N-(α-Hydroxyalkanoyl) Derivatives of Leu-Val-Phe-OCH_3 as Inhibitors of Renin

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The following N-(α-hydroxyalkanoyl) derivatives of Leu-Val-Phe-OCH_3 were synthesized and tested for their ability to inhibit human amniotic renin: D- and L-α-hydroxyisocaproyl-Leu-Val-Phe-OCH_3, D- and L-α-hydroxyisovaleryl-Leu-Val-Phe-OCH_3, L-2-hydroxy-3-phenylpropanoyl-Leu-Val-Phe-OCH_3, and D- and L-α-hydroxyphenylacetyl-Leu-Val-Phe-OCH_3. Analysis of the compounds through the use of Dixon plots showed all of the compounds to be competitive inhibitors of renin. All but D-α-hydroxyisovaleryl-Leu-Val-Phe-OCH_3 were found to be more active than the known tetrapeptide inhibitor Leu-Leu-Val-Phe-OCH_3 (1). The two most active compounds of the series were L-α-hydroxyisocaproyl-Leu-Val-Phe-OCH_3 (K_i = 0.23 mM) and L-α-hydroxyisovaleryl-Leu-Val-Phe-OCH_3 (K_i = 0.3 mM).

The renin–angiotensin system plays an important role in the regulation of blood pressure and blood volume in both physiological and pathophysiological states.[1-3] This system consists of a series of active polypeptides which are generated through a sequence of enzymatic reactions. Renin is the rate-limiting enzyme in this sequence of enzymatic reactions. It specifically cleaves the Leu^{10}–Leu^{11} peptide bond of angiotensinogen to give angiotensin I. Converting enzyme subsequently cleaves this decapeptide to give the potent vasoconstrictor, angiotensin II, which is in turn cleaved by an aminopeptidase to give the potent stimulator of aldosterone release, angiotensin III.

Because of the potential value that antagonists of the renin–angiotensin system have as pharmacological tools and as therapeutic agents, there has been an ongoing effort to develop inhibitors of the various components of this system. The reaction between renin and angiotensinogen has long been thought to offer a particularly attractive site for inhibiting the renin–angiotensin system, since an inhibitor of renin would provide a means of completely blocking this system. This would, in turn, help to clarify the ambiguities which have arisen in studies where converting enzyme inhibitors and angiotensin II receptor antagonists have been used. Although several inhibitors of renin have been developed,[4,5] their usefulness as either pharmacological tools or as therapeutic agents has been limited.

The present investigation was undertaken as part of an effort to develop new types of renin inhibitors. The approach which has been taken involves the synthesis of compounds which might mimic the postulated transition state of the renin–angiotensinogen reaction. Although renin's precise mechanism of catalysis remains unknown, studies which have been conducted on renin and related aspartyl proteases have resulted in a hypothetical model being proposed.[6-8] This model, which is depicted in Figure 1, postulates that a carboxyl group of one of the aspartic acid residues known to be present within the active site of the enzyme attacks the carbonyl group of the Leu^{10}–Leu^{11} peptide bond of angiotensinogen to give a tetrahedral intermediate wherein the Leu^{10} carbonyl group is transformed into a hydroxyl group and the Leu^{11} amide nitrogen begins to take on the characteristics of an amino nitrogen.

In one attempt to mimic this postulated renin–angiotensinogen transition state, the N-terminal leucyl residue of the known substrate analogue inhibitor Leu-Leu-Val-Phe-OCH_3 was replaced with the L-α-hydroxyisocaproyl residue. It was felt that the α-hydroxy moiety of this residue might simulate the hydroxy moiety which is thought to be formed when the aspartyl carboxyl group attacks the Leu^{10} carbonyl group during the enzymatic reaction. When preliminary studies showed this derivative to be a more active inhibitor of renin than the tetrapeptide after which it was modeled, several additional N-(α-hydroxyalkanoyl) derivatives of Leu-Val-Phe-OCH_3 were synthesized and tested in order to determine the stereochemical and structural requirements of this type of renin inhibitor.

Results and Discussion

Syntheses. The α-hydroxyalkanoyl derivatives 2–8 were prepared by coupling Leu-Val-Phe-OCH_3 to L- and D-α-hydroxyisocaproic acid, L- and D-α-hydroxyisovaleric acid, L-2-hydroxy-3-phenylpropanoic acid, and L- and D-α-hydroxyphenylacetic acid, respectively, using the 1-hydroxybenzotriazole/dicyclohexylcarbodiimide method of coupling. The α-hydroxyalkanoic acids which were not obtained from commercial sources were synthesized from the corresponding amino acids using nitrous acid.[9] The tetrapeptide Leu-Leu-Val-Phe-OCH_3 (1) was synthesized by first coupling benzyloxycarbonyl-L-leucine to Leu-Val-Phe-OCH_3 using the mixed anhydride method of coupling and then removing the benzyloxycarbonyl protecting group by hydrogenolysis. Although Ide et al.[10] reported a specific rotation of −69.8° for 1, the material which was obtained in this study gave a specific rotation of −37°. The reason for this discrepancy is not known. Racemization during the synthesis of 1 does not appear to be a problem, since the specific rotation of several of the intermediates, including the immediate precursor Z-Leu-Leu-Val-Phe-OCH_3, agreed with the values reported in the literature.[10]

Biological. Compounds 1–8 were all tested for their ability to inhibit human amniotic renin. Human amniotic prorenin which had been partially purified and activated with pepsin as described previously[11] served as the source of renin in this study. A double-reciprocal plot of the reaction velocity of the preparation at various concentrations of porcine angiotensinogen indicated that this renin

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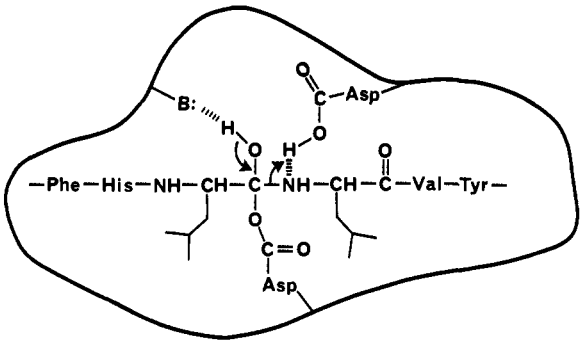
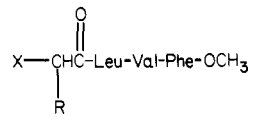


Figure 1. Hypothetical transition state of the renin–angiotensinogen reaction.

Table I. Renin Inhibitory Properties of N-(α-Hydroxyalkanoyl) Derivatives of Leu-Val-Phe-OCH_3



no.	X	R	chirality[a]	K_i, mM
1	NH_2	$CH_2CH(CH_3)_2$	L	1.14
2	OH	$CH_2CH(CH_3)_2$	L	0.23
3	OH	$CH_2CH(CH_3)_2$	D	0.60
4	OH	$CH(CH_3)_2$	L	0.30
5	OH	$CH(CH_3)_2$	D	1.78
6	OH	$CH_2C_6H_5$	L	0.67
7	OH	C_6H_5	L	0.53
8	OH	C_6H_5	D	0.98

[a] Chirality of the α-hydroxyalkanoyl portion of the polypeptide.

preparation had a V_{max} of 0.0426 μmol of angiotensin I mL^{-1} h^{-1} and a K_i of 0.4 μM.

All of the compounds tested were found to inhibit renin to some extent. The nature of each compound's inhibitory activity and its inhibitory constant (K_i) were determined with Dixon plots.[12] The Dixon plot for L-α-hydroxyisocaproyl-Leu-Val-Phe-OCH_3 (**2**) is shown in Figure 2 and illustrates the type of plots that were obtained. All of the compounds were found to be competitive inhibitors with inhibitory constants ranging from 0.23 to 1.78 mM (Table I).

Previous studies with the tetrapeptide inhibitor Leu-Leu-Val-Phe-OCH_3 (**1**) have yielded conflicting results. While Kokubu et al.[13] found **1** to be a competitive inhibitor of renin, Workman et al.[14] found it to be a noncompetitive inhibitor. In the present study, **1** was found to be a competitive inhibitor of human amniotic renin with a K_i of 1.14 mM. This result supports the original findings of Kokubu et al.[13]

The replacement of the N-terminal leucyl residue of **1** with α-hydroxyalkanoyl residues led to more active inhibitors in every case except one. The sole exception was D-α-hydroxyisovaleryl-Leu-Val-Phe-OCH_3 (**5**). This derivative had only two-thirds the activity of **1**. The two most active compounds were L-α-hydroxyisocaproyl-Leu-Val-Phe-OCH_3 (**2**) and L-α-hydroxyisovaleryl-Leu-Val-Phe-OCH_3 (**4**). The inhibitory constants of these two

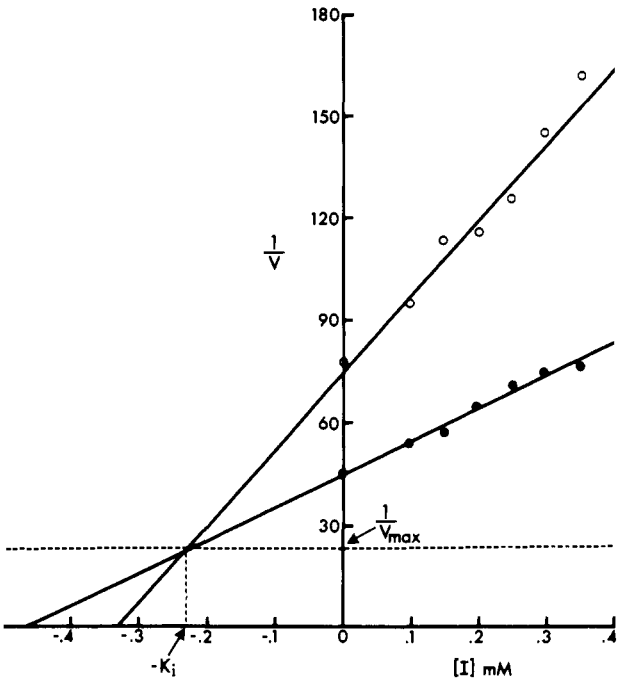


Figure 2. Dixon plot of the inhibition of human amniotic renin by L-α-hydroxyisocaproyl-Leu-Val-Phe-OCH_3 (**2**). V is the reaction velocity (μmol of angiotensin I mL^{-1} h^{-1}) determined at two concentrations of porcine angiotensinogen [0.2 μM (●) and 0.1 μM (○)] in the presence of varying concentrations of inhibitor. V_{max} = 0.0426 μmol of angiotensin I mL^{-1} h^{-1} and K_i = 0.23 mM.

derivatives were 0.23 and 0.30 mM, respectively. D-α-Hydroxyisocaproyl-Leu-Val-Phe-OCH_3 (**3**), L-2-hydroxy-3-phenylpropanoyl-Leu-Val-Phe-OCH_3 (**6**), and L-α-hydroxyphenylacetyl-Leu-Val-Phe-OCH_3 (**7**) were found to possess approximately twice the activity of **1**, while D-α-hydroxyphenylacetyl-Leu-Val-Phe-OCH_3 (**8**) was only slightly more active than **1**. This series of compounds did exhibit some degree of stereoselectivity in that those derivatives which contained L-α-hydroxyalkanoyl residues were more active than those with the respective D-α-hydroxyalkanoyl residues. Surprisingly, however, two of the D-α-hydroxyalkanoyl derivatives, compounds **8** and **3**, showed greater renin inhibitory activity than compound **1**.

Although previous studies[13] with several tetrapeptide substrate analogues suggested that a Leu–Leu bond with L-Leu at the N-terminal end was needed for renin inhibitory activity, the present study has shown that this is not the case in that substitutions with α-hydroxyalkanoyl residues can provide compounds which are even more active than the tetrapeptide inhibitors. For example, if one compares the activity of the α-hydroxyalkanoyl derivatives **2**, **3**, and **4** with their corresponding tetrapeptide derivatives Leu-Leu-Val-Phe-OCH_3 (**1**), D-Leu-Leu-Val-Phe-OCH_3, and Val-Leu-Val-Phe-OCH_3, one finds that in every case greater activity is seen with the α-hydroxyalkanoyl derivative. Compound **2** is five times as active as its corresponding tetrapeptide, compound **1**, and compounds **3** and **4** show better renin inhibitory activity than **1**, even though their corresponding tetrapeptides D-Leu-Leu-Val-Phe-OCH_3 and Val-Leu-Val-Phe-OCH_3 show no renin inhibitory activity.[13] Since the only difference between the α-hydroxyalkanoyl derivatives **2**–**4** and their corresponding tetrapeptide derivatives is the substitution of a hydroxyl moiety for the α-amino group, it is clear from these comparisons that the enhanced renin inhibitory activity seen with the α-hydroxyalkanoyl derivatives is due to the presence of the α-hydroxyl moiety. Whether or not

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this is because the α-hydroxyl moiety is actually simulating the proposed tetrahedral transition state of the renin–angiotensinogen reaction or because the hydroxyl group is able to interact with a group within the active site of renin with which the amino group cannot remains unknown. Regardless, the results indicate that modifications of the N-terminal amino acid residue of the tetrapeptide substrate analogue Leu-Leu-Val-Phe-OCH_3 (1) can yield compounds with greater renin inhibitory activity and suggest that even more active inhibitors can be developed through further modifications, modifications which will perhaps yield compounds that resemble even more closely the transition state of the renin–angiotensinogen reaction.

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. All analytical results were within ±0.4% of the theoretical values. NMR spectra were recorded on a Varian A-60D spectrometer. IR spectra were recorded on a Perkin-Elmer 281 spectrophotometer. Unless stated otherwise, all amino acids are of L configuration.

Materials. D-α-Hydroxy- and L-α-hydroxyphenylacetic acid, L-α-hydroxyisocaproic acid, and porcine angiotensinogen were obtained from Sigma Chemical Co., St. Louis, MO. [^{125}I]Angiotensin I, rabbit angiotensin I antibody, and angiotensin I standards were obtained from New England Nuclear, Boston, MA.

α-Hydroxyalkanoic Acids. L-α-Hydroxy- and D-α-hydroxyisovaleric acid, D-α-hydroxyisocaproic acid, and L-2-hydroxy-3-phenylpropanoic acid were synthesized from L- and D-valine, D-leucine, and L-phenylalanine, respectively, using a modification of the procedure of Iselin and Zeller.[9] In each instance, the appropriate amino acid (20 mmol) was dissolved in 30 mL of 1 N H_2SO_4. This solution was cooled in an ice bath and then treated gradually with a solution of $NaNO_2$ (30 mmol) in H_2O. The solution was stirred at 0 °C for 3 h and then at room temperature for 2 h, after which time the solution was extracted with Et_2O (3 × 10 mL). The combined ether extracts were washed with saturated NaCl solution and then dried ($MgSO_4$). Removal of the ether in vacuo yielded an oil which crystallized upon cooling in an ice bath. The crystals were suspended in petroleum ether (30–60 °C) and collected by suction filtration.

D-α-Hydroxyisocaproic acid: yield 70%, mp 75–77 °C; $[\alpha]^{25}_D$ +25.3° (c 1.1, 1 N NaOH) [lit.[9] mp 79–80 °C; $[\alpha]^{18}_D$ +26.1° (c 1.95, 1 N NaOH)].

L-α-Hydroxyisovaleric acid: yield 59%; mp 63–64 °C; $[\alpha]^{25}_D$ +16.2° (c 1.13, $CHCl_3$) [lit.[15] mp 65 °C; $[\alpha]_D$ +16.9° ($CHCl_3$)].

D-α-Hydroxyisovaleric acid: yield 47%; mp 61–63 °C; $[\alpha]^{25}_D$ −16.2° (c 1.27, $CHCl_3$).

L-2-Hydroxy-3-phenylpropanoic acid: yield 42%; mp 124–126 °C; $[\alpha]^{25}_D$ −21.4° (c 1.04, H_2O) [lit.[9] mp 125 °C].

Leu-Val-Phe-OCH_3·HCl. Benzyloxycarbonyl-L-leucine (2.75 g, 10.4 mmol), 1-hydroxybenzotriazole (2.8 g, 20.8 mmol), *N*-methylmorpholine (1.05 g, 10.4 mmol), and Val-Phe-OCH_3·HCl (3.5 g, 10.4 mmol) [mp 197–199 °C, $[\alpha]^{25}_D$ +19.9° (c 1.2, H_2O); lit.[16] mp 196.5–199 °C, $[\alpha]_D$ +19° (H_2O)] were dissolved in DMF (40 mL). The solution was cooled in an ice bath and then treated with a solution of DCC (2.14 g, 10.4 mmol) in DMF (20 mL). The solution was stirred at 0 °C for 1 h and then at room temperature for 3 h. After the mixture was cooled, the dicyclohexylurea was removed by filtration and the filtrate was stripped of solvent in vacuo. The residue obtained was triturated with 1 M $NaHCO_3$. The precipitate was collected, washed successively with H_2O, 10% HCl, and H_2O, and then dried under vacuum over P_2O_5 to give 4.7 g (86%) of Z-Leu-Val-Phe-OCH_3: mp 185–188 °C; $[\alpha]^{25}_D$ −10.5° (c 1.0, DMF).

The above material was dissolved in 150 mL of MeOH containing 250 mg of 5% Pd/C and 0.85 mL of concentrated HCl. Hydrogen was bubbled into the mixture until TLC showed no starting material remaining. After the mixture was filtered, the filtrate was stripped of solvent in vacuo. The residue which remained was triturated with Et_2O and collected to give 3.4 g (89%) of crude product, mp 205–207 °C. This material was recrystallized from isopropyl alcohol–Et_2O to give 2.6 g of Leu-Val-Phe-OCH_3·HCl: mp 206–208 °C; $[\alpha]^{25}_D$ −3.5° (c 2.0, EtOH). Anal. ($C_{21}H_{34}N_3O_4Cl$) C, H, N.

Leu-Leu-Val-Phe-OCH_3·HCl (1). Benzyloxycarbonyl-L-leucine (310 mg, 1.2 mmol) and *N*-methylmorpholine (120 mg, 1.2 mmol) were dissolved in THF (25 mL), and the solution was cooled to −30 °C. A solution of isobutyl chloroformate (160 mg, 1.2 mmol) in THF (5 mL) was added dropwise, being sure to maintain the temperature of the mixture below −20 °C. A solution of NEt_3 (120 mg, 1.2 mmol) and Leu-Val-Phe-OCH_3·HCl (500 mg, 1.2 mmol) in a 1:1 mixture of THF and H_2O was added next. The resulting mixture was stirred until the temperature reached 25 °C. The THF was removed in vacuo to give the product Z-Leu-Leu-Val-Phe-OCH_3 suspended in the acidic aqueous phase. The protected tetrapeptide was collected, washed with 10% $NaHCO_3$ and H_2O, and dried under vacuum to give 622 mg (81%) of Z-Leu-Leu-Val-Phe-OCH_3: mp 208–210 °C; $[\alpha]^{25}_D$ −26° (c 1.0, DMF) [lit.[10] mp 209.5–210 °C; $[\alpha]^{20}_D$ −25.4° (c 1.0, DMF)].

A portion of this material (600 mg, 0.94 mmol) was dissolved in MeOH (50 mL) and then added to a flask containing 100 mg of 5% Pd/C and 0.08 mL of concentrated HCl. Hydrogen was bubbled into the mixture until TLC analysis showed no starting material remaining (2 h). The mixture was filtered and the filtrate evaporated to dryness under reduced pressure. The residue was triturated with Et_2O and collected to give 447 mg (88%) of **1**: mp 251–252 °C (dec); $[\alpha]^{25}_D$ −37° (c 1.0, MeOH) [lit.[10] mp 250 °C (dec); $[\alpha]^{20}_D$ −69.8° (c 1.0, MeOH)]. TLC analysis on silica gel using a solvent system consisting of *n*-propyl alcohol–NH_3 (4:1) gave only a single spot (R_f 0.70) after either UV or ninhydrin visualization. Anal. ($C_{27}H_{45}N_4O_5Cl$) C, H, N.

***N*-(α-Hydroxyalkanoyl) Derivatives of Leu-Val-Phe-OCH_3.** L-α-Hydroxy- and D-α-hydroxyisocaproic acid, L- and D-α-hydroxyisovaleric acid, L-2-hydroxy-3-phenylpropanoic acid, and L- and D-α-hydroxyphenylacetic acid were coupled to Leu-Val-Phe-OCH_3 using the general procedure described below to give compounds **2–8**, respectively. To a cooled solution (0 °C) of the α-hydroxyalkanoic acid (0.8 mmol), Leu-Val-Phe-OCH_3·HCl (0.7 mmol), 1-hydroxybenzotriazole (0.8 mmol), and *N*-methylmorpholine (0.7 mmol) in THF (10 mL) was added a solution of dicyclohexylcarbodiimide (0.7 mmol) in THF (2 mL). The mixture was stirred at 0 °C for 24 h, after which time the dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness, and the residue obtained was partitioned between $CHCl_3$ and 1 M $NaHCO_3$. The $CHCl_3$ extract was washed with 1 M $NaHCO_3$ (10 mL), 10% HCl (10 mL), and saturated NaCl solution (10 mL). After the $CHCl_3$ layer was dried ($MgSO_4$), it was stripped of solvent in vacuo to give the desired *N*-(α-hydroxyalkanoyl) derivative. The chemical and physical properties of the *N*-(α-hydroxyalkanoyl) derivatives **2–8** are summarized below.

L-α-Hydroxyisocaproyl-Leu-Val-Phe-OCH_3 (2): yield 53% after recrystallization from EtOAc; mp 165–168 °C; $[\alpha]^{25}_D$ −25.8° (c 1.17, DMF). Anal. ($C_{27}H_{43}N_3O_6$) C, H, N.

D-α-Hydroxyisocaproyl-Leu-Val-Phe-OCH_3 (3): yield 62% after recrystallization from EtOAc; mp 160–162 °C; $[\alpha]^{25}_D$ +1.0° (c 1.12, DMF). Anal. ($C_{27}H_{43}N_3O_6$) C, H, N.

L-α-Hydroxyisovaleryl-Leu-Val-Phe-OCH_3 (4): yield 80%; mp 203–206 °C; $[\alpha]^{25}_D$ −27.7° (c 1.0, $CHCl_3$). Anal. ($C_{26}H_{41}N_3O_6$) C, H, N.

D-α-Hydroxyisovaleryl-Leu-Val-Phe-OCH_3 (5): yield 66% after recrystallization from a mixture of EtOAc and petroleum ether; mp 170–173 °C; $[\alpha]^{25}_D$ +3.9° (c 1.0, $CHCl_3$). Anal. ($C_{26}H_{41}N_3O_6$) C, H, N.

L-2-Hydroxy-3-phenylpropanoyl-Leu-Val-Phe-OCH_3 (6): yield 57% after recrystallization from a mixture of MeOH–H_2O; mp 171–172 °C; $[\alpha]^{25}_D$ −77.3° (c 0.83, MeOH). Anal. ($C_{29}H_{41}N_3O_6$) C, H, N.

L-α-Hydroxyphenylacetyl-Leu-Val-Phe-OCH_3 (7): yield 45% after recrystallization from EtOAc; mp 180–182 °C; $[\alpha]^{25}_D$ −33.8° (c 1.0, MeOH). Anal. ($C_{28}H_{39}N_3O_6$) C, H, N.

D-α-Hydroxyphenylacetyl-Leu-Val-Phe-OCH_3 (8): yield 77% after recrystallization from MeOH–H_2O; mp 125–126 °C;

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$[\alpha]^{25}_D$ −78.5° (c 0.8, MeOH). Anal. ($C_{28}H_{39}N_3O_6$) C, H, N.

Inhibition Studies. The ability of compounds 1–8 to inhibit renin was measured by determining the inhibitory constant (K_i) of each compound. Activated human amniotic prorenin served as the source of renin for these studies. The procedures for the purification and pepsin activation of human amniotic prorenin have been described previously.[11] The K_i of each compound was determined through the use of Dixon plots.[12] Data for these plots were obtained by measuring the reaction velocities of renin at two concentrations of porcine angiotensinogen (0.1 and 0.2 μM) in the presence of varying concentrations of each inhibitor ([1] = 0.25–1.25 mM; [2] = 0.1–0.35 mM; [3], [4], [6], and [7] = 0.3–0.9 mM; [5] and [8] = 0.5–1.5 mM).

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 inhibitor at 37 °C for 30 min. The renin and substrate solutions were made up in 0.1 M sodium phosphate buffer (pH 7.0), 10 mM EDTA, while the inhibitor solutions were made up in a 1:1 mixture of MeOH and H_2O. At the end of the incubation period, the enzymatic reaction was stopped by placing the incubation mixtures 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 using the procedure described below.

Reaction velocities were expressed as the number of micromoles of angiotensin I generated per milliliter per hour. Three determinations were made for each inhibitor concentration at each substrate level. A plot of $1/V$ vs. inhibitor concentration was made for each of the compounds tested. The lines were calculated by linear regression analysis. The $-[I]$ value at the intersection of the two substrate lines and the horizontal line at the height of $1/V_{max}$ gave the K_i of the compound (Table I).

The V_{max} of the renin preparation used was determined by measuring the reaction velocity of the preparation at various substrate concentrations (0.25–2 μM) and then plotting the results in a Lineweaver–Burk plot of $1/V$ vs. $1/S$.

Radioimmunoassay of Angiotensin I. A modification of the method described by Haber et al.[17] was used in this study. Samples (50 μL) of the incubation mixture to be assayed were mixed with the 50 μL of [^{125}I]angtioensin I (6500 cpm) in 0.1 M Tris–acetate buffer, pH 7.4, containing bovine serum albumin (4 mg/mL). Rabbit antiserum (100 μL) was added, and the mixture was allowed to equilibrate at 4 °C for 18 h. After this time, the mixture was treated with 0.8 mL of a charcoal suspension containing 4 g/L of charcoal and 0.39 g/L of dextran (average M_r 80 700) in 0.1 M Tris–acetate buffer, pH 7.4. The mixtures were thoroughly mixed and then centrifuged at 7000 rpm for 10 min. The supernatants were decanted and counted in a Packard 5230 gamma scintillation counter. Known amounts of angiotensin I varying from 5 to 250 pg were treated in a similar manner to produce a standard curve.

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Metabolic Depropargylation and Its Relationship to Aldehyde Dehydrogenase Inhibition in Vivo

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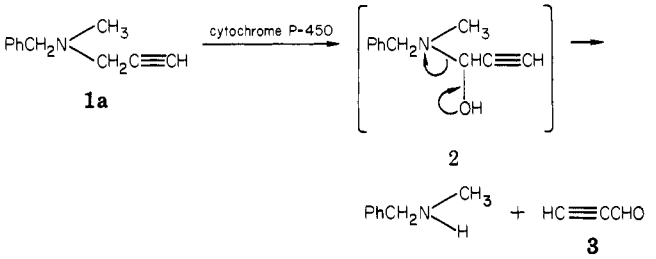
The relationship between metabolic depropargylation in vitro to inhibition of the low K_m aldehyde dehydrogenase (AlDH) of rat liver mitochondria in vivo was determined for a number of compounds bearing a propargyl substituent on nitrogen or oxygen. Only those compounds which enzymatically released the highly reactive α,β-acetylenic aldehyde, propiolaldehyde, when incubated in vitro with phenobarbital-induced rat liver microsomes, e.g., tripropargylamine (**4**), pargyline (1a), and *N*-propargylbenzylamine (**1b**), significantly elevated blood acetaldehyde levels when administered in vivo. Mitochondrial AlDH activity in these animals was correspondingly reduced to ≤20% that of control animals. Compounds that did not inhibit mitochondrial AlDH activity to this degree did not produce significant levels of propiolaldehyde when incubated with microsomes. Thus, for this series of compounds, metabolic depropargylation is a requirement for AlDH inhibitory activity in vivo.

The pargyline–ethanol reaction observed in vivo[1a,b] mimics the disulfiram–ethanol interaction[2] in the underlying biochemical–pharmacological mechanism, viz., the inhibition of the enzyme aldehyde dehydrogenase (AlDH). The consequence of this AlDH inhibition is the elevation of blood acetaldehyde (AcH) to levels that can trigger a catecholamine response.[3] Unlike disulfiram which is a direct inhibitor of this enzyme and is therefore effective both in vivo and in vitro, pargyline requires metabolic activation before inhibition can occur.[1b] We have identified this metabolic process as an N-depropargylation reaction mediated by the hepatic cytochrome P-450 enzymes.[4] The result of this N-depropargylation of pargyline is the formation of propiolaldehyde, a highly reactive α,β-acetylenic aldehyde which irreversibly inhibits the low

Scheme I



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