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SYNTHESIS OF SIX ANALOGUES AND THEIR TWO FRAGMENTS RELATED TO L-α-HYDROXYISOVALERYL-Leu-Val-Phe-OMe AS RENIN INHIBITOR*

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SUMMARY

Six analogues of Hyd-Leu-Val-Phe-OMe as renin inhibitor, in which the Phe residue was replaced respectively by Phe(NO_2), Tyr(Me) and Phe(NH_2), and L-α-hydroxyisovaleric acid was replaced by L-α-lactic acid, and their two fragments, H-Leu-Val-Tyr(Me)-OMe and H-Leu-Val-Phe(NO_2)-OMe, were synthesized and their renin-inhibitory activities also described. One of these analogues, Hyd-Leu-Val-Tyr(Me)-OMe was more potent than that of Hyd-Leu-Val-Phe-OMe. However, all of the other analogues showed diminished biological activity compared to synthetic Hyd-Leu-Val-Phe-OMe. Unfortunately, their two fragments could not be tested for the renin-inhibitory activity because of almost insoluble in aqueous solvent.

INTRODUCTION

Renin, primarily from kidney, hydrolyzes angiotensinogen in plasma to produce a decapeptide, angiotensin I (1). The renin-angiotensin system plays an important role in the regulation of blood pressure and blood volume in both physiological and pathophysiological state (2,3). Recently, new types of renin inhibitors (L-α-hydroxyalkanoyl-derivatives of Leu-Val-Phe-OMe) have been reported by Johnson (4). In this report, it described to us that the two most active compounds of the series were L-α-hydroxy-

* Symbols for amino acid derivatives and peptides are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: (1972) Biochem. J. 126, 773. Other abbreviations: WSCI, water-soluble carbodiimide; DMF, dimethylformamide; TFA, trifluoroacetic acid; HOBT, N-hydroxybenzotriazole; Et_3N, triethylamine; Hyd, L-α-hydroxyisovaleric acid; Lac, L-α-lactic acid; Tyr(Me), o-methytyrosine; Phe(NO_2), p-nitrophenylalanine; Phe(NH_2), p-aminophenylalanine; DCC, dicyclohexylcarbodiimide; EDTA, ethylenediamine tetraacetic acid.

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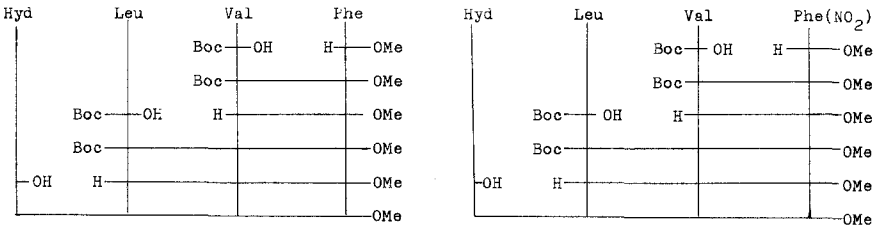

Fig. 1. Synthetic Scheme for Hyd-Leu-Val-Phe-OMe and Hyd-Leu-Val-Phe(NO_2)-OMe

isocaproyl-Leu-Val-Phe-OMe and L-α-hydroisovaleryl-Leu-Val-Phe-OMe. The present paper describes further synthetic studies to obtain additional knowledge on the structure-activity relationship on the renin-inhibitory activity. Chemically, these two active derivatives (4) have phenyl group in common. In conjunction with this point, it occured to us that substitution of Phe with Phe(NO_2), Tyr(Me) and Phe(NH_2) might invoke more active than that of Hyd-Leu-Val-Phe-OMe. In addition, dipole moments created by substituent group and the stereochemical structure of the substituted group. We have synthesized this biologically active renin inhibitor (Hyd-Leu-Val-Phe-OMe), and the following six analogues and two fragments: Hyd-Leu-Val-Phe(NO_2)-OMe, Hyd-Leu-Val-Tyr(Me)-OMe, Hyd-Leu-Val-Phe(NH_2)-OMe, Lac-Leu-Val-Phe(NO_2)-OMe, Lac-Leu-Val-Tyr(Me)-OMe, Lac-Leu-Val-Phe(NH_2)-OMe, H-Leu-Val-Phe(NO_2)-OMe and H-Leu-Val-Tyr(Me)-OMe. Relative potencies of these synthetic compounds are also reported in this paper. The synthetic routes to Hyd-Leu-Val-Phe-OMe and Hyd-Leu-Val-Phe(NO_2)-OMe are illustrated in Fig. 1 as examples.

H-Phe-OMe HCl was condensed with Boc-Val-OH by HOBT-DCC procedure (5) to afford Boc-Val-Phe-OMe I. The protected dipeptide I was treated with TFA-anosole to remove the Boc group and the product was condensed with Boc-Leu-OH by the HOBT-DCC procedure to give Boc-Leu-Val-Phe-OMe II. The Boc group of II was similarly removed and the free base was condensed with Hyd by the HOBT-DCC procedure

Table I. Relative Potencies of six Analogues of Hyd-Leu-Val-Phe-OMe and their two Fragments on Renin-Inhibitory Activity

Compounds	Relative potency (molar basis)
Hyd-Leu-Val-Phe-OMe	1.00
Hyd-Leu-Val-Tyr(Me)-OMe	1.17
Hyd-Leu-Val-Phe(NO_2)-OMe	0.93
Hyd-Leu-Val-Phe(NH_2)-OMe	0.96
H-Leu-Val-Tyr(Me)-OMe	—[a]
H-Leu-Val-Phe(NO_2)-OMe	—[a]
Lac-Leu-Val-Tyr(Me)-OMe	0.92
Lac-Leu-Val-Phe(NO_2)-OMe	0.90
Lac-Leu-Val-Phe(NH_2)-OMe	0.80

a) could not be tested for the biological activity because of poor solubility in incubation buffer.

to give Hyd-Leu-Val-Phe-OMe III. The other four analogues, Hyd-Leu-Val-Tyr(Me)-OMe, Hyd-Leu-Val-Phe(NO_2)-OMe, Lac-Leu-Val-Tyr(Me)-OMe and Lac-Leu-Val-Phe(NO_2)-OMe and two fragments, H-Leu-Val-Tyr(Me)-OMe and H-Leu-Val-Phe(NO_2)-OMe, were prepared by essentially same approach that had been used to obtain III. Hyd-Leu-Val-Phe(NO_2)-OMe X was hydrogenated over 10% Pd-C in MeOH-10% AcOH for 36 hr to change from Phe(NO_2) residue to Phe(NH_2) residue. The Phe(NH_2) in the acid hydrolesate of this hydrogenated product XII was as expected by amino acid analysis. Lac-Leu-Val-Phe(NH_2)-OMe XV was prepared by essentially same approach that had been used to obtained XII. Compounds III-XV were all tested for their ability to inhibit porcine renin. One of these analogues, Hyd-Leu-Val-Tyr(Me)-OMe, was more potent than that of Hyd-Leu-Val-Phe-OMe (Table I). But, all of the other compounds tested were lower than that of Hyd-Leu-Val-Phe-OMe except H-Leu-Val-Tyr(Me)-OMe and H-Leu-Val-Phe(NO_2)-OMe (Table I).

These compounds, H-Leu-Val-Tyr(Me)-OMe and H-Leu-Val-Phe(NO_2)-OMe could not be tested for biological activity because of almost insoluble in water as compared with the other six analogues.

EXPERIMENTAL

All melting points are uncorrected. Rotations were determined with an Atago Polax machine. For paper chromatography, the protected peptides were deblocked with TFA and resulting amino compounds were chromatographed on a filter paper Toyo Roshi No. 51, at room temperature. Rf^1 values refer to Partridge system (6) and Rf^2 values to the system of BuOH-pyridine-AcOH-H_2O (30:20:6:24) (7). The amino acid compositions of the acid hydrolysates were determined by JEOL JLC-8AH amino acid analyzer according to the directions given by Moore et al (8). Concentration was carried out in a rotary evaporator under reduced pressure at a temperature of 35-40°. Analytical samples were dried over P_2O_5 at 50-60° overnight under highly reduced pressure. Phe(NO_2) and Phe(NH_2) were purchased from Kokusan Chemical Works LTD as standards for amino acid analysis. Renin (from porcine kidney) and L-α-lactic acid were purchased from Sigma Chemical Company and angiotensinogen (from procine plasma) was purchased from ICN Pharmaceuticals, Inc., Life Science Group, Cleveland. H-Tyr(Me)-OMe HCl and H-Phe(NO_2)-OMe HCl were prepared from Tyr(Me) and Phe(NO_2) in the usual manner using thionyl chloride and MeOH (9).

Boc-Val-Phe-OMe I: H-Phe-OMe (9) (1.1 g) was dissolved in DMF (11 ml) together with Et_3N (0.77 ml). Boc-Val-OH (1.2 g), HOBT (0.744 g) and WSCI (0.854 g) at 0° for 16 hr. EtOAc was then added and the EtOAc solution was washed successively with 1 N $NaHCO_3$, H_2O, 1 N citric acid and H_2O. The solution was dried over $MgSO_4$ and concentrated to a small volume, then petroleum ether was added to the residue. The precipitates were reprecipitated from EtOAc and n-hexane. Yield 1.4 g (74%), mp 102-103°, $(\alpha)_D^{31}$ - 90.0° (c= 1.0, DMF), Rf^1 0.72, Rf^2 0.87, single ninhydrin-positive spot. Anal. Calcd. for $C_{20}H_{30}N_2O_5$: C, 63.47; H, 8.00; N, 7.40. Found: C, 63.36; H, 8.30; N, 7.39.

Boc-Leu-Val-Phe-OMe II: The protected dipeptide ester I (757 mg) was dissolved in TFA (3 ml) in the presence of anisole (0.3 ml) and the solution was kept at room temperature for 20 min, then concentrated. The residue was dried over KOH pellets in vacuo. HOBT (297 mg), Boc-Leu-OH (542 mg) and WSCI (341 mg) were added to an ice-cold solution of H-Val-Phe-OMe TFA in DMF (5 ml), followed by addition of Et_3N to keep the solution slightly alkaline. After 16 hr at 0°, the reaction mixture was diluted with EtOAc and the solution was washed succesively with 1 N $NaHCO_3$, H_2O, 1 N citric acid and H_2O. The solution was dried over $MgSO_4$ and concentrated to a small volume, then petroleum ether was added. The precipitates were reprecipitated from EtOAc and n-hexane. Yield 890 mg (91%), mp 124°, $(\alpha)_D^{31}$ - 31.4° (c= 1.0, DMF), Rf^1 0.83, Rf^2 0.91, single ninhydrin-positive spot. Anal. Calcd.for $C_{26}H_{41}N_3O_6$: C, 63.52; H, 8.41; N, 8.55. Found: C, 63.76; H, 8.49; N, 8.96.

Hyd-Leu-Val-Phe-OMe III: II (328 mg) was treated with TFA (2 ml)-anisole (0.2 ml) as described above. The resulting tripeptide ester

was condensed with Hyd (prepared from 4.6 g of Val in the similar manner (3) using 4.1 g of $NaNO_2$ and 60 ml of 1 N H_2SO_4) (87 mg) in the presence of HOBT (100 mg) and WSCI (114 mg) essentially as described for the preparation of I. The product was recrystallized from hot EtOAc. Yield 234 mg (71%), mp 186-192°, $(\alpha)_D^{31}$ - 40.2° (c= 1.0, DMF), Rf^1 0.69, Rf^2 0.80, single chlorine-tolidine-positive spot. Anal. Calcd. for $C_{26}H_{41}N_3O_6 \cdot H_2O$: C, 61.27; H, 8.51; N, 8.25. Found: C, 61.19; H, 8.67; N, 8.03. Amino acid ratios in the acid hydrolysate: Val 0.96, Leu 1.12, Phe 0.94 (average recovery 88%).

Boc-Val-Tyr(Me)-OMe IV: This compound was prepared from H-Tyr(Me)-OMe HCl (1.2 g), Boc-Val-OH (1.2 g), HOBT (0.744 g) and WSCI (0.854 g) essentially as described for the preparation of I. Needles; yield 1.1 g (52%), mp 120°, $(\alpha)_D^{31}$ - 10.3° (c= 1.0, DMF), Rf^1 0.75, Rf^2 0.88, single ninhydrin-positive spot. Anal. Calcd. for $C_{21}H_{32}N_2O_7$: C, 59.42; H, 7.60; N, 6.60. Found: C, 59.52; H, 7.88; N, 6.66.

Boc-Leu-Val-Tyr(Me)-OMe V: This compound was prepared from IV (408 mg), Boc-Leu-OH (274 mg), HOBT (149 mg) and WSCI (171 mg) essentially as described for the preparation of II. Yield 410 mg (76%), mp 108-109°, $(\alpha)_D^{31}$ - 24.3° (c= 1.0, DMF), Rf^1 0.76, Rf^2 0.91, single ninhydrin-positive spot. Anal. Calcd. for $C_{27}H_{43}N_3O_8 \cdot H_2O$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.49; H, 8.39; N, 7.38.

Hyd-Leu-Val-Tyr(Me)-OMe VI: This compound was prepared from V (261 mg), Hyd (65 mg), HOBT (75 mg) and WSCI (86 mg) essentially as described for the preparation of II. Yield 202 mg (77%), mp 201°, $(\alpha)_D^{31}$ - 35.2° (c= 1.0, DMF). Rf^1 0.67, Rf^2 0.89, single chlorine-tolidine-positive spot. Anal. Calcd. for $C_{27}H_{43}N_3O_7$: C, 62.24; H, 8.32; N, 8.06. Found: C, 62.34; H, 7.93; N, 7.74. Amino acid ratios in the acid hydrolysate: Val 0.84, Leu 0.96, Tyr 0.83 (average recovery 91%).

H-Leu-Val-Tyr(Me)-OMe VII: V (50 mg) was dissolved in TFA (1 ml)-anisole (0.1 ml) and the solution was kept at room temperature for 20 min, then dry ether was added. The precipitate formed was dried over KOH pellets in vacuo. The resulting tripeptide ester was treated with Amberlite CG-4B (acetate form 1 g) for convert to acetate type for 30 min and then filtered in vacuo. The filtrate was evaporated in vacuo and then the residue was recrystallized from MeOH and ether. Yield 38 mg (88%), mp 177-178°, $(\alpha)_D^{28}$ + 20.0° (c= 1.0, DMF), Rf^1 0.78, Rf^2 0.85, single ninhydrin-positive spot. Anal. Calcd. for $C_{24}H_{39}N_3O_7$: C, 59.85; H, 8.16; N, 8.73. Found: C, 59.61; H, 8.29; N, 8.44. Amino acid ratios in the acid hydrolysate: Val 0.89, Leu 0.88, Tyr 0.85 (average recovery 85%).

Boc-Val-Phe(NO_2)-OMe VIII: This compound was prepared from H-Phe(NO_2)-OMe HCl (869 mg), Boc-Val-OH (796 mg), HOBT (495 mg) and WSCI (570 mg) essentially as described for the preparation of II. The product was recrystallized from EtOAc. Yield 1.1 g (79%), mp 208°, $(\alpha)_D^{31}$ - 20.0° (c= 1.0, DMF), Rf^1 0.52, Rf^2 0.71, single ninhydrin-positive spot. Anal. Calcd. for $C_{20}H_{29}N_3O_7$: C, 56.72; H, 6.90; N, 9.92. Found: C, 56.33; H, 6.93; N, 9.97.

Boc-Leu-Val-Phe(NO_2)-OMe IX: This compound was prepared from VIII (706 mg), Boc-Leu-OH (458 mg), HOBT (248 mg) and WSCI (285 mg) essentially as described for the preparation of II. The product was recrystallized from EtOAc. Yield 784 mg (80%), mp 191°, $(\alpha)_D^{31}$ - 33.5° (c= 1.0, DMF), Rf^1 0.87, Rf^2 0.92, single ninhydrin-positive spot. Anal. Calcd. for $C_{26}H_{40}N_4O_8$: C, 61.89; H, 6.85; N, 9.52. Found: C, 61.73; H, 7.15; N, 9.51.

Hyd-Leu-Val-Phe(NO_2)-OMe X: This compound was prepared from IX (294 mg), Hyd (65 mg), HOBT (75 mg) and WSCI (86 mg) essentially as described for the preparation of II. Yield 186 mg (69%), mp 229-235°, $(\alpha)_D^{31}$ - 40.1° (c= 1.0, DMF), Rf^1 0.88, Rf^2 0.90, single chlorine-tolidine-positive spot. Anal. Calcd. for $C_{26}H_{40}N_4O_8$: C, 58.19; H, 7.15; N, 10.44. Found: C, 57.72; H, 7.35; N, 10.44. Amino acid ratios in the acid hydrolysate: Val 1.10, Leu 0.92, Phe(NO_2) 0.89 (average recovery 90%).

H-Leu-Val-Phe(NO_2)-OMe XI: This compound was prepared from IX (50 mg) essentially as described for the preparation of VII. Yield 35 mg (83%), mp 180-182°, $(\alpha)_D^{28}$ 0° (c= 1.0, DMF), Rf^1 0.85, Rf^2 0.91, single ninhydrin-positive spot. Anal. Calcd. for $C_{23}H_{36}N_4O_8$: C, 55.63; H, 7.31; N, 11.28. Found: C, 55.29; H, 7.58; N, 10.94. Amino acid ratios in the acid hydrolysate: Leu 0.88, Val 1.19, Phe(NO_2) 0.86 (average recovery 90%).

Hyd-Leu-Val-Phe(NH_2)-OMe XII: X (130 mg) was hydrogenated in MeOH (25 ml)-10% AcOH (5 ml) over 10% Pd-C for 36 hr. The catalyst was removed using aid of cellite. The filtrate was evaporated to dryness and the reisdue was dried over KOH pellets *in vacuo*. The hydrogenated product thus obtained was dissolved in MeOH-BuOH (3:1) (3 ml) and applied to a column of Sephadex LH-20 (2.8 X 60 cm), eluting with the same solvent. Individual fractions (4 ml each) were collected and the absorbancy at 230 nm was determined. Fractions corresponding to the main peak (tube Nos. 48-55) were combined and the solvent was evaporated off *in vacuo*. The residue was reprecipitated from EtOAc and n-hexane. Yield 79 mg (57%), mp 109-117°, $(\alpha)_D^{31}$ - 28.9° (c= 1.0, DMF), Rf^1 0.86, Rf^2 0.91, single chlorine-tolidine-positive spot. Anal. Calcd. for $C_{26}H_{42}N_4O_6$: C, 61.64; H, 8.36; N, 11.06. Found: C, 61.38; H, 8.50; N, 10.85. Amino acid ratios in the acid hydrolysate: Val 0.89, Leu 0.93, Phe(NH_2) 0.90 (average recovery 83%).

Lac-Leu-Val-Tyr(Me)-OMe XIII: This compound was prepared from V (179 mg), Lac (33 mg), HOBT (50 mg) and WSCI (57 mg) essentially as described for the preparation of II. The product was recrystallized from EtOAc. Yield 138 mg (81%), mp 198-200°, $(\alpha)_D^{31}$ - 25.3° (c= 1.0, DMF). Rf^1 0.86, Rf^2 0.91, single chlorine-tolidine-positive spot. Anal. Calcd. for $C_{25}H_{39}N_3O_8$: C, 58.92; H, 7.72; N, 8.25. Found: C, 59.14; H, 7.81; N, 8.55. Amino acid ratios in the acid hydrolysate: Val 0.94, Leu 1.23, Tyr 0.82 (average recovery 86%).

Lac-Leu-Val-Phe(NO_2)-OMe XIV: This compound was prepared from IX (196 mg), Lac (33 mg), HOBT (50 mg) and WSCI (57 mg) essentially as described for the preparation of II. Yield 148 mg (88%), mp 158-161°, $(\alpha)_D^{31}$ - 42.1° (c= 1.0, DMF), Rf^1 0.84, Rf^2 0.86, single chlorine-tolidine-positive spot. Anal. Calcd. for $C_{24}H_{36}N_4O_8$: C, 56.68; H, 7.14; N, 11.02. Found: C, 56.42; H, 7.37; N, 10.77. Amino acid ratios in the acid hydrolysate: Val 1.08, Leu 0.88, Phe(NO_2) 0.81 (average recovery 85%).

Lac-Leu-Val-Phe(NH_2)-OMe XV: This compound was prepared from XIV (80 mg) essentially as described for the preparation of XII. Fractions corresponding to the main peak (tube Nos. 57-60) were combined and the solvent was evaporated to dryness *in vacuo*. Yield 28 mg (37%), mp 189-196°, $(\alpha)_D^{29}$ - 33.2° (c= 0.3, DMF), Rf^1 0.83, Rf^2 0.89, single chlorine-tolidine-positive spot. Anal. Calcd. for

$C_{24}H_{38}N_4O_6$: C, 60.23; H, 8.00; N, 11.71. Found: C, 60.30; H, 8.24; N, 11.58. Amino acid ratios in the acid hydrolysate: Val 1.15, Leu 1.21, Phe(NH_2) 0.84 (average recovery 90%).

Renin-Inhibitory Activity Test (4): The enzymatic assay was carried out by incubating 50 μl of a 250 fold dilution of the renin preparation with 50 μl of angiotensinogen and 10 μl of synthetic analogue or fragment at 37° for 30 min. The renin and substrate solutions were made up in 0.1 M sodium phosphate buffer (pH 7.01), 10 mM EDTA, while the inhibitor solutions were made up in a 2:1 mixture of MeOH and H_2O. At the end of the incubation period, the enzymatic reaction was stopped by placing the incubation mixture on ice and diluting them with 0.8 ml of 0.1 M Tris-acetate buffer, pH 7.4. The angiotensin I produced was measured by radioimmunoassay.

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