Inhibition of Renin by Substrate Analogue Inhibitors Containing the Olefinic Amino Acid 5(*S*)-Amino-7-methyl-3(*E*)-octenoic Acid

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The olefinic dipeptide 5(*S*)-amino-7-methyl-3(*E*)-octenoic acid (1) was synthesized and used to make the olefinic peptides Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3 (2) and His-Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3 (3). These olefinic peptides were found to exhibit renin inhibitory activity against both hog kidney renin and human amniotic renin that was comparable to the substrate analogue inhibitors Leu-Leu-Val-Phe-OCH_3 and His-Leu-Leu-Val-Phe-OCH_3.

In the design of peptide analogues increasing use is being made of isosteric replacements for the peptide amide bond.[1] An example of one such substitution is the replacement of the amide linkage of a peptide [CONH] with a trans carbon–carbon double bond (ψ[*E*-CH=CH]).[2] The trans carbon–carbon double bond appears to be a good mimic of the trans amide bond since comparison of the spatial disposition of substituents attached to either of these moieties has shown the disposition to be quite similar.[3-5] However, unlike the amide bond, which can also exist in a cis conformation, the trans carbon–carbon double bond locks the molecule in a trans conformation. In addition, this type of substitution should stabilize the peptide to the action of peptidases. Isosteric replacements of this type have been used successfully in the synthesis of analogues of enkephalin and substance P.[3,4,6] This type of substitution has also been carried out on the angiotensin-converting enzyme inhibitor Bz-Phe-Gly-Pro where it was found that the olefinic analogue Bz-Pheψ[*E*-CH=CH]Gly-Pro possessed about one-fifth the inhibitory activity of Bz-Phe-Gly-Pro.[7]

As part of an effort to determine the structural features required for the inhibition of renin by substrate analogues, the synthesis of analogues of the known renin substrate analogue inhibitors Leu-Leu-Val-Phe-OCH_3 and His-Leu-Leu-Val-Phe-OCH_3 was carried out wherein the Leu-Leu dipeptide unit was replaced with the olefinic dipeptide mimic 5(*S*)-amino-7-methyl-3(*E*)-octenoic acid (1). This paper describes the synthesis of residue 1 and the renin inhibitory activity of peptides in which this residue has been incorporated.

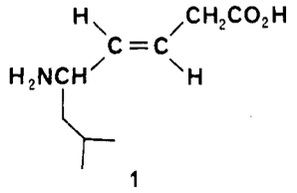

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Table I. Renin Inhibitory Activity of Olefinic Peptides

peptide	K_i, 10^{-4} M (type of inhibn[a]) hog kidney	human amniotic
Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3 (2)	8.0 (NC)	4.0 (C)
His-Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3 (3)	1.46 (C)	5.0 (C)
Leu-Leu-Val-Phe-OCH_3	5.0 (NC)	11.4 (C)
Leu-Gly-Val-Phe-OCH_3	ND[b]	10.0 (C)
His-Leu-Leu-Val-Phe-OCH_3	0.8 (C)	5.4 (C)

[a] C = competitive, NC = noncompetitive. [b] Not determined.

Results and Discussion

Chemistry. The olefinic dipeptide isostere 5(*S*)-amino-7-methyl-3(*E*)-octenoic acid (1) used in the synthesis of the olefinic peptides Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3 (2) and His-Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3 (3) was synthesized by using a method analogous to that recently described by Hann et al.[4] (Scheme I). The starting material for this synthesis was propargyl alcohol. This material was treated sequentially with *n*-butyllithium, chlorotrimethylsilane, and aqueous ammonium chloride to give 3-(trimethylsilyl)-2-propyn-1-ol (4) in a 75% yield. The yield of 4 obtained by this procedure represents an improvement over previously reported methods.[4,8] The major difference between the method used in this study and previous methods is the use of *n*-butyllithium instead of Grignard reagent to generate the dianion of propargyl alcohol.

Alcohol 4 was converted to the bromo derivative 3-bromo-1-(trimethylsilyl)prop-1-yne (5) by use of carbon tetrabromide and triphenylphosphine.[9] This material was in turn transformed into the phosphonium salt 6. Compound 6 was used in a Wittig reaction with *N*-*tert*-butoxycarbonyl-L-leucinal[10] to provide enyne 7. Analysis of the above Wittig reaction by ^1H NMR spectroscopy indicated that only the trans enyne was formed. Treatment of 7 with dicyclohexylborane followed by oxidative workup with hydrogen peroxide[11] gave the desired *tert*-butoxycarbonyl β,γ-unsaturated amino acid 8 in good yield. Since 8 was obtained as an oil, the dicyclohexylammonium salt of this material was obtained in order to more fully characterize this material. Also, 8 was deprotected with HCl in dioxane to give 1.

The *tert*-butoxycarbonyl-protected β,γ-unsaturated amino acid 8 was coupled to the dipeptide Val-Phe-OCH_3 by using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole to give the protected olefinic tetrapeptide Boc-

(1) Spatola, A. F. "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins"; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, Chapter 5.
(2) The ψ[ ] nomenclature used for describing peptide backbone modifications is that proposed by A. F. Spatola in ref 1.
(3) Hann, M. M.; Sammes, P. G. *J. Chem. Soc., Chem. Commun.* **1980**, 234.
(4) Hann, M. M.; Sammes, P. G.; Kennewell, P. O.; Taylor, J. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 307.
(5) Marshall, G. R.; Humblet, C.; Van Opdenbosch, N.; Fabrocki, R. J. "Peptides: Synthesis–Structure–Function"; Rich, D.; Gross, E., Ed.; Pierce Chemical Co.: Rockford, IL, 1981; pp 669–672.
(6) Cox, M. T.; Gormley, J. J.; Hayward, C. F.; Petter, N. N. *J. Chem. Soc., Chem. Commun.* **1980**, 800.
(7) Natarajan, S.; Condon, M. E.; Nakane, M.; Reid, J.; Gordon, E. M.; Cushman, S. W.; Ondetti, M. A. "Peptides: Synthesis–Structure–Function"; Rich, D., Gross, E., Ed.; Pierce Chemical Co.: Rockford, IL, 1981; pp 429–433.
(8) Denmark, S. E.; Jones, T. K. *J. Org. Chem.* **1982**, *47*, 4595.
(9) Hooz, J.; Gilani, S. S. H. *Can. J. Chem.* **1968**, *46*, 86.
(10) Rich, D. H.; Sun, E. T.; Bopawrai, A. S. *J. Org. Chem.* **1978**, *43*, 3624.
(11) Zweifel, G.; Backlund, S. J. *J. Am. Chem. Soc.* **1977**, *99*, 3184.

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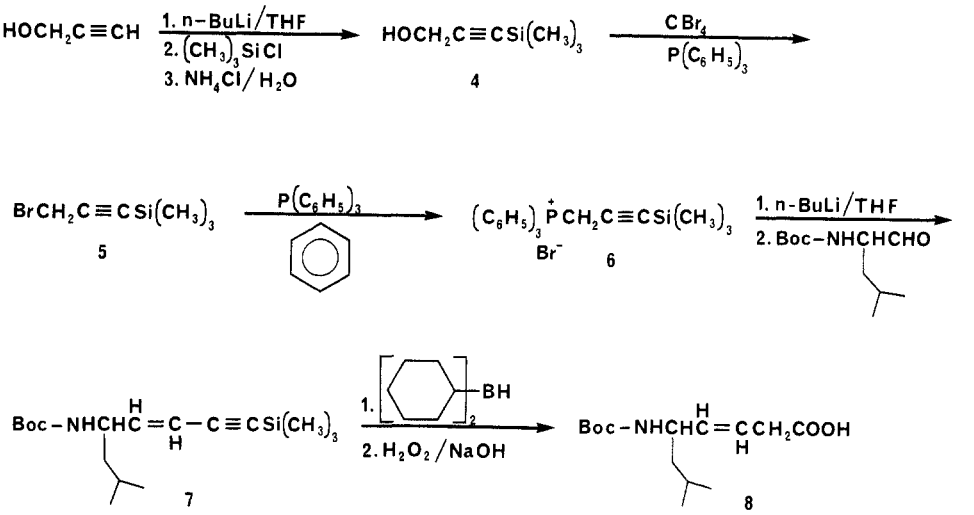
Scheme I



Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3 (**9**). Deprotection of **9** with HCl in dioxane afforded the olefinic peptide Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3 (**2**). Peptide **2** was coupled with *tert*-butoxycarbonyl-L-histidine by using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole to give Boc-His-Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3 (**10**). Removal of the *tert*-butoxycarbonyl protecting group with HCl in dioxane provided the olefinic peptide His-Leuψ-[*E*-CH=CH]Gly-Val-Phe-OCH_3 (**3**).

Renin Inhibition Studies. Olefinic peptides **2** and **3** were tested for their ability to inhibit both hog kidney renin and human amniotic renin[12] by using an in vitro renin assay system described previously.[13,14] Each compound's inhibitory constant (K_i) was determined through the use of Dixon plots. The results obtained for olefinic peptides **2** and **3** are summarized in Table I. Also included in this table for comparison are the results obtained previously for the peptides Leu-Leu-Val-Phe-OCH_3,[13] Leu-Gly-Val-Phe-OCH_3,[15] and His-Leu-Leu-Val-Phe-OCH_3.[15]

Olefinic peptide **2** was found to be a competitive inhibitor of human amniotic renin with a K_i equal to 4×10^{-4} M. The inhibitory effectiveness of **2** was approximately 2.5 times that of the corresponding renin substrate analogues Leu-Leu-Val-Phe-OCH_3 ($K_i = 11.4 \times 10^{-4}$ M) and Leu-Gly-Val-Phe-OCH_3 ($K_i = 10 \times 10^{-4}$ M). Like **2** these peptides also inhibited human amniotic renin competitively. Olefinic peptide **2** also inhibited hog kidney renin with a $K_i = 8 \times 10^{-4}$ M. In this case, however, noncompetitive inhibition was observed. The corresponding renin substrate analogue Leu-Leu-Val-Phe-OCH_3 also inhibited hog kidney renin noncompetitively with a K_i similar to that of **2**.

Olefinic peptide **3** inhibited hog kidney renin and human amniotic renin in a competitive fashion. As shown in Table I the K_i values for **3** were comparable to those obtained for the corresponding renin substrate analogue His-Leu-Leu-Val-Phe-OCH_3.

The above results indicate that the olefinic amino acid 5(*S*)-amino-7-methyl-3(*E*)-octenoic acid (**1**) can be substituted for the Leu-Leu dipeptide segment of the renin substrate analogue inhibitors Leu-Leu-Val-Phe-OCH_3 and His-Leu-Leu-Val-Phe-OCH_3 to give olefinic peptide analogues with very similar renin inhibitory activities. Furthermore, these results suggest that the Leu-Leu amide bond of Leu-Leu-Val-Phe-OCH_3 and His-Leu-Leu-Val-Phe-OCH_3 is in a trans conformation when these peptides bind to and inhibit renin.

Another example of a peptide amide bond modification that has been carried out on the tetrapeptide Leu-Leu-Val-Phe-OCH_3 is the replacement of the Leu-Leu amide bond with the reduced amide function [CH_2NH] to give the renin inhibitor Leuψ[CH_2NH]Leu-Val-Phe-OCH_3.[16] This analogue has been reported to have a greater affinity for renin than the substrate analogue inhibitor Leu-Leu-Val-Phe-OCH_3.[17] Since the olefinic peptides synthesized in this study did not show enhanced affinity for renin but rather exhibited affinity constants that were of the same magnitude as the affinity constants of the substrate analogue inhibitors after which they were modeled, it would appear that there is a qualitative difference in this case between the olefinic peptide bond modifications [CH=CH] and the reduced amide peptide bond modification [CH_2NH]. A probable explanation for this relative difference is that while the trans carbon–carbon double bond is a very good mimic of the trans form of the peptide amide bond,[3-5] the reduced amide function mimics the tetrahedral transition state formed during renin's hydrolysis of angiotensinogen.[18]

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt. Specific rotations were measured with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Unless otherwise indicated, all analytical results were within ±0.4% of the theoretical values. NMR spectra were recorded on either a JEOL FX 90-MHz or a Brucker 250-MHz spectrometer. Low-pressure chromatography (20–40 psi) was carried out on Silica Woelm (32–63 μm) from ICN Nutritional Biochemicals. Thin-layer chromatography (TLC) was carried out on Analtech 250-μm silica gel GF uniplates. Visualization was done with UV, I_2, and Pauly's reagent. Hog kidney renin (lot no. 41F-8550) and procine angiotensinogen (lot no. 102F-8584) were obtained from the Sigma Chemical Co., St. Louis, MO.

(12) Johnson, R. L.; Poisner, A. M.; Crist, R. D. *Biochem. Pharmacol.* **1979**, *28*, 1791.
(13) Johnson, R. L. *J. Med. Chem.* **1980**, *23*, 666.
(14) Johnson, R. L. *J. Med. Chem.* **1982**, *25*, 605.
(15) Johnson, R. L.; Verschoor, K. *J. Med. Chem.* **1983**, *26*, 1457.
(16) Parry, M. J.; Russell, A. B.; Szelke, M. "Chemistry and Biology of Peptides"; Meienhofer, J., Ed.; Ann Arbor Science: Ann Arbor, MI, 1972; pp 541–544.
(17) Szelke, M.; Leckie, B. J.; Tree, M.; Brown, A.; Grant, J.; Hallettl, A.; Hughes, M.; Jones, D. M.; Lever, A. F. *Hypertension* **1982**, *4*, Suppl. II, 59.
(18) Szelke, M.; Leckie, B.; Hallett, A.; Jones, D. M.; Sueivas, J.; Atrash, B.; Lever, A. F. *Nature (London)* **1982**, *299*, 555.

3-(Trimethylsilyl)-2-propyn-1-ol (4). Propargyl alcohol (4 g, 71.3 mmol) was added to a three-necked round-bottom flask fitted with two addition funnels and containing 300 mL of dry, freshly distilled THF. The system was flushed with argon and the temperature of the flask lowered to −78 °C. *n*-Butyllithium (88.9 mL, 143 mmol) was added dropwise to the stirring reaction mixture. After the addition of *n*-butyllithium was complete, the reaction mixture was stirred for 1 h at −78 °C. A solution of chlorotrimethylsilane (25.8 mL, 215 mmol) in dry THF (30 mL) was then added dropwise to the reaction mixture. The reaction mixture was allowed to warm up to room temperature where it was stirred for 2 h. Saturated NH_4Cl (50 mL) was then slowly added to the reaction mixture. The THF was removed in vacuo and the remaining solution extracted with Et_2O (100 mL). The ether extract was washed with saturated NaCl solution and then dried ($MgSO_4$). Removal of the ether in vacuo gave a yellow oil, which when vacuum distilled at 32 °C (1 mmHg) provided 6.92 g (76%) of **4** as a colorless liquid [lit.[4] 60–62 °C (12 mmHg)]: IR (NaCl) 2175 cm^{-1} (C≡C).

3-Bromo-1-(trimethylsilyl)prop-1-yne (5). To a solution of **4** (6.75 g, 52.5 mmol) and CBr_4 (34.5 g, 104 mmol) in Et_2O (100 mL) was added in small portions triphenylphosphine (27.3 g, 104 mmol). The reaction mixture was stirred for 90 min and then filtered. The precipitate that was collected was washed several times with Et_2O. The Et_2O from the combined washings and filtrate was removed in vacuo and the residue obtained was passed through a small column of silica gel using petroleum ether (bp 60–70 °C) as the eluting solvent. The elutant was stripped of solvent under reduced pressure and the ligand that was obtained was distilled to give a 65% yield of **5**: bp 32 °C (2 mmHg) [lit.[4] bp 43 °C 6 mmHg)]; IR (NaCl) 2170 cm^{-1} (C≡C).

Triphenyl[3-(trimethylsilyl)prop-2-ynyl]phosphonium Bromide (6). 3-Bromo-1-(trimethylsilyl)prop-1-yne (**5**; 6.22 g, 32.5 mmol) was treated with a solution of triphenylphosphine (8.52 g, 32.5 mmol) in benzene (70 mL) by using the same method as that described by Ahmed et al.[19] A yield of 9.49 g (64.4%) of the title compound was obtained: mp 159–161 °C (lit.[16] mp 154–156 °C); IR (KBr) 2240 cm^{-1} (C≡C); NMR ($CDCl_3$) δ 8.04–7.58 (m, 15 H, PPh_3), 5.15 (d, 2 H, *J* = 14.9 Hz, CH_2P), 0.1 (s, 9 H, $Si(CH_3)_3$).

5(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-7-methyl-1-(trimethylsilyl)-3(*E*)-octen-1-yne (7). The phophonium salt **6** (1.27 g, 2.8 mmol) was suspended in dry, freshly distilled THF (40 mL). The system was flushed with N_2 and the temperature lowered to −78 °C. *n*-Butyllithium in hexane (2.80 mmol) was added dropwise to the cold suspension. After the addition of the *n*-butyllithium was complete, the resulting solution was stirred for 1 h. A solution of (*tert*-butoxycarbonyl-L-leucinal[10] (0.6 g, 2.8 mmol) in dry THF (20 mL) was then added in a dropwise manner. The reaction mixture was stirred while it was slowly allowed to warm up to room temperature (ca. 2 h). The solvent was removed in vacuo and the residue triturated with Et_2O. The Et_2O extract was evaporated to dryness and the residue partitioned between ethyl acetate and 1 M $NaHCO_3$. The ethyl acetate layer was washed with 10% HCl followed by saturated NaCl solution and then dried over $MgSO_4$. Removal of the EtOAc in vacuo afforded an orange oil. This material was purified by passing it through a 1.5 × 75 cm silica gel column using CH_2Cl_2 as the eluting solvent. Recrystallization of the material obtained from the column chromatographic purification from a mixture of acetone and H_2O yielded 0.4 g (53%) of **7**: mp 89–90 °C; $[\alpha]^{24}_D$ −53.2° (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$) δ 6.04 (dd, *J* = 6.14 and 15.9 Hz, 1 H, C*H*=CHC≡), 5.61 (dd, *J* = 1.3 and 15.9 Hz, 1 H, CH=C*H*C≡), 4.23–4.34 (m, 1 H, NH), 4.03–4.2 (m, 1 H, NCH), 1.56–1.69 (m, 1 H, CH), 1.41 (s, 9 H, $C(CH_3)_3$), 1.23–1.35 (m, 2 H, CH_2), 0.89 (d, 6 H, $(CH_3)_2$), 0.15 (s, 9 H, $Si(CH_3)_3$). Anal. ($C_{17}H_{31}O_2Si$) C, H, N.

5(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-7-methyl-3(*E*)-octenoic Acid (8). The procedure used was based on the method of Zweifel and Backlund[11] and similar to that described by Hann et al.[3] To a suspension of dicyclohexylborane (11.2 mmol), prepared according to the method of Uchida et al.,[20] at 0 °C was added a solution of **7** (3.15 g, 10.2 mmol) in THF (25 mL). The reaction mixture was allowed to warm up to room temperature where it was stirred for 1 h. The resulting solution was next cooled to 0 °C where it was treated sequentially with MeOH (5 mL), 30% H_2O_2 (5.5 mL, 48 mmol), and 10% NaOH (5.5 mL). The mixture was stirred for 30 min at 0 °C after which time an additional 5.5 mL of 10% NaOH was added. The mixture was washed with Et_2O. The aqueous layer was acidified with 10% citric acid and then extracted with EtOAc (3 × 25 mL). The combined EtOAc extracts were washed with saturated NaCl solution and then dried (Na_2SO_4). Removal of the EtOAc under reduced pressure provided 2.7 g (98%) of the β,γ-unsaturated acid as an oil: TLC (1-propanol/NH_4OH, 4:1) R_f 0.58, ($MeOH/CHCl_3$, 9:1) R_f 0.68; NMR ($CDCl_3$) δ 9.8–10.4 (br, 1 H, COOH), 5.4–5.9 (m, 2 H, CH=CH), 3.8–4.28 (m, 1 H, NCH), 3.1 (d, *J* = 5.5 Hz, CH_2CO), 1.0–1.8 (m with singlet at 1.44 for $C(CH_3)_3$, 12 H, CH_2CH), 0.9 (d, 6 H, $(CH_3)_2$).

A portion of the above oil was converted to its dicyclohexylammonium salt: mp 129–130 °C; $[\alpha]^{22}_D$ −17.6° (*c* 0.5, MeOH). Anal. ($C_{26}H_{38}N_2O_4$) C, H, N.

5(*S*)-Amino-7-methyl-3(*E*)-octenoic Acid (1). The protected β,γ-unsaturated amino acid **8** (0.27 g, 1 mmol) was treated with 10 mL of 4 N HCl in dioxane. The solution was stirred for 1 h and then stripped of HCl and dioxane under reduced pressure. The residue that was obtained was dissolved in H_2O and this solution placed on a 23.5 × 1.5 cm AG 50W-X8 (50–100 mesh) cation-exchange column. The amino acid was eluted from the column using 2 N NH_4OH. The fractions containing the desired product were stripped of NH_4OH and H_2O in vacuo. The residue that remained was triturated with acetone and collected to give 80 mg (47%) of **1**: mp 194–196 °C dec; $[\alpha]^{24}_D$ +44.8° (*c* 0.5, H_2O); NMR (D_2O) δ 6.0 (dt, *J* = 7.2 and 15.3 Hz, 1 H, CH=C*H*CH_2), 5.46 (dd, *J* = 8.1 and 15.3 Hz, 1 H, NCHC*H*=CH), 3.68–4.0 (m, 1 H, NCH), 2.98 (d, *J* = 7.2 Hz, 2 H, CH_2CO) 1.4–1.8 (m, 3 H, CH_2CH), 0.9 (m, 6 H, $(CH_3)_2$). Anal. ($C_9H_{17}NO_2$) C, H, N.

[5(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-7-methyl-3(*E*)-octenoyl]-L-valyl-L-phenylalanine Methyl Ester (9). The β,γ-unsaturated acid **8** (2.43 g, 8.96 mmol), 1-hydroxybenzotriazole (2.42 g, 17.9 mmol), and Val-Phe-OCH_3·HCl (2.82 g, 8.96 mmol) were dissolved in THF (30 mL). *N*-Methylmorpholine (0.9 g, 8.96 mmol) was added to the above solution. The resulting solution was cooled in an ice bath and then treated with a solution of dicyclohexylcarbodiimide (1.85 g, 8.96 mmol) in THF (20 mL). The solution was slowly allowed to warm up to room temperature where it was stirred overnight. The mixture was cooled in an ice bath and the precipitated dicyclohexylurea removed by filtration. The filtrate was stripped of THF in vacuo and the residue obtained partitioned between 1 M $NaHCO_3$ and EtOAc. The EtOAc layer was washed with 10% citric acid (two times) and then with saturated NaCl solution. The EtOAc layer was dried ($MgSO_4$) and then stripped of solvent under reduced pressure. The crude product obtained was recrystallized from a mixture of EtOAc/Et_2O to give 2.73 g (57%) of pure product: mp 170–171 °C; $[\alpha]^{22}_D$ −48.8° (*c* 1.04, CH_3OH); TLC ($CH_3OH/CHCl_3$, 1:9) R_f 0.81, ($CHCl_3$/EtOAc, 1:1) R_f 0.69; NMR ($CDCl_3$) δ 5.68 (dt, *J* = 15.7 and 6.58 Hz, 1 H, HC=C*H*CH_2), 5.54 (dd, *J* = 15.7 and 5.85 Hz, 1 H, NCHC*H*=CH), 2.99 (d, *J* = 6.94 Hz, 2 H, CH_2CO). Anal. ($C_{29}H_{45}N_3O_6$) C, H, N.

[5(*S*)-Amino-7-methyl-3(*E*)-octenoyl]-L-valyl-L-phenylalanine Methyl Ester Hydrochloride (Leuψ[*E*-CH=CH]-Gly-Val-Phe-OCH_3, 2). Protected peptide **9** (2.73 g, 5.13 mmol) was treated with 60 mL of 4 N HCl in dioxane for 1 h. The HCl and dioxane were removed in vacuo, and the residue was obtained dried in vacuo over KOH. The dried residue was triturated with Et_2O and collected to give 2.3 g (96%) of **2**: mp 115–118 °C; $[\alpha]^{22}_D$ −9.9° (*c* 0.89, CH_3OH); TLC ($CH_3OH/CHCl_3$, 1:9) R_f 0.51, (1-propanol/NH_4OH, 4:1) R_f 0.77; NMR (Me_2SO-d_6) δ 5.88 (dt, *J* = 15.43 and 6.9 Hz, 1 H, HC=C*H*CH_2), 5.47 (dd, *J* = 15.43 and 8.5 Hz, 1 H, NCHC*H*=CH). Anal. ($C_{24}H_{38}N_3O_4Cl$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-histidinyl-[5(*S*)-amino-7-methyl-3(*E*)-octenoyl]-L-valyl-L-phenylalanine Methyl Ester**

(19) Ahmed, M.; Barley, G. C.; Hearn, M. T. W.; Jones, E. R. H.; Thaller, V.; Yates, J. A. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1974.

(20) Uchida, K.; Utimoto, K.; Nozaki, H. *J. Org. Chem.* **1976**, *41*, 297.

(10). Boc-His-OH (0.32 g, 1.28 mmol), 1-hydroxybenzotriazole (0.34 g, 2.56 mmol), and 2 (0.6, 1.28 mmol) were dissolved in DMF (7 mL). The solution was cooled in an ice bath and then treated with *N*-methylmorpholine (0.13 g, 1.28 mmol) followed by a solution of dicyclohexylcarbodimide (0.26 g, 1.28 mmol) in CH_2Cl_2 (7 mL). The mixture was allowed to come to room temperature where it was stirred overnight. The mixture was filtered and the filtrate stripped of solvents in vacuo. The residue was partitioned between 1 M $NaHCO_3$ and EtOAc. The EtOAc layer was washed with 1 M $NaHCO_3$ followed by saturated NaCl solution. The EtOAc layer was then dried ($MgSO_4$). Removal of the solvent in vacuo yielded 0.74 g (86%) of the crude product. Recrystallization of this material from a mixture of EtOAc/MeOH provided 0.45 g of pure **10**: mp 191–192.5 °C; $[\alpha]^{25}_D$ −43.2° (*c* 1.23, CH_3OH); TLC ($CHCl_3$/MeOH, 9:1) R_f 0.36; (1-propanol/NH_4OH, 4:1) R_f 0.93; NMR ($CDCl_3$) δ 5.39 (br s, 2 H, CH=CH). Anal. ($C_{35}H_{52}N_6O_7$) C, H, N.

L-Histidinyl-[5(*S*)-amino-7-methyl-3(*E*)-octenoyl]-L-valyl-L-phenylalanine Methyl Ester Hydrochloride (His-Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH_3, 3). The *tert*-butoxycarbonyl group was removed from **10** (0.21 g, 0.31 mmol) by using 10 mL of 4 N HCl in dioxane. Workup in the same manner as described above for **2** yielded 188 mg (94%) of hygroscopic product: $[\alpha]^{22}_D$ −2.1° (*c* 1.04, CH_3OH); TLC (1-propanol/NH_4OH, 4:1) R_f 0.82; (butanol/HOAc/pyridine/H_2O, 5:1:3:4) R_f 0.74; NMR (Me_2SO-d_6) δ 5.48 (dt, *J* = 15.4 and 6.7 Hz, 1 H, CH=C*H*CH_2), 5.31 (dd, *J* = 15.4 and 6.7 Hz, 1 H, NCHC*H*=CH). Anal. ($C_{30}H_{46}N_6O_5Cl_2 \cdot {}^1/_2H_2O$) C, H, N.

Renin Inhibition Studies. The ability of compounds **2** and **3** to inhibit either hog kidney renin or human amniotic renin was measured by determining the inhibitory constant (K_i) of each compound. The K_i and the type of inhibition of each compound were determined through the use of Dixon plots.[21] Data for these plots were obtained by measuring the reaction velocities of hog kidney renin or human amniotic renin at two concentrations of porcine angiotensinogen (0.1 and 0.05 μM) in the presence of varying concentrations of each inhibitor.

The enzymatic assay was carried out in a manner identical with that described previously.[13,14] Reaction velocities for hog kidney renin were expressed as the number of nanomoles of angiotensin I generated per unit of enzyme per minute, while the reaction velocities for human amniotic renin were expressed as the number of nanomoles of angiotensin I generated per milliliter per hour. The average values of three determinations for each inhibitor concentration at each substrate level were used to generate a Dixon plot ($1/v$ vs. inhibitor concentration) for each compound tested. All lines were calculated by linear regression analysis. The −[I] value at the intersection of the two substrate lines gave the K_i value of each analogue. The competitive and noncompetitive nature of each inhibitor was assessed by whether the point of intersection of the two lines was above or on the *x* axis, respectively.

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(21) Dixon, M. *Biochem. J.* **1953**, *55*, 1970.

Antihypertensive Activities of Phenyl Aminoethyl Sulfides, a Class of Synthetic Substrates for Dopamine β-Hydroxylase

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Four sulfur-containing analogues of phenylpropylamine were synthesized and evaluated as substrates for dopamine β-hydroxylase (DBH) and monoamine oxidase (MAO). All four phenyl aminoethyl sulfides were shown to be good substrates for DBH whereas only the two analogues not possessing a methyl group α to the terminal amino group were substrates for MAO. All four analogues were tested for acute antihypertensive activity in an animal model for hypertension, the spontaneously hypertensive rat (SHR). Two of the analogues, both of which should partition readily across the blood–brain barrier, did not appreciably reduce systemic blood pressure in the 6-h testing period. However, the two analogues that were designed to be relatively restricted to peripheral sites of action caused a dramatic drop in blood pressure in SHR of 25% within 1–1.5-h postinjection, with the analogue designed to be both restricted to the periphery and MAO inactive, causing a more prolonged antihypertensive activity.

Phenyl 2-aminoethyl sulfide (PAES, **4a**) was designed, synthesized, and characterized in our laboratories as a synthetic substrate for dopamine β-hydroxylase (DBH; EC 1.14.17.1). May and Phillips[1] have previously shown that DBH readily oxygenates PAES to the corresponding sulfoxide (PAESO), and the kinetics and mechanism of this reaction have been investigated.[2] We have also demonstrated that PAES possesses indirect sympathomimetic activity in vivo and inhibits the reflexive tachycardia induced by vasodilatory antihypertensives.[3] These findings suggested that PAES or structurally-similar derivatives might be effective in reversibly modifying peripheral adrenergic function, and thus might be useful in the control of hypertension.

In the present study, we have synthesized three additional PAES derivatives designed to provide information on structure–activity relationships of this class of sulfur-containing compounds (Scheme I). Our rationale was to prepare MEPAES, **4b**, which would be protected from catabolism by amine oxidase enzymes, HOPAES, **4c**, which would be relatively restricted to peripheral sites of action by virtue of its reduced ability to penetrate the blood–brain barrier,[4] and HOMEPAES, **4d**, possessing both of these

(1) May, S. W.; Phillips, R. S. *J. Am. Chem. Soc.* **1980**, *102*, 5981–5983.
(2) May, S. W.; Phillips, R. S.; Mueller, P. W.; Herman, H. H. *J. Biol. Chem.* **1981**, *256*, 8470–8475.
(3) Herman, H. H.; Pollock, S. H.; Padgette, S. R.; Lange, J. R.; Han, J. H.; May, S. W. *J. Cardiovasc. Pharmacol.* **1983**, *5*, 725–730.
(4) Weiner, N. "The Pharmacological Basis of Therapeutics"; Goodman, A. G., Goodman, L. S., Gilman, A., Eds.; Macmillan; New York, 1980; pp 163–164.

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