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Short communication

N-[2(*S*)-Hydroxy-4-methylpentyl] tripeptide derivatives as inhibitors of renin

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Summary — The following *N*-2*(S)*-hydroxy-4-methylpentyl derivatives of Gly—Val—Phe—OMe and Leu—Val—Phe—OMe were designed as analogues of the transition state of the renin—angiotensinogen reaction: *N*-[2*(S)*-hydroxy-4-methylpentyl] Gly—Val—Phe—OMe **2** and *N*-[2*(S)*-hydroxy-4-methylpentyl] Leu—Val—Phe—OMe **3.** Once synthesized they were tested for their abilities to inhibit hog kidney renin. The two compounds were found to have comparable activity to the known tetrapeptide inhibitors Leu—Gly—Val—Phe—OMe and Leu—Leu—Val—Phe—OMe. The type of kinetic behavior for each of the synthesized compounds was the same as that of the respective parent tetrapeptide; being competitive in the case of **2** and non-competitive in the case of **3.**

Résumé — **Dérivés de *N*-[hydroxy-2(*S*)méthyl-4 pentyl] tripeptide comme inhibiteurs de la rénine.** N-[*Hydroxy-2 méthyl-4 pentyl* (2S)] *Gly—Val—Phe—OMe* **2** *et* N-[*hydroxy-2 methyl-4 pentyl* (2S)] *Leu—Val—Phe—OMe* **3** *ont été préparés afin de mimer l'état de transition de la réaction rénine—angiotensinogène. Ils ont été testés pour leur capacité d'inhiber l'activité de la rénine de rein de porc. Ces deux composés ont montré une activité comparable à celle d'inhibiteurs connus tels que Leu—Gly—Val—Phe—OMe et Leu—Leu—Val—Phe—OMe. La cinétique de chacun de ces dérivés est identique à celle de son peptide de référence, de type compétitif pour* **2** *et non-compétitif pour* **3.**

renin inhibitors / transition state analogues / amino alcohol / trifluoromethanesulfonate intermediate

Introduction

The renin—angiotensin system plays an important role in the regulation of blood pressure and blood volume in both physiological and pathological states [1—3]. The inhibition of renin has been thought to be one means by which the renin—angiotensin system could be blocked, since the reaction between renin and its substrate, angiotensinogen, is the rate-limiting step in the sequence of enzymatic reactions that make up the renin—angiotensin system.

The present investigation was undertaken as part of an effort to develop new types of renin inhibitors. The approach that we have taken involves the synthesis of compounds that might mimic the postulated transition state of the renin—angiotensinogen reaction. This transition state is believed to be tetrahedral in nature wherein the carbonyl group of the scissile amide bond is transformed into a hydroxyl group and the amide nitrogen atom of the scissile amide bond begins to take on the characteristics of an amino nitrogen [4].

In a previous study aimed at mimicking this postulated tetrahedral intermediate, we modified the known substrate analogue inhibitor Leu—Leu—Val—Phe—OCH_3 by replacing the N-terminal leucyl residue with various α-hydroxyalkanoyl residues [5]. This modification yielded peptides such as **1** that were 5—10 times more active as inhibitors of renin than the tetrapeptide Leu—Leu—Val—Phe—OCH_3. Since Szelke *et al.* [6] had shown previously that incorporation of the reduced amide modification into renin substrate analogues gives rise to very potent inhibitors of renin, we felt that the renin inhibitory activity of α-hydroxyalkanoyl derivatives such as **1** might be enhanced if the α-hydroxyisocaproyl—leucyl amide bond was replaced with the reduced amide isostere (—CH_2NH—). To test this hypothesis, the aminoalcohols **2** and **3** were synthesized and evaluated as inhibitors of renin.

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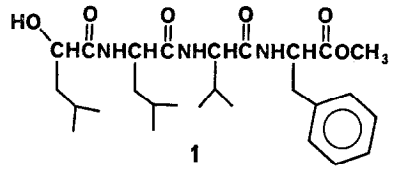

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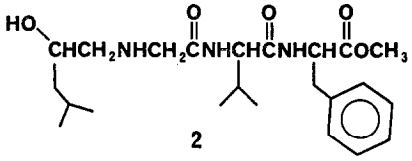

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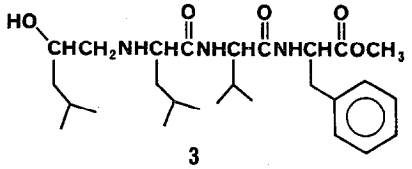

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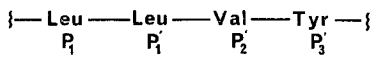
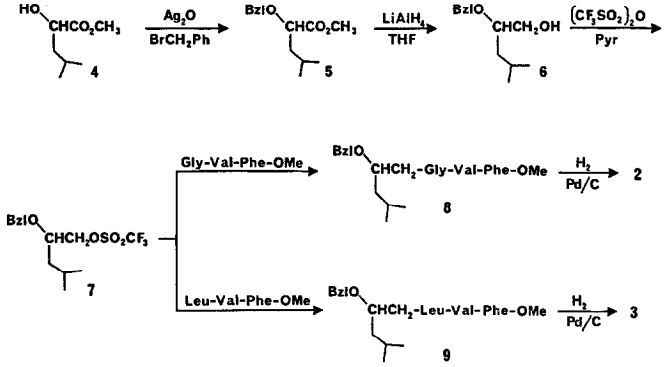


Fig. 1. Corresponding sequence of the N-terminal region of porcine angiotensinogen.

Results and Discussion

Chemistry

Compounds **2** and **3** were prepared as outlined in Scheme 1. The hydroxyester, methyl L-α-hydroxyisocaproate **4**, which was prepared by esterifying L-α-hydroxyisocaproic acid [5] using diazomethane, served as the starting material for this synthesis. Benzylation of **4** was carried out in an 85% yield, using a modification of the procedure of Mislow *et al.* [7, 8], whereby **4** was treated with benzyl bromide in the presence of silver oxide. The benzyl ether **5** thus obtained was reduced with $LiAlH_4$ in refluxing tetrahydrofuran (THF) to give 2-benzyl-oxy-4-methyl-1-pentanol **6**. The



Scheme 1.

optical purity of **6** was determined by converting this material into its *R*-(+)-α-methoxy-α-(trifluoromethyl)phenyl acetate (MTPA) derivative [9], using the esterification procedure of Dhaon *et al.* [10]. NMR analysis of the MTPA derivative of **6** showed the presence of only one diastereoisomer thereby indicating that no significant racemization of the chiral carbon atom took place during the conversion of **4** into **6**. The conversion of **6** into triflate **7** was accomplished by treating the primary alcohol **6** with trifluoromethanesulfonic anhydride and pyridine [11—13]. This material was used immediately after being synthesized, since it decomposed to a brown solid upon standing at room temperature.

The tripeptides Gly—Val—Phe—OCH_3 and Leu—Val—Phe—OCH_3, which had been synthesized as described previously [5], were each reacted with the reactive triflate **7** using a modification of the procedure that Effemberger *et al.* [14] have described for the efficient synthesis of N-substituted α-amino acids. The reactions were followed by monitoring the disappearance of starting materials using thin—layer chromatography (TLC). In the case of the reaction of **7** with Gly—Val—Phe—OCH_3 the reaction was complete in 2 h at room temperature. The desired product methyl *N*-[2*(S)*-benzyloxy-4-methylpentyl]glycyl-L-valyl-L-phenylalaninate **8** was obtained in a 40% yield after purification by flash chromatography. For the reaction between **7** and Leu—Val—Phe—OCH_3, refluxing in CH_2Cl_2 was required. The product, methyl *N*-[2*(S)*-benzyloxy-4-methylpentyl]-L-leucyl-L-valyl-L-phenylalaninate **9** was obtained in a 50% yield. The removal of the benzyl group from **8** and **9** was initially attempted, using catalytic transfer hydrogenation with ammonium formate in 20% acetic acid in MeOH. This method proved unsuccessful, however. Catalytic hydrogenation of **8** and **9** over Pd/C in 5% HCl—MeOH provided the desired amino alcohol peptides *N*-[2*(S)*-hydroxy-4-methylpentyl]glycyl-L-valyl-L-phenylalaninate **2** and methyl *N*-[2*(S)*-hydroxy-4-methylpentyl]-L-leucyl-L-valyl-L-phenylalaninate **3**, respectively.

Renin inhibition studies

Compounds **2** and **3** were tested for their abilities to inhibit hog kidney renin. Renin activity was determined by measuring the amount of angiotensin I formed in the reaction of hog kidney renin with porcine angiotensinogen using an assay system we described previously [5]. The nature of each compound's inhibitory activity and its inhibitory constant (K_i) was determined by the Lineweaver—Burk method of plotting [15].

Peptide analogue **3** was found to be the most active inhibitor of renin of the two 2-hydroxy-4-methylpentyl peptide derivatives synthesized in this study. This analogue was found to have an inhibitory constant of 4.8×10^{-4} M. In contrast, compound **2** was found to be about four times weaker with a $K_i = 1.98$ mM. This is not too surprising, since it is well known that renin prefers amino acid residues with lipophilic side chains at the P_1' position. There were also other differences between these two compounds. While **2** inhibited hog kidney renin competitively, analogue **3** inhibited renin in a non-competitive fashion. In compa-

rison, the tetrapeptide Leu—Leu—Val—Phe—OMe was found to inhibit renin with a $K_i = 5 \times 10^{-4}$ M. Like its corresponding amino alcohol peptide inhibitor, analogue **3**, this tetrapeptide inhibited hog kidney renin in a non-competitive manner.

The renin inhibitory activity observed for **3** in this study was comparable to that previously observed for the α-alkanoyl peptide analogue **1** [5], as well as for that observed with the reduced amide tetrapeptide analogue Leuψ[CH_2-NH]Leu—Val—Phe—OCH_3 [16]. It is thus clear that the combination of the α-alkanoyl residue with the reduced amide bond to give an amino alcohol does not bring about an enhancement of renin inhibitory activity of the tetrapeptide substrate analogue inhibitors, as was originally hoped. Recently, Dann *et al.* [17] have incorporated a slightly different type of amino alcohol grouping into substrate analogue inhibitors of renin. In this case, the scissile amide bond has been replaced with the following peptide bond surrogate (—$CH(OH)CH_2NH$—). High renin inhibitory activity was observed when this moiety was utilized in octapeptide substrate analogues. As these analogues were shortened, however, the renin inhibitory activity diminished dramatically. Thus the low activity seen with **3** was probably not due to the inability of the particular amino alcohol used in this study to serve as a scissile amide bond surrogate, but rather to the fact that **3** lacked the N-terminal amino acid residues that apparently are important in positioning the peptide bond surrogates, such as the amino alcohol moiety, so that they can maximally interact with renin.

Experimental protocols

Chemical methods

Melting points were determined in capillaries using a Thomas Hoover capillary melting point apparatus and are uncorrected. TLC was performed on Analtech silica gel-GF 250 μm plates and visualized by either UV light, iodine, ninhydrin spray (amines), 2,2-diphenylpicrylhydrazyl (alcohols) or 2,6-dichlorophenol indophenol (acids). Column chromatography was accomplished either by flash chromatography on columns packed with silica gel 60 (40—63 μm) obtained from E. M. Reagents (F.R.G.), or by a medium pressure chromatographic system consisting of Altex 15 × 500 mm or 25 × 500 mm glass columns dry packed with Woelm silica gel (32—63 μm). Optical rotations were measured on a Perkin—Elmer 141 polarimeter at the 589 nm Na D-line. Nuclear magnetic resonance (NMR) spectroscopy was carried out on either a JEOL FX-90 MHz, a Bruker WM-250 MHz, or a Nicolet 300 MHz spectrometer, using tetramethylsilane (TMS) as the internal standard. Elemental analyses were determined by M-H-W Laboratories, Phœnix, Arizona. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ± 0.4% of the theoretical values. Hog kidney renin and porcine angiotensinogen were obtained from the Sigma Chemical Co., St. Louis, MO. Rabbit angiotensin I antibody, [^{125}I]-angiotensin I, and angiotensin I standards were obtained from New England Nuclear, Boston, MA.

Methyl L-*(α)-hydroxyisocaproate* ***4***

L-*(α)*-Hydroxyisocaproic acid (30 g, 0.23 mol) was dissolved in Et_2O (200 ml). To this solution was added dropwise at 0°C an equivalent amount of diazomethane. After the addition of diazomethane was complete, the solvent was removed under reduced pressure. The residue was dissolved in Et_2O and this solution was washed with 1 M $NaHCO_3$ and saturated NaCl solution. The ether layer was dried over $MgSO_4$ and then stripped of solvent *in vacuo* to give 31 g (93.4%) of the desired ester as a colorless liquid: $[\alpha]_D^{25}$ + 2.70° (*c* 1.0, $CHCl_3$). TLC: R_f = 0.8 (EtOAc: MeOH, 9:1). NMR ($CDCl_3$) δ 4.20 (m, 1H, αCH); 3.80 (s, 3H, OCH_3); 3.40 (d, 1H, *J* = 7 Hz, OH); 1.5 (m, 3H, βCH_2 and γCH); 0.90 (d, 6H, *J* = 6 Hz, $(CH_3)_2$). Anal. ($C_7H_{14}O_3$) C, H.

Methyl L-*α-benzyloxyisocaproate* ***5***

To a solution of **4** (5 g, 34.2 mmol) and benzylbromide (8.7 g, 34.5 mmol) in Et_2O was added in portions Ag_2O (8 g, 34.5 mmol). After the addition of an initial portion of Ag_2O, the mixture was heated to initiate refluxing. From then on, refluxing was maintained by the addition of Ag_2O. After all of the Ag_2O had been added, the mixture was refluxed for 2 h. The silver oxide was removed by filtration and the solvent removed *in vacuo*. The residue was distilled under vacuum to give 6.87 g (85%) of the desired product as a colorless oil: bp: 105—110°C (0.5 mm Hg); $[\alpha]_D^{25}$ −67.9° (neat). NMR ($CDCl_3$) δ 7.30 (s, 5H, C_6H_5); 4.20—4.80 (m, 2H, CH_2Ph); 4.00 (m, 1H, αCH); 3.80 (s, 3H, OCH_3); 1.40—1.90 (m, 3H, βCH_2 and γCH); 0.90 (dd, 6H, $(CH_3)_2$). Anal. ($C_{14}H_{20}O_3$) C, H.

2(S)-Benzyloxy-4-methyl-1-pentanol ***6***

A solution of 1 M $LiAlH_4$ (50 ml, 50 mmol) in 7 ml of freshly dried THF was placed in a 25 ml three-necked flask equipped with a reflux condenser, dropping funnel and stirrer and protected from moisture until completion of the reaction by $CaCl_2$ tubes attached to the openings. Through the dropping funnel, **5** (10 g, 42.32 mmol) was introduced and the mixture refluxed for 3 h. With continuous stirring, water was added dropwise cautiously to the cooled flask. The mixture was then poured into ice water (12 ml). To this mixture was added 10% H_2SO_4 (20 ml). The aqueous layer was extracted with two 40 ml portions of EtOAc. The combined EtOAc extracts were washed with saturated NaCl solution and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure afforded 8.68 g of the crude alcohol which was purified by flash chromatography using hexane:EtOAc (3:1). The desired pure alcohol **6** was obtained as a colorless oil in a yield of 6.68 g (76%): $[\alpha]_D^{25}$ + 75.4° (*c* 1.0, MeOH). TLC: R_f = 0.4 (hexane:EtOAc, 3:1). NMR ($CDCl_3$) δ 7.30 (m, 5H, C_6H_5); 4.50 (s, 2H, CH_2Ph); 3.40—3.80 (m, 3H, OCH and CH_2O); 1.00—2.00 (m, 3H, CH_2CH); 0.90 (dd, 6H, $(CH_3)_2$). Anal. ($C_{13}H_{20}O_2$) C, H.

A portion of **6** (50 mg, 0.24 mmol) was converted to its MTPA derivative using 4-dimethylaminopyridine (15 mg, 0.12 mmol), *R*-(+)-α-methoxy-α-(trifluormethyl)phenylacetic acid (56 mg, 0.24 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride [10]. The methoxy resonance of the MTPA derivative appeared at 3.46 ppm.

2(S)-Benzyloxy-4-methyl-1-pentanol trifluoromethanesulfonate ***7***

To a solution of pyridine (1.47 g, 18.48 mmol, distilled from BaO and stored over KOH) in CH_2Cl_2 (50 ml, distilled from P_2O_5 and stored over 4 Å molecular sieves) at −22°C was slowly added freshly opened trifluoromethanesulfonic anhydride (5.25 g, 18.48 mmol). The white suspension which formed was stirred vigorously for 5 min and then **6** (3.50 g, 16.80 mmol) in CH_2Cl_2 (7 ml) was slowly added. Stirring was continued for 15 min at −22°C. A 20°C water bath was used to warm the reaction mixture quickly to ambient temperature. The reaction turned light tan at this point and the precipitate was filtered and washed with more CH_2Cl_2. After evaporating the combined filtrates, the residue was redissolved in Et_2O and filtered, dried over Na_2SO_4 and passed through a 3 cm silica gel plug. The plug was thoroughly washed with Et_2O. Evaporation of the eluent gave 4.02 g (70%) of a colorless liquid which decomposed on standing at room temperature (*i.e.*, became a dark brown solid). The same decomposition took place at 4°C in a week. This material thus was used without further purification: $[\alpha]_D^{25}$ −41.5° (*c* 1.0, MeOH). TLC: R_f = 0.8 (hexane:EtOAc, 3:2). NMR ($CDCl_3$) δ 7.40 (m, 5H, C_6H_5); 4.50 (m, 4H, CH_2Ph and CH_2O); 3.80 (m, 1H, OCH); 1.10—2.0 (m, 3H, CH_2CH); 0.96 (dd, 6H, *J* = 6 Hz, $(CH_3)_2$).

Methyl N-[*2(S)-benzyloxy-4-methylpentyl*]*glycyl*-L-*valyl*-L-*phenylalaninate* ***8***

A solution of **7** (1.37 g, 4.02 mmol) in CH_2Cl_2 (20 ml) was added dropwise within 5 min at room temperature to a stirred solution of Gly—Val—Phe—OMe (2.70 g, 8.05 mmol) in CH_2Cl_2 (20 ml). Stirring was continued for 2 h at room temperature. The reaction was monitored by TLC (EtOAc) during this period. The reaction mixture became extremely viscous at this time. The reaction mixture was extracted

with 1 M $NaHCO_3$, washed with saturated NaCl solution and dried over Na_2SO_4. Evaporation of the solvent afforded 2.78 g of a yellow oil which was purified by flash chromatography, using EtOAc:hexane (2:1) to yield a colorless oil which crystallized at 4°C overnight. The solid was isolated with petroleum ether (60—70°C) to afford 810 mg (40%) of **8** as a white solid: mp: 64—65°C; $[\alpha]_D^{25}$ + 8.1° (*c* 1.0, MeOH). TLC: R_f = 0.3 (hexane:EtOAc, 2:1). NMR ($CDCl_3$) δ 7.76 (d, 1H, amide NH); 7.00—7.33 (m, 10H, C_6H_5); 6.56 (d, 1H, amide NH); 4.83 (dd, 1H, *J* = 13.6 and 6.2 Hz, Phe αCH); 4.58 (d, 1H, *J* = 11.53 Hz, OCH_2Ph); 4.50 (d, 1H, *J* = 11.5 Hz, OCH_2Ph); 4.25 (m, 1H, Val αCH); 3.73 (s, 3H, OCH_3); 3.60 (broad, 1H, OCH); 3.21 (s, 2H, Gly αCH_2); 3.10 (m, 2H, Phe βCH_2); 2.66 (dd, 2H, CH_2N); 2.16 (m, 1H, Val βCH); 1.83 (br, 1H, amine NH); 1.66 (m, 1H, CH); 1.33 (m, 2H, CH_2); 0.90 (m, 6H, $(CH_3)_2$). Anal. ($C_{30}H_{43}N_3O_5$) C, H, N.

Methyl N-*(2(S)-benzyloxy-4-methylpentyl)*-L-*leucyl*-L-*valyl*-L-*phenylalaninate* **9**

A solution of **7** (0.87 g, 2.55 mmol) in CH_2Cl_2 (20 ml) was added dropwise within 5 min at room temperature to a stirred solution of Leu—Val—Phe—OMe (2 g, 5.11 mmol) in CH_2Cl_2 (20 ml). Refluxing was maintained for 2 h. The reaction was monitored by TLC (EtOAc) during this time. The reaction mixture was extracted with 1 M $NaHCO_3$, washed with saturated NaCl solution and dried over Na_2SO_4. Evaporation of the solvent afforded 2.66 g of a yellow oil which was purified by a combination of flash and mesh column chromatography, using hexane:EtOAc (3:2) to yield a colorless oil which crystallized at 4°C overnight. The solid was isolated with petroleum ether (60—70°C) to afford 790 mg (53%) of the desired product as a white material: mp: 88—89°C; $[\alpha]_D^{25}$ —24.2° (*c* 1.0, MeOH). TLC: R_f = 0.4 (hexane:EtOAc, 3:2). NMR ($CDCl_3$) δ 7.80 (d, 1H, amide NH); 7.05—7.33 (m, 10H, C_6H_5); 6.45 (d, 1H, amide NH); 4.83 (dd, 1H, *J* = 13.6 and 6.2 Hz, Phe α-CH); 4.61 (d, 1H, *J* = 11.7, OCH_2Ph); 4.50 (d, 1H, *J* = 11.7 Hz, OCH_2Ph); 4.25 (m, 1H, Val αCH); 3.73 (s, 3H, OCH_3); 3.60 (br, 1H, OCH); 3.10 (m, 3H, Leu αCH and Phe βCH_2); 2.66 (m, 2H, CH_2N); 2.16 (m, 1H, Val βCH); 1.45—1.76 (m, 3H, Leu γCH and amine NH); 1.33 (m, 4H, Leu βCH_2 and CH_2); 0.90 (m, 12H, $(CH_3)_2$). Anal. ($C_{34}H_{51}N_3O_5$) C, H, N.

Methyl N-[*2(S)-hydroxy-4-methylpentyl*]-*glycyl*-L-*valyl*-L-*phenylalaninate* **2**

A mixture of benzyl ether **8** (100 mg, 0.19 mmol) and 10% Pd/C (10 mg) in 5% HCl in MeOH was hydrogenated at 40 psi at room temperature for 4 h. The mixture was filtered through Celite and the solvent evaporated under reduced pressure. Trituration of the remaining solid with Et_2O afforded 70 mg (75%) of the title compound as its hydrochloride salt: mp: 116—118°C; $[\alpha]_D^{25}$ —2.7° (*c* 1.0, MeOH). TLC: R_f = 0.95 (*n*-propanol:NH_4OH, 4:1).

The free amine was obtained by partitioning the hydrochloride salt (70 mg, 0.15 mmol) between 1 M $NaHCO_3$ and EtOAc. The organic layer was washed again with 1 M $NaHCO_3$, then saturated NaCl solution and dried over $MgSO_4$. Evaporation of the solvent afforded a yellow oil which was purified by flash chromatography using EtOAc:hexane (2:1). Crystallization from petroleum ether gave 60 mg of the pure free amine as a white solid material: $[\alpha]_D^{25}$ + 15.6° (*c* 1.0, MeOH). TLC: R_f = 0.4 (EtOAc:hexane, 2:1). NMR ($CDCl_3$) δ 8.44 (d, 1H, amide NH); 7.10—7.31 (m, 5H, C_6H_5); 6.46 (d, 1H, amide NH); 4.85 (dd, 1H, *J* = 16.5 and 9.9 Hz, Phe αCH); 4.33 (dd, 1H, *J* = 10.7 and 6.6 Hz, Val αCH); 4.10 (br, 1H, OCH); 3.70 (s, 3H, OCH_3); 3.39 (d, 1H, *J* = 17.7 Hz, Gly αCH_2); 3.18 (d, 1H, *J* = 17.7 Hz, Gly αCH_2); 3.09 (dd, 2H, *J* = 4.1 and 2.5 Hz, Phe βCH_2); 2.42—2.63 (m, 2H, CH_2N); 2.10 (m, 1H, Val βCH); 1.8 (m, 1H, CH); 1.35 (m, 1H, CHH); 1.08 (m, 1H, CHH); 0.90 (dd, 6H, $(CH_3)_2$). Anal. ($C_{23}H_{37}N_3O_5 \cdot 1.5\ C_4H_8O_2$) C, H, N.

Methyl N-[*2(S)-hydroxy-4-methylpentyl*]-L-*leucyl*-L-*valyl*-L-*phenylalaninate* **3**

A mixture of the benzyl ether **9** (125 mg, 0.21 mmol) and 10% Pd/C (200 mg) in 5% HCl/MeOH was hydrogenated at 30 psi at room temperature overnight. The mixture was filtered through Celite and the solvent evaporated under reduced pressure. Trituration with Et_2O of the remaining solid afforded 96 mg (85%) of the desired product as its hydrochloride salt: mp: 207—208°C; $[\alpha]_D^{25}$ —1.4° (*c* 1.0, MeOH); $[\alpha]_D^{25}$ + 4.4° (*c* 1.0, DMSO); TLC: R_f = 0.7 (EtOAc); R_f = 0.9 (*n*-propanol:NH_4OH, 4:1).

The free amine was obtained by partitioning the hydrochloride salt (85 mg, 0.16 mmol) between 1 M $NaHCO_3$ and EtOAc. The organic layer was washed with 1 M $NaHCO_3$, then saturated NaCl solution and dried over $MgSO_4$. Evaporation of the solvent and trituration of the residue with Et_2O afforded 71 mg (90%) of the pure free amine as a white solid material: mp: 72—74°C; $[\alpha]_D^{25}$ —29.8° (*c* 1.0, MeOH). NMR ($CDCl_3$) δ 7.98 (d, 1H, amide NH); 7.10—7.30 (m, 5H, C_6H_5); 6.52 (d, 1H, amide NH); 4.87 (dd, 1H, *J* = 16.5 and 6.6 Hz, Phe αCH); 4.23 (dd, 1H, *J* = 10.7 and 6.6 Hz, Val αCH); 3.70 (s, 3H, OCH_3); 3.65 (br, 1H, OCH); 3.05—3.10 (m, 3H, Leu αCH and Phe βCH_2); 2.50—2.61 (m, 2H, CH_2N); 2.07 (m, 1H, Val βCH); 1.05—1.78 (m, 6H, CH_2CH and Leu βCH_2 and γCH); 0.90 (m, 12H, $(CH_3)_2$). Anal. ($C_{27}H_{45}N_3O_5$) C, H, N.

Biological methods

The abilities of compounds **2** and **3** to inhibit hog kidney renin were measured by determining the inhibitory constant (K_i) of each compound. The K_i and the type of inhibition of **2** and **3** were determined through the use of Lineweaver—Burk plots [15]. Data for these plots were obtained by measuring the reaction velocities of hog kidney renin at several concentrations of porcine angiotensinogen (0.05—1 μM for **2** and 0.1—1 μM for **3**) in the presence and absence of a given concentration of each inhibitor (7.5×10^{-4} M for **2** and 10^{-3} M for **3**).

The enzymatic assay was carried out in a manner identical with that described by us previously [5]. Reaction velocities for hog kidney renin were expressed as the number of nmol of angiotensin I generated/unit of enzyme/min. The average values of three determinations for each substrate concentration at each inhibitor level were used to generate a Lineweaver—Burk plot. The reciprocal of the reaction velocities ($1/v$) *vs* the reciprocal of the substrate concentration $1/[S]$ was made for each of the compounds tested. All lines were calculated using linear regression analysis. The $-1/[S]$ value at the intersection of the line of the abscisa and the $1/V_{max}$ at the ordinate gave the K_i of each compound when calculated from the following expressions: for competitive inhibitors, $K_m\text{app} = K_m(1 + [I]/K_i)$; for non-competitive inhibitors, $1/V_{max}\text{app} = (1 + [I]/K_i)/V_{max}$.

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