Studies on Angiotensin Converting Enzyme Inhibitors. VI.1) Synthesis and Angiotensin Converting Enzyme Inhibitory Activities of theDicarboxylic Acid Derivative of Imidapril and Its Diastereoisomers2)

Hitoshi KUBOTA, a Ken-ichi NUNAMI, a,d Kimiaki HAYASHI, a Yoshikatsu HASHIMOTO, a Noriko OGIKU, b Yuzo MATSUOKA, b and Ryuichi ISHIDA b

Research Laboratory of Applied Biochemistry a and Biological Research Laboratory, b Tanabe Seiyaku Co., Ltd., 16-89 Kashima-3-chome, Yodogawa-ku, Osaka 532, Japan. Received November 18, 1991

All possible diastereoisomers of the dicarboxylic acid (10a), the biologically active form of imidapril (1), were synthesized, and their inhibitory activity against angiotensin converting enzyme (ACE) was examined. The in vitro ACE inhibitory activity of these compounds greatly depended on the configurations of the three asymmetric carbons in each molecule. The (S,S,S) isomer (10a) showed much more potent activity than the others.

**Keywords** ACE inhibitor; imidapril; enalapril; biologically active form

Angiotensin converting enzyme (ACE) inhibitors such as enalapril 5) have recently received attention as effective drugs for hypertension. Most of them have three asymmetric carbons, with each carbons being having an S configuration. Although the configurations of the three asymmetric carbons would greatly contribute to the inhibitory activity, the structure–activity relationships of all possible diastereoisomers have not yet been systematically studied.

In the series of our synthetic studies on ACE inhibitors, we previously reported that imidapril (1), 4(S)-3-{[2(S)-2-[N-[(1S)-1-ethoxycarbonyl-3-phenylpropyl]amino]-propionyl]-1-methyl-2-oximidazolidine-4-carboxylic acid, is a potent and long-lasting antihypertensive drug for oral use. 4) The biologically active form of imidapril is known to be a dicarboxylic acid derivative (10a). In this paper, we wish to report the synthesis and ACE inhibitory activity of all possible diastereoisomers of the biologically active form of imidapril.

**Chemistry** The requisite chiral building blocks for the synthesis of the diastereoisomers of the biologically active form of imidapril were synthesized as shown in Chart 1. The 2-oximidazolidine derivatives (2a, b) were prepared from L- or D-asparagine according to the reported method. 4) The propionic acid derivatives (3a, b) were prepared from L- or D-lactate, 1) and subsequently converted to the requisite acid chlorides (4a, b). Both (2S) and (2R)-2-amino-4-phenylbutyric acid esters (7a, b) were enzymatically prepared from N-acetyl-2-amino-4-phenylbutyric acid (5). Thus, enantioselective decylation of 5 by using aminocyanase originated from *Aspergillus oryzae* afforded the L-amino acid (6a), while the recovered D-acetyl derivative (5b) was chemically decylated to give the D-amino acid (6b). Each amino acid was then converted to the corresponding benzyl ester by the usual method.

By using the above three chiral building blocks, all possible diastereoisomers (10a–h) of the biologically active form of imidapril were synthesized (Chart 2). Thus,
**Table I. Physical Properties and in Vitro ACE Inhibitory Activities of the Dicarboxylic Acids (10a–h)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(±), (±), (±)</th>
<th>Yield (%) (from 9)</th>
<th>mp (°C)</th>
<th>[α]_D^25 (c = 0.5, solv.)*</th>
<th>Formula</th>
<th>IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>(S, S, S)</td>
<td>81</td>
<td>239–241</td>
<td>-90.4 (A)</td>
<td>C_{11}H_{13}N_2O_7</td>
<td>1.8 × 10^{-9}</td>
</tr>
<tr>
<td>10b</td>
<td>(S, S, R)</td>
<td>75</td>
<td>197–198</td>
<td>-112.0 (A)</td>
<td>C_{11}H_{13}N_2O_7</td>
<td>9.6 × 10^{-8}</td>
</tr>
<tr>
<td>10c</td>
<td>(S, R, S)</td>
<td>60</td>
<td>157–159</td>
<td>-33.2 (A)</td>
<td>C_{11}H_{13}N_2O_7</td>
<td>2.3 × 10^{-3}</td>
</tr>
<tr>
<td>10d</td>
<td>(S, R, R)</td>
<td>58</td>
<td>Amorphous powder</td>
<td>-53.2 (B)</td>
<td>C_{11}H_{13}N_2O_7·HCl</td>
<td>2.2 × 10^{-4}</td>
</tr>
<tr>
<td>10e</td>
<td>(R, S, S)</td>
<td>60</td>
<td>Amorphous powder</td>
<td>+52.0 (B)</td>
<td>C_{11}H_{13}N_2O_7·HCl</td>
<td>4.9 × 10^{-5}</td>
</tr>
<tr>
<td>10f</td>
<td>(R, S, R)</td>
<td>65</td>
<td>158–160</td>
<td>+52.8 (A)</td>
<td>C_{11}H_{13}N_2O_7</td>
<td>6.6 × 10^{-5}</td>
</tr>
<tr>
<td>10g</td>
<td>(R, R, S)</td>
<td>72</td>
<td>212–213</td>
<td>+112.5 (A)</td>
<td>C_{11}H_{13}N_2O_7</td>
<td>7.2 × 10^{-4}</td>
</tr>
<tr>
<td>10h</td>
<td>(R, R, R)</td>
<td>84</td>
<td>239–240</td>
<td>+89.5 (A)</td>
<td>C_{11}H_{13}N_2O_7</td>
<td>&gt; 10^{-3}</td>
</tr>
</tbody>
</table>

Enalaprilat

* a) Solvent: A, 5% NaHCO_3; B, MeOH. b) All compounds exhibited satisfactory C, H, and N elemental analyses. c) Hydrochloride.

**Biological Results** The *in vitro* ACE inhibitory activity of the dicarboxylic acid derivatives (10) was examined in the presence of hippurylhistidylleucine as a substrate using ACE originated from swine kidney cortex. The results are summarized in Table I. The inhibitory activity of (S,R,S)-10c and (R,S,S)-10e was 10^4 times less potent than that of (S,S,S)-10a, which was comparable to the activity of enalaprilat. Accordingly, both carbons *±1 and *±2 should be S configurations for exhibiting the inhibitory activity. On the other hand, the inhibitory activity of (S,S,R)-10b was only about 50 times less potent than that of 10a, indicating that the configuration of the carbon *±3 plays a less important role than carbon *±1 and *±2. From the above results, it is concluded that the (S,S,S) isomer showed much more potent activity than the others. Furthermore, these results will give useful information for analysis of the tertiary structure of the active site of ACE.

**Experimental**

Melting points (mp) were measured by the use of a Yamato MP-21 melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 1640 spectrophotometer. Proton nuclear magnetic resonance (1H-NMR) spectra were recorded on a Bruker AC-200 instrument, using tetramethylsilane as an internal standard. Mass spectra (MS) were taken on a Hitachi M-200A mass spectrometer. Specific rotations were measured with a Perkin-Elmer 243 polarimeter. High-performance liquid chromatography (HPLC) was done on a Shimadzu LC-6A instrument. For silica gel column chromatography, Kieselgel 60 (0.040–0.063 mm, E. Merck) was employed.

The synthetic procedure of 10a is given below as an example. The other compounds were prepared according to this procedure from appropriate starting materials, and yields and physical properties are described.

(2S,2-Amino-4-phenylbutyric Acid (6a) and (2R)-2-Amino-4-phenylbutyric Acid (6b) Aminoacylase (1295 mg) obtained from *Aspergillus oryzae* was added to a suspension of 5 (68.1 g, 308 mmol) in water (1800 ml) containing CoCl_2·6H_2O (305 mg). The mixture was adjusted to pH 9.0 with aqueous NaOH and incubated at 37 °C for 24 h. The crystalline precipitates were collected by filtration and washed with water and with MeOH to afford 6a (20.9 g, 38%) as colorless leaflets. mp > 240 °C. [α]_D^25 + 47.5° (c = 1, 1 N HCl). IR (KBr): 3400, 1655, 1625, 1582 cm⁻¹. 1H-NMR (D_2O + TFA) δ: 2.10–2.45 (2H, m), 2.68–2.97 (2H, m), 4.10 (1H, t, J = 6.3 Hz), 7.20–7.48 (5H, m). MS m/z: 179 (M⁺). Anal. Calcd for C_{11}H_{13}NO_2: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.08; H, 7.26; N, 7.77.
The filtrate after isolation of crystals (6a) was acidified with concentrated 
HCl, and extracted with AcOEt. The extract was washed with 
brine, dried over MgSO₄, and concentrated in vacuo. The resulting 
residue was recrystallized from AcOEt-MeOH to give 6b (25.3 g, 37%) as 
colourless needles. mp 170—172°C.

A mixture of 5b (20.0 g, 90.4 mmol) and 6H (300 ml) was 
heated under reflux overnight. After cooling, the mixture was concentrated 
to a half of its original volume, and the resulting mixture was neutralized with 
concentrated NaOH. The crystalline mass was collected by filtration 
and washed with water and with MeOH to afford 6b (88.7 g, 96%) as 
colourless leaflets. mp >240°C. [x]D 325° = 46.0° (c = 1, 1HCl).

Benzyl (2S)-2-Amino-4-phenylbutyrate (7a) A mixture of 6a (20.5 g, 
114 mmol), benzyl alcohol (175 ml), p-toluenesulfonic acid monohydrate 
(28.0 g) and toluene (175 ml) was heated under reflux for 4h with a 
Dean-Stark apparatus. The crude product was recrystallized from 
thf and with isopropanol to afford 7a p-toluenesulfonic acid (40.7 g, 79%) 
as colourless needles. mp 139—140°C. IR (KBr): 1749, 1600 cm⁻¹.

1H-NMR (DMSO-d₆) δ: 1.95—2.18 (2H, m), 2.29 (3H, s), 2.43—2.81 
(2H, m), 4.13 (1H, t, J = 6Hz), 5.22, 5.28 (2H, ABq, J = 12Hz), 
7.05—7.56 (4H, m), 8.46 (3H, s). MS m/z: C63 (M⁺, 26%). Anal. Caled. 
C₁₄H₁₂N₂O₂C₂H₅O₂S₄; C 56.59; H 6.17; N 3.15. Compound 7a 
p-toluenesulfonic acid dissolves in DMSO was neutralized with saturated 
aqueous NaHCO₃ and extracted with AcOEt. The extract was washed 
with brine, dried over MgSO₄, and evaporated to dryness. The crude 
crystalline residue was triturated with acetone and recrystallized from 
AcOEt. The resulting crystals were recrystallized from DMSO to afford 10a (0.89%, 81%) as colourless needles. IR (KBr): 3470, 1745, 1730, 1695 cm⁻¹. 1H-NMR (DMSO-d₆) δ: 1.25 
(3H, d, J = 7Hz), 1.70—1.95 (2H, m), 2.55—2.73 (2H, m), 2.76 (3H, s), 
3.15 (1H, t, J = 6Hz). MS m/z: 370 (M⁺, 77%). Anal. Caled. C₁₇H₁₆N₂O₄ 
C 65.39; H 6.14; N 11.13. Found: C 65.69; H 6.09; N 11.03.

10b: IR (KBr): 3450, 3200, 1745, 1730, 1690, 1635 cm⁻¹. 1H-NMR 
(DMSO-d₆) δ: 1.19 (3H, d, J = 7Hz), 1.70—1.95 (2H, m), 2.43—2.73 
(2H, m), 3.07 (3H, s), 3.07 (1H, d, J = 6Hz). 3.37 (1H, dd, J = 4, 10Hz), 
3.70 (1H, t, J = 10Hz), 4.53—4.71 (2H, m), 7.12—7.34 (5H, m). MS 
m/z: 377 (M⁺, 77%).

In Vitro ACE Inhibitory Activity The partially purified ACE was 
prepared from swine kidney cortex by the method of Oshima et al.³¹ ACE 
inhibitory activity was determined by the following methods. The 
reaction mixture contained 1 mm hippuryllhistidylleucine, 50 mm NaCl, 50 mm 
Tris-Cl (pH 7.4), 6 mg enzyme protein (specific activity: 14.2 mmol 
hippuric acid/mg protein/h) and each final concentration of the samples. 
Incubation was carried out for 20 min at 37°C. The reaction was 
stopped by adding ice cold methanol. After centrifugation of these solutions 
for 5min at 3000 rpm, the supernatant was filtrated through a membrane 
filter (pore size: 0.45 mm). The liberated hippuric acid was measured by 
the HPLC technique. Column: Nucleosil 5C₁₈ 4.0 x 200 mm (Chromco), 
column temperature: 40°C, mobile phase: 0.1M K₂HPO₄—H₃PO₄ (pH 
3.0): CH₂CN—80:20, flow rate: 1.0 ml/min, detection wave length: 
228 nm, detection sensitivity: 0.04 AUS, injection volume: 10 μl.
Activity was designated in terms of the IC₅₀, which was the molar 
concentration of the test inhibitor causing 50% inhibition of the control 
converting enzyme activity.

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References and Notes
1) Part V: H. Kubota, K. Nunami, M. Yamagishi, S. Nishimoto, and 
2) A part of this work was presented at the 111th Annual Meeting of

