62 7.44)
19[b]	Et	Et	H	157—161 (dec.)	+11.7 (1, MeOH, 23)	$C_{36}H_{46}CaN_4O_{10} \cdot 1/2H_2O$	58.12 (58.10	6.37 6.43	7.53 7.54)
20[b]	Et	Bu	H	135—136 (dec.)	+11.0 (1, MeOH, 23)	$C_{40}H_{54}CaN_4O_{10}$	60.74 (60.76	6.88 6.98	7.08 7.15)
21[b]	iso-Pr	Bu	H	91—94 (dec.)	+0.5 (1, MeOH, 23)	$C_{38}H_{50}CaN_4O_{10} \cdot 5H_2O$	55.49 (55.34	7.54 7.18	6.16 6.24)

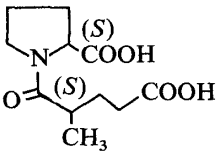
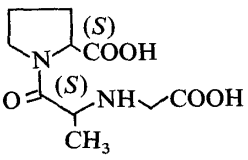
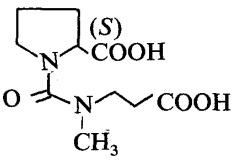
a) Calcium salt. *b*) 1/2 Calcium salt.

termined using ACE obtained from pig renal cortex, with hippuryl-histidyl-leucine as a substrate.

Table II shows the IC_{50} value of compound **1** in comparison with those of SQ-14102 and Merck's *N*-carboxymethyl-L-alanyl-L-proline. In the latter compounds, the chiral carbon atom in the side chain should possess the *S*-configuration, the reverse *R*-configuration reducing the activity markedly.[2b] On the other hand, in compound **1** the replacement of the chiral carbon atom with the achiral nitrogen atom did not lower the activity, but rather increased the inhibitory potency. The difference of the activities observed here presumably reflects the relative hydrogen bond acceptor strength of the ureido carbonyl and the amide carbonyl, because the compounds have the same binding sites.

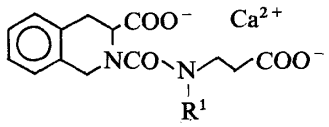
Table III shows the IC_{50} values of the tetrahydroisoquinoline compounds (V) containing various substituents at the ureido nitrogen. The enhanced inhibitory activities observed here can be explained in terms of cumulative binding interactions of the hydrophobic tetrahydroisoquinoline moiety and of the ureido carbonyl to the active sites of the enzyme. Among the *N*-substituents, the inhibitory activity was in the order Me < Et > iso-Pr > Pr > Bu > CH_2Ph, and compounds **13** (*N*-ethyl) and **14** (*N*-isopropyl) exhibited IC_{50} values of 1.1×10^{-8}

TABLE II. Comparison of *in Vitro* ACE Inhibitory Activities of Proline Analogs

Inhibitor	SQ-14102	*N*-Carboxymethyl-L-Ala-L-Pro	Compound **1**
Structure			
IC_{50} (M)[a]	4.9×10^{-6} [b]	2.4×10^{-6} [c]	7.9×10^{-7}

a) Molar concentration for 50% inhibition.
b) Value is from reference 2*b*; rabbit lung ACE, Hip-His-Leu substrate.
c) Value is from reference 5*a*; hog plasma ACE, Cbz-Phe-His-Leu substrate.

TABLE III. *In Vitro* ACE Inhibitory Activities of the Dicarboxylic Acids (V)



Compd. No.	R^1	IC_{50} (M)
12	Me	5.1×10^{-7}
13	Et	1.1×10^{-8}
14	iso-Pr	7.7×10^{-8}
15	Pr	5.5×10^{-7}
16	Bu	9.2×10^{-7}
17	CH_2Ph	2.2×10^{-6}

TABLE IV. *In Vivo* ACE Inhibitory Activities of the Dicarboxylic Acids (V)

Compd. No.	% Inhibition[a]		
	i.v. (mg/kg)	*p.o.* (mg/kg)	*i.d.* (mg/kg)
13	78% (0.1)	32% (1.0)	60% (0.3)
14	70% (0.1)	15% (1.0)	

a) Per cent inhibition of pressor responses to angiotensin I.

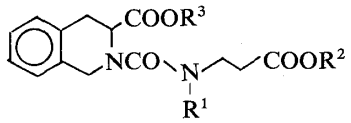
and 7.7×10^{-8} M, respectively.

The *in vivo* ACE inhibitory activities of **13** and **14** in normotensive rats are listed in Table IV. These two compounds produced marked inhibition of the pressor responses to angiotensin I at an intravenous dose of 0.1 mg/kg. The compounds showed only weak inhibition after oral administration at a dose of 1 mg/kg, but compound **13** showed somewhat greater activity after intraduodenal administration.

In Vivo ACE Inhibitory Activities of Ester Derivatives (IV, VI, and VII)

In vivo ACE inhibitory activities of the ester derivatives (**3**, **6**, **18**—**21**) are listed in Table

TABLE V. *In Vivo* ACE Inhibitory Activities of the Ester Derivatives (IV, VI and VII)



Compd. No.	R^1	R^2	R^3	% Inhibition[b]	
				i.v. (mg/kg)	*p.o.* (mg/kg)
3	Et	Me	Me	20% (0.1)	
6	iso-Pr	Me	Me	20% (0.1)	27% (1.0)
18[a]	iso-Pr	H	Me	25% (0.1)	21% (1.0)
19[a]	Et	Et	H	74% (0.1)	71% (1.0)
20[a]	Et	Bu	H	80% (0.1)	82% (1.0)
21[a]	iso-Pr	Bu	H	77% (0.1)	58% (1.0)

a) 1/2 Calcium salt.
b) Per cent inhibition of pressor responses to angiotensin I.

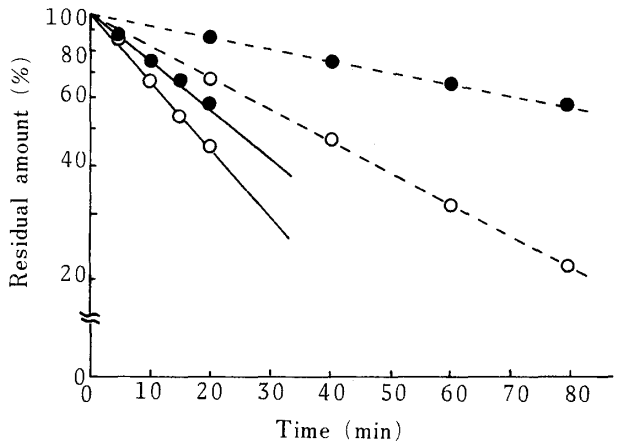


Fig. 1. First Order Plot for the Degradation of Compounds **13** and **20** in MeOH–McIlvaine Buffer (1 : 4) Solution at 37 °C

○, **13**; ●, **20**. ——, pH 2.23; ----, pH 4.37.

V. Of these esters, the diester compounds (**3** and **6**) and the monoester (**18**) at the 3-position of tetrahydroisoquinoline were found to be less active than the corresponding parent acids (**13** and **14**) when given intravenously, and showed only weak inhibition after oral administration. These results suggested that the ester group at the 3-position of tetrahydroisoquinoline was poorly hydrolyzed by esterase. On the other hand, the other monoester compounds (**19**—**21**) having the ester group at the side chain showed almost the same activities as the corresponding parent acids (**13** and **14**) on intravenous administration. Moreover, they were found to be orally active; in particular, compounds **19** and **20** inhibited the pressor responses to angiotensin I by up to 71 and 82%, respectively, at an oral dose of 1.0 mg/kg.

The difference in activities between the monoesters (**19**—**21**) and their parent dicarboxylic acids (**13** and **14**) on oral administration may be attributable to differences in the stability in digestive fluids and the absorbability from the gastro-intestinal tract.

To obtain some information on the degradation mechanism of the monoester **20** and the parent acid **13** during oral administration, the chemical stability of these compounds under solubilized conditions was investigated. When **13** and **20** were dissolved in acidic solution, both were decomposed spontaneously to 3-ethylaminopropionic acid or its butyl ester, and 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid with liberation of carbon dioxide at room temperature. In a methanol–McIlvaine buffer (1 : 4) solution at 37 °C, the degradation rates of

the two compounds (**13** and **20**) were obtained by the periodic determination of the residual amounts by high-performance liquid chromatography (HPLC). Semilog plots of the degradation of **13** and **20** *versus* time are shown in Fig. 1, in which the rates follow pseudo-first order kinetics. The results clearly show that in acidic solution the monoester (**20**) is more stable than the dicarboxylic acid (**13**).

As has been described above, the monoester (**20**) was found to be the most potent and orally active ACE inhibitor among the compounds tested in the present study. Since the effectiveness of the carbamoyl group in ACE inhibitors has been proved, the design of further analogs is in progress.

Experimental

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

Starting Materials——Methyl and benzyl (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylates (III) were prepared from L-phenylalanine by the usual method.[8a,b]

3-Alkylaminopropionates (II) were prepared by addition of primary amines to acrylic esters (I) according to the general procedure.[9a,b] Boiling points of new compounds are as follows. Butyl 3-ethylaminopropionate: bp 115 °C/33 mmHg. Methyl 3-isopropylaminopropionate: bp 80—81 °C/29 mmHg. Butyl 3-isopropylaminopropionate: bp 126—127 °C/33 mmHg. Benzyl 3-isopropylaminopropionate: bp 104 °C/2 mmHg. Benzyl 3-butylaminopropionate: bp 142 °C/5 mmHg. Benzyl 3-benzylaminopropionate: bp 140 °C/2 mmHg.

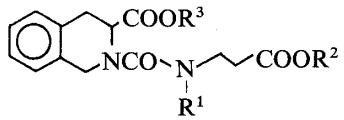
Benzyl 1-[*N*-Methyl-*N*-(2-benzyloxycarbonylethyl)carbamoyl]-L-prolinate——A solution of benzyl 3-methylaminopropionate (1.74 g, 9 mmol) and triethylamine (1.1 g, 10.8 mmol) in CH_2Cl_2 (20 ml) was added dropwise to a solution of phosgene (1.2 g, 12 mmol) in CH_2Cl_2 (20 ml) with stirring at −30 °C. The mixture was further stirred at the same temperature for 30 min and concentrated to dryness *in vacuo*. The residue (benzyl *N*-chlorocarbonyl-3-methylaminopropionate) was dissolved in CH_2Cl_2 (20 ml) and a suspension of benzyl L-prolinate hydrochloride (2.2 g, 9 mmol) and triethylamine (2.1 g, 20.7 mmol) in CH_2Cl_2 (20 ml) was added to the above solution. The mixture was stirred at room temperature overnight and concentrated *in vacuo*. The residue was diluted with AcOEt and the mixture was washed successively with dilute hydrochloric acid, water, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The residue thus obtained was purified by column chromatography on silica gel with toluene–AcOEt (3 : 1) as an eluent to give the title compound (2.3 g, 60.2%) as a colorless syrup. IR ν_{max}^{film} cm^{-1}: 1735, 1635. ^{1}H NMR ($CDCl_3$) δ: 1.60—2.20 (4H, m, CH_2CH_2CH), 2.59 (2H, t, J=7 Hz, CH_2COO), 2.81 (3H, s, NCH_3), 3.20—3.70 (4H, m, CH_3NCH_2, $CONCH_2$), 4.52 (1H, t, J=6 Hz, NCH), 5.08 (4H, s, $CH_2Ph \times 2$), 7.28 (10H, s, aromatic H). MS *m/e*: 424 (M^+).

1-[*N*-Methyl-*N*-(2-carboxyethyl)carbamoyl]-L-proline (1)——A mixture of benzyl 1-[*N*-methyl-*N*-(2-benzyloxycarbonylethyl)carbamoyl-L-prolinate (1.0 g, 2.36 mmol), $Ca(OH)_2$ (170 mg), and palladium-black (40 mg) in 50% aqueous EtOH (30 ml) was stirred under a stream of hydrogen for 3 h at room temperature. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. After addition of EtOH, the resulting crystalline precipitate was collected by filtration, washed with EtOH, and dried to give **1** calcium salt (0.62 g, 84.2%) as colorless needles, mp > 279 °C, $[\alpha]_D^{24}$ +10.4° (c=1, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3300, 1590. *Anal.* Calcd for $C_{10}H_{14}CaN_2O_5 \cdot 5/3H_2O$: C, 38.42; H, 5.67; N, 8.96. Found: C, 38.70; H, 5.74; N, 8.65.

Typical Procedure for the Preparation of (3*S*)-2-[*N*-Substituted *N*-(2-Alkoxycarbonylethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylates (2—11)——(a) Route A: A solution of benzyl (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (5.35 g, 20 mmol) and triethylamine (2.63 g, 26 mmol) in CH_2Cl_2 (10 ml) was added to a solution of butyl *N*-chlorocarbonyl-3-ethylaminopropionate [prepared from butyl 3-ethylaminopropionate (3.22 g, 20 mmol) and triethylamine (2.63 g, 26 mmol), phosgene (2.6 g, 26 mmol), and CH_2Cl_2 (20 ml) in the same manner as described for the preparation of benzyl 1-[*N*-methyl-*N*-(2-benzyloxycarbonylethyl)carbamoyl]-L-prolinate] in CH_2Cl_2 (20 ml). The mixture was stirred at room temperature overnight and concentrated *in vacuo*. The residue was diluted with AcOEt and the mixture was washed successively with dilute hydrochloric acid, water, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The residue thus obtained was purified by column chromatography on silica gel with toluene–AcOEt (8 : 1) as an eluent to give benzyl (3*S*)-2-[*N*-ethyl-*N*-(2-butyloxycarbonylethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**5**) (7.9 g, 84.7%) as a colorless viscous oil.

(b) Route B: A solution of methyl (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (3.82 g, 20 mmol) and triethylamine (2.8 g, 28 mmol) in CH_2Cl_2 (20 ml) was added dropwise to a solution of phosgene (2.8 g, 28 mmol) in CH_2Cl_2 (20 ml) with stirring at −30 °C. After being stirred at the same temperature for 30 min, the mixture was

TABLE VI. Yield and Physical Data for IV



Compd. No.	R^1	R^2	R^3	Prepn. route	Yield (%)	mp (°C)	MS (M^+)	IR ν_{max}^{film} cm^{-1}
2	Me	Me	Me	B	57.2	Oil	334	1740, 1640
3	Et	Me	Me	B	71.8	53—55	348	1738, 1725, 1635[a]
4	Et	Et	CH_2Ph	A	70.6	Oil	438	1735, 1640
5	Et	Bu	CH_2Ph	A	84.7	Oil	466	1730, 1640
6	iso-Pr	Me	Me	B	70.8	78—79	362	1735, 1640[a]
7	iso-Pr	CH_2Ph	Me	A	50.9	Oil	438	1740, 1640
8	iso-Pr	Bu	CH_2Ph	B	47.9	Oil	480	1735, 1640
9	Pr	Me	Me	B	71.7	Oil	362	1740, 1640
10	Bu	CH_2Ph	CH_2Ph	A	68.0	Oil	528	1730, 1640
11	CH_2Ph	CH_2Ph	CH_2Ph	A	65.5	Oil	562	1730, 1640

a) Taken in Nujol.

Compd. No.	^1H NMR ($CDCl_3$) δ
2	2.59 (2H, t, J=7 Hz), 2.93 (3H, s), 3.17 (2H, d, J=6 Hz), 3.45 (2H, t, J=7 Hz), 3.63 (6H, s), 4.50 (2H, s), 4.76 (1H, t, J=6 Hz), 7.05—7.20 (4H, m).
3	1.20 (3H, t, J=7 Hz), 2.57 (2H, t, J=7 Hz), 3.09—3.50 (4H, m), 3.19 (2H, d, J=6 Hz), 3.64 (3H, s), 3.66 (3H, s), 4.52 (2H, s), 4.80 (1H, t, J=6 Hz), 7.0—7.20 (4H, m).
4	1.17 (3H, t, J=7 Hz), 1.21 (3H, t, J=7 Hz), 2.53 (2H, t, J=7 Hz), 3.0—3.80 (6H, m), 4.08 (2H, q, J=7 Hz), 4.53 (2H, s), 4.89 (1H, t, J=6 Hz), 5.10 (2H, s), 6.90—7.40 (m, 9H).
5	0.89 (3H, t, J=6 Hz), 1.17 (3H, t, J=7 Hz), 1.0—1.80 (4H, m), 2.46 (2H, t, J=7 Hz), 3.0—3.80 (6H, m), 4.06 (2H, t, J=7 Hz), 4.57 (2H, s), 4.93 (1H, t, J=6 Hz), 5.14 (2H, s), 6.90—7.50 (9H, m).
6	1.16 (3H, d, J=7 Hz), 1.26 (3H, d, J=7 Hz), 2.48 (2H, t, J=7 Hz), 3.20 (2H, d, J=6 Hz), 3.27 (2H, t, J=7 Hz), 3.50—4.10 (1H, m), 3.59 (3H, s), 3.66 (3H, s), 4.52 (2H, s), 4.79 (1H, t; J=6 Hz), 7.0—7.20 (4H, m).
7	1.16 (3H, d, J=7 Hz), 1.25 (3H, d, J=7 Hz), 2.55 (2H, t, J=7 Hz), 3.18 (2H, d, J=6 Hz), 3.32 (2H, t, J=7 Hz), 3.50—4.0 (1H, m), 3.63 (3H, s), 4.50 (3H, s), 4.78 (1H, t, J=6 Hz), 5.04 (2H, s), 6.90—7.35 (9H, m).
8	0.89 (3H, t, J=6 Hz), 1.10 (3H, d, J=7 Hz), 1.22 (3H, d, J=7 Hz), 1.10—1.70 (4H, m), 2.46 (2H, t, J=7 Hz), 3.22 (2H, d, J=6 Hz), 3.27 (2H, t, J=7 Hz), 3.48—4.0 (1H, m), 3.98 (2H, t, J=7 Hz), 4.52 (2H, s), 4.92 (1H, t, J=6 Hz), 5.09 (2H, s), 6.90—7.40 (9H, m).
9	0.90 (3H, t, J=6 Hz), 1.30—2.0 (2H, m), 2.59 (2H, t, J=7 Hz), 3.14 (2H, d, J=6 Hz), 3.20—3.60 (4H, m), 3.65 (3H, s), 3.68 (3H, s), 4.53 (2H, s), 4.80 (1H, t, J=6 Hz), 7.10—7.20 (4H, m).
10	0.89 (3H, t, J=6 Hz), 1.05—1.70 (4H, m), 2.62 (2H, t, J=7 Hz), 3.22 (2H, d, J=6 Hz), 3.10—3.70 (4H, m), 4.52 (2H, s), 4.86 (1H, t, J=6 Hz), 5.09 (4H, s), 7.0—7.40 (10H, m).
11	2.59 (2H, t, J=7 Hz), 3.20 (2H, d, J=6 Hz), 3.25—3.60 (2H, m), 4.36—4.47 (2H, m), 4.50 (2H, s), 4.85 (1H, t, J=6 Hz), 5.01 (2H, s), 5.05 (2H, s), 7.0—7.40 (15H, m).

concentrated to dryness *in vacuo*. The residue was dissolved in CH_2Cl_2 (20 ml), and a solution of methyl 3-ethylaminopropionate (2.62 g, 20 mmol) and triethylamine (2.42 g, 24 mmol) in CH_2Cl_2 (10 ml) was added to the above solution at room temperature. The mixture was stirred at room temperature overnight and worked up as described for the preparation of **5**. The crude product was chromatographed on a silica gel column using toluene–AcOEt (3 : 1) as an eluent to give methyl (3*S*)-2-[*N*-ethyl-*N*-(2-methoxycarbonylethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**3**) (5.0 g, 71.8%) as colorless crystals, which were recrystallized from a mixture of AcOEt and hexane to give colorless prisms.

Other diester derivatives (**2**, **4**, **6**—**11**) were prepared in the same manner, and the yields and physical data are summarized in Table VI.

Typical Procedure for the Preparation of (3*S*)-2-[*N*-Substituted *N*-(2-carboxyethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acids (12—17)——(a) Alkaline Hydrolysis: A solution of KOH (1.2 g) in water (10 ml) was added to a solution of **3** (2.1 g, 6 mmol) in MeOH (10 ml), and the mixture was stirred at room temperature for 2 h. The mixture was acidified with dilute hydrochloric acid and extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$, and then evaporated *in vacuo*. Calcium hydroxide (430 mg) was added to a solution of the above residue in 50% aqueous EtOH (30 ml), and the mixture was stirred at room temperature for 40 min. The reaction mixture was concentrated *in vacuo*, and the resulting crystalline precipitate was triturated with EtOH, collected by filtration, and dried to give (3*S*)-2-[*N*-ethyl-*N*-(2-carboxyethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**13**) calcium salt (2.5 g, 66.4%). IR ν_{max}^{KBr} cm^{-1}: 3400, 1600, 1570.

(b) Catalytic Hydrogenation: The hydrogenation of **10** (900 mg, 1.7 mmol) was carried out according to the procedure described for the preparation of **1** to give (3*S*)-2-[*N*-butyl-*N*-(2-carboxyethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**16**) calcium salt as colorless crystals (490 mg, 69.6%). IR ν_{max}^{KBr} cm^{-1}: 3400, 1580.

Other dicarboxylic acids (**12**, **14**, **19**, **21**) were prepared similarly, and their physical and analytical data are summarized in Table I.

Typical Procedure for the Preparation of (3*S*)-2-[*N*-Substituted *N*-(2-alkoxycarbonylethyl)carbamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acids (18—21)——A mixture of **5** (5.0 g, 10.7 mmol) and palladium-black (90 mg) in MeOH (50 ml) was stirred under a stream of hydrogen for 2 h at room temperature. After the catalyst had been filtered off, $Ca(OH)_2$ (385 mg) and water (10 ml) were added to the filtrate. The mixture was stirred for 30 min at room temperature, and the insoluble materials were filtered off. The filtrate was concentrated *in vacuo*, and the resulting syrup was crystallized from a small amount of water under ice-cooling. The crystals were collected by filtration, recrystallized from aqueous EtOH, and dried to give (3*S*)-2-[*N*-ethyl-*N*-(2-butoxycarbonylethyl)carmamoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**20**) as colorless prisms (2.83 g, 66.9%). IR ν_{max}^{KBr} cm^{-1}: 3400, 1730, 1610, 1580.

Other monoester derivatives (**18**, **19**, **21**) were obtained similarly, and the physical constants and analytical data are summarized in Table I.

Biological Methods——The *in vitro* ACE inhibitory activity was determined according to the procedure reported previously.[7]

The *in vivo* ACE inhibitory activity was investigated by the following methods. Male Wistar rats weighing 300 to 400 g were anesthetized with urethane (1.5 g/kg, *s.c.*). The right carotid artery and right femoral vein were canulated for the recording of arterial pressure and for the injection of angiotensin I (300 ng/kg), respectively. The pressor responses to angiotensin I were measured before and after administration (*i.v.*, *p.o.*, or *i.d.*) of the test compounds.

Degradation of 13 or 20 in Acidic Solution (Kinetic Measurements)——Solutions of **13** calcium salt (5×10^{-3} M) and **20** calcium salt (2.5×10^{-3} M) in MeOH–McIlvaine buffer (1 : 4) solution (pH 2.23 or 4.37) were prepared. The solution was maintained at 37 °C in a circulating water bath. At appropriate time intervals, aliquots were pipetted out, and their pH was adjusted to 7.5 to quench the reaction by addition of aqueous Na_2HPO_4. The samples were assayed for **13** or **20** by HPLC.

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References and Notes

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