Sanborn recorder. Control blood-pressure readings were taken prior to and at 1, 3, 6, and 24 h after drug administration. All compounds were administered orally in acacia (6%). Six to twelve rats were used for each drug. Data were analyzed for statistical significance using Student's t test (paired comparison; $p < 0.05$).

Renal hypertensive dogs were surgically prepared by removing one kidney and wrapping the contralateral kidney in cellophane.[20] All dogs were used 6–8 weeks after recovery from the anesthesia.

(20) Page, I. *J. Am. Med. Assoc.* **1939**, *113*, 2046.

Acknowledgment. We gratefully acknowledge the contributions made by members of the pharmacology department, including J. Sztokalo, S. Urbano, E. Hinsch, A. Davidson, D. Hane, and E. Boff for providing data for the cardiovascular and behavioral studies. We thank the members of the physical chemistry department for the spectral and microanalytical results.

Synthesis and Biological Activity of a Ketomethylene Analogue of a Tripeptide Inhibitor of Angiotensin Converting Enzyme

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An analogue of a tripeptide inhibitor of angiotensin converting enzyme, Bz-Phe-Gly-Pro, has been synthesized in which the amide bond connecting phenylalanine and glycine has been replaced by a ketomethylene group. This nonpeptide analogue, **20**, shows more potent converting enzyme inhibiting activity, I_{50} = 0.07 μM, than Bz-Phe-Gly-Pro, I_{50} = 9.4 μM, or than the orally active D-3-mercapto-2-methylpropanoyl-L-proline (captopril, 1), I_{50} = 0.30 μM. Compound **20** has a K_i of 1.06×10^{-7} and either competitive or noncompetitive enzyme kinetics depending on what substrate is used in the converting enzyme assay. In tests for inhibition of angiotensin I induced contractions in the guinea pig ileum, **20** has one-tenth the activity of 1.

Angiotensin converting enzyme (ACE) is responsible for the conversion of the decapeptide angiotensin I to the potent vasopressor angiotensin II (an octapeptide). In addition, the enzyme's ability to hydrolyze the potent vasodepressor bradykinin is considered one of bradykinin's major pathways for inactivation.[1] These combined actions of the converting enzyme may play a role in blood pressure regulation.

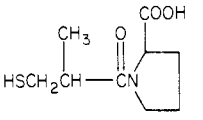
Inhibition of this enzyme by pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (SQ 20881) isolated from snake venom[2,3] lowers blood pressure in animal models of renovascular hypertension[4,5] and in humans with various forms of hypertension.[6-9] The lack of oral activity of this nonapeptide, however, has limited its use as a therapeutic drug for the treatment of hypertension.

Recently, Ondetti et al.[10] reported on the development of an extremely potent orally active inhibitor of the converting enzyme D-3-mercapto-2-methylpropanoyl-L-proline (SQ 14225 or captopril, 1). This compound has shown similar but more potent activity in angiotensin converting enzyme assays,[10,11] as well as in animal studies,[10,12,13] than its predecessor pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro.

Table I. Inhibition Results with Porcine Plasma Angiotensin Converting Enzyme

inhibitor	I_{50}, μM[a]
pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (SQ 20881)	0.22
pGlu-Lys-Trp-Ala-Pro (SQ 20475)	0.73
Phe-Ala-Pro (3)	1.4
Phe-Gly-Pro (11)	20
Bz-Phe-Gly-Pro (12)	9.4
Ts-Phe-Gly-Pro (8)	67
1	0.30
20	0.07

[a] All values are the average of results obtained in two or more experiments.



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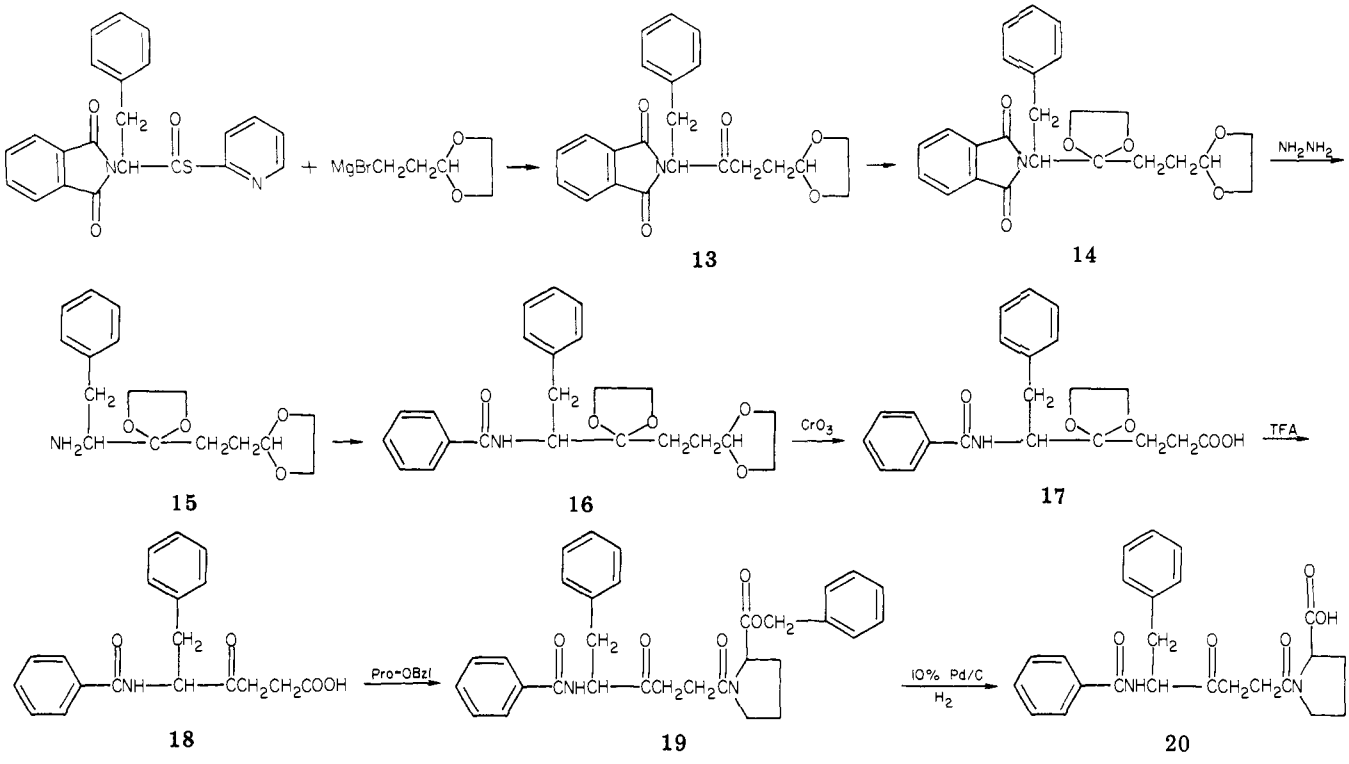
Scheme I

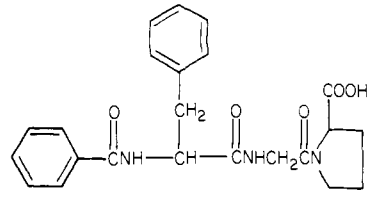


This new converting enzyme inhibitor holds great promise for treatment of hypertension in humans.[14,15]

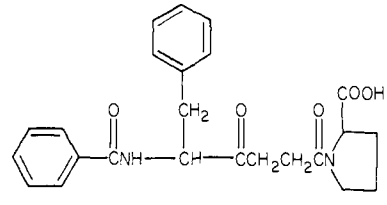
When we began our studies on the development of angiotensin converting enzyme inhibitors, two of the most potent inhibitors known were pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, previously mentioned, and pGlu-Lys-Trp-Ala-Pro (SQ 20475) that had also been isolated from snake venom.[2,3] Our approach for producing an orally active inhibitor was to use a peptide inhibitor as a model for the synthesis of a nonpeptide analogue. The new compound would have the orally labile peptide amide bonds of the model peptide replaced by ketomethylene moieties. It was hoped that these changes would not drastically alter the enzyme inhibition activity of the new analogue below the activity of the model peptide.

Our search for an appropriate peptide model for this approach began with examination of an article by Cushman et al.[16] describing the rabbit lung converting enzyme inhibition activity of numerous synthetic peptide analogues of both pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro and pGlu-Lys-Trp-Ala-Pro. We focused on a simple tripeptide, Phe-Ala-Pro, That in their enzyme assay had $^1/_{28}$th the inhibition activity of pGlu-Lys-Trp-Ala-Pro and about the same activity as pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro. When these three peptides were retested in our porcine plasma converting enzyme assay (Table I), the absolute and relative inhibition activities were somewhat different from those reported by Cushman et al.[16] These differences can probably be attributed to the different source of enzyme and a somewhat different assay procedure used in our case.

The I_{50} value obtained for Phe-Ala-Pro in our assay system showed it to be a good initial model peptide for our studies. A few peptide analogues of this tripeptide were made and tested against porcine plasma converting enzyme (Table I) to find an active tripeptide model with one less optical center. Such a change would simplify the synthesis of a ketomethylene analogue. Replacement of L-alanine with glycine yielded a tripeptide (11) with $^1/_{14}$th the inhibition activity of Phe-Ala-Pro. The benzoylated derivative of 11, Bz-Phe-Gly-Pro, was twice as active as 11 and



Bz-Phe-Gly-Pro



20

was chosen as the desired peptide model for our work. Since the amide bond between glycine and L-proline was a teriary amide bond and therefore unreactive with the various peptidases of the gut, it was not changed in our nonpeptide analogue. The target compound (**20**) had the amide bond between L-phenylalanine and glycine replaced by a ketomethylene group.

Chemistry. Peptide Syntheses. pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro and pGlu-Lys-Trp-Ala-Pro were gifts from the Squibb Institute. The other peptide derivatives (**3**, **8**, **11**, and **12**) were made by standard classical peptide synthesis techniques. Details are presented under Ex-

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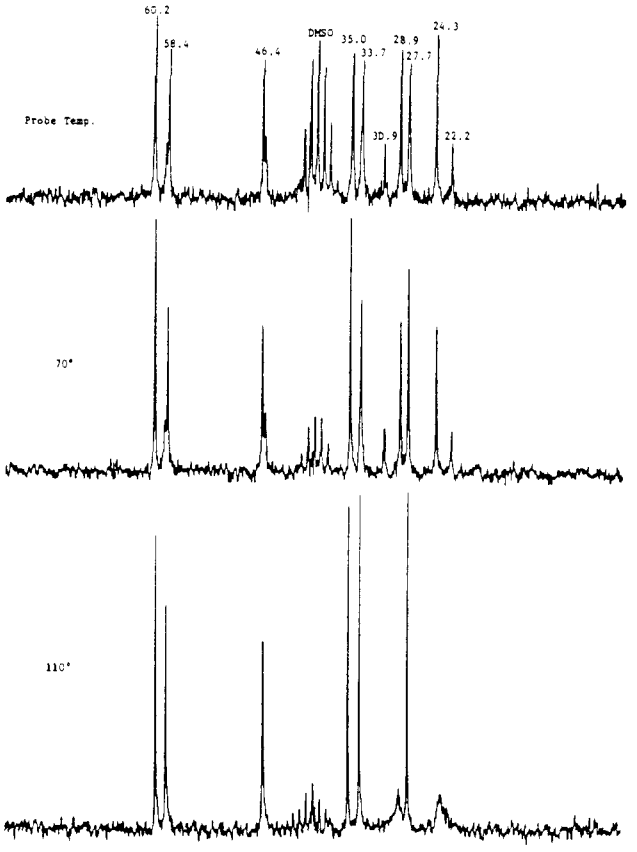

Figure 1. ^{13}C NMR spectra of **20** in Me_2SO-d_6 at probe temperature, 70 and 110 °C. The spectra were obtained on a Varian XL-100 FT (25.17 MHz) spectrometer.

perimental Section. D-3-Mercapto-2-methylpropanoyl-L-proline was also a gift from the Squibb Institute.

Synthesis of 20 (Scheme I). The key step in the synthesis was the preparation of an optically active ketomethylene analogue of the dipeptide L-phenylalanylglycine. The preparation of ketones by the reaction of Grignard reagents with 2-pyridyl thioates had been described by Araki et al.[17] Using this procedure the 2-pyridyl thioester of *N*-phthaloyl-L-phenylalanine[18] was reacted with 2-(2-magnesio-2-bromoethyl)-1,3-dioxolane to yield the keto-acetal **13**. Attempts to use the 2-pyridyl thioester of *N*-benzoyl-L-phenylalanine in this reaction gave a very low yield (1.5%) of the desired ketoacetal derivative of **13**. A similar low yield was obtained with the 2-pyridyl thioester of *N*-(carbobenzyloxy)-L-phenylalanine. It appears that thiopyridine esters with amide NH's are unstable to the Grignard reagent.

The ketone **13** was converted to its ethylene ketal **14** using a procedure described by W. S. Johnson et al.[19] Hydrazinolysis of the phthaloyl group of **14**, followed by benzoylation of the resulting amine **15**, gave the benzamido ketal **16**. Jones oxidation of **16** yielded the intermediary ketal acid **17** whose ketal protecting group was removed in 90% trifluoroacetic acid to give the keto acid **18**. Condensation of **18** with L-proline benzyl ester using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole yielded compound **19**. The target compound **20** was obtained by hydrogenolysis of the benzyl ester of **19**.

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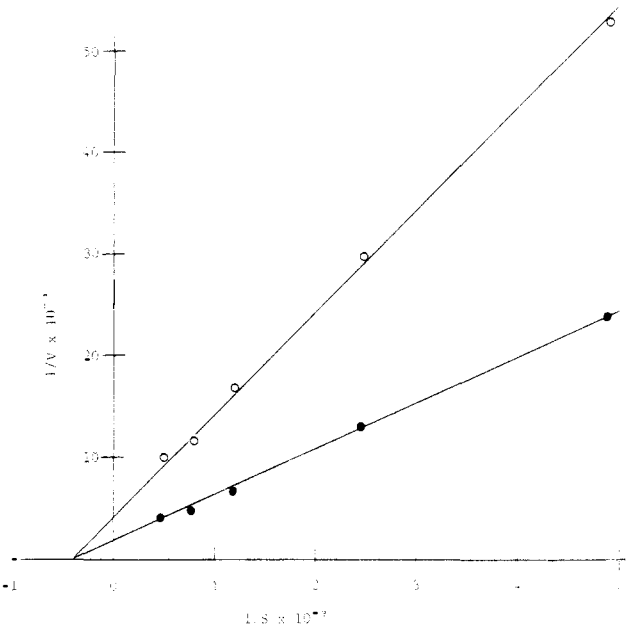

Figure 2. Double-reciprocal plots of the effect of **20** of porcine plasma converting enzyme activity using hippurylhistidylleucine as substrate: (●–●) enzyme activity without inhibitor; (○–○) enzyme activity in the presence of 0.9×10^{-7} M **20**. S = substrate concentration; V = reaction velocity.

Examination of the ^{13}C and ^1H NMR of both **19** and **20** showed the presence of extraneous peaks adjacent to the expected peaks for these compounds. Both compounds appeared pure by TLC and analytical high-performance LC in numerous solvent systems. Since the elemental analyses of these compounds were as expected, it was suspected that racemization had occurred either before or during the proline coupling, and two diastereomers were obtained.

A second explanation for the presence of the extraneous peaks was that the proline amide linkage in **19** and **20** exists in cis and trans forms as discussed by Dorman and Bovey.[20] If this was correct, then heating the sample should increase the exchange rate between the cis and trans forms, and a corresponding change in the ^{13}C NMR should be seen. When ^{13}C NMR spectra of **20** were taken in Me_2SO-d_6 at increasing temperatures (Figure 1), the peaks at δ 22.2 and 24.3, as well as at δ 28.9 and 30.9, began to collapse toward one another until at 110 °C they appeared as very broad peaks on either side of the peak at δ 27.7. The cis–trans isomerism of the proline amide bond is therefore responsible for the extraneous peaks observed.

Biological Activity and Discussion. The I_{50} values for **1** and **20** are included in Table I. It is important to note that **20** has an I_{50} 130 times lower than the tripeptide derivative **12** that it was modeled after. The fact that **12** can act as a substrate and be cleaved by angiotensin converting enzyme may be one contributing factor to its lower activity. Compound **20** of course cannot be cleaved by the converting enzyme.

Compound **20** caused a 50% inhibition of the enzyme at a fourfold lower molar concentration than **1**. Enzyme kinetic studies of the two inhibitors using Hip-His-Leu as the substrate gave K_i values for **1** and **20** of 7.15×10^{-8} and 1.06×10^{-7} M, respectively. The I_{50} and K_i values obtained in our enzyme assay system for **1** are higher than those reported by Cushman et al.[11] As explained previously, we attribute this discrepancy to the differences in enzyme

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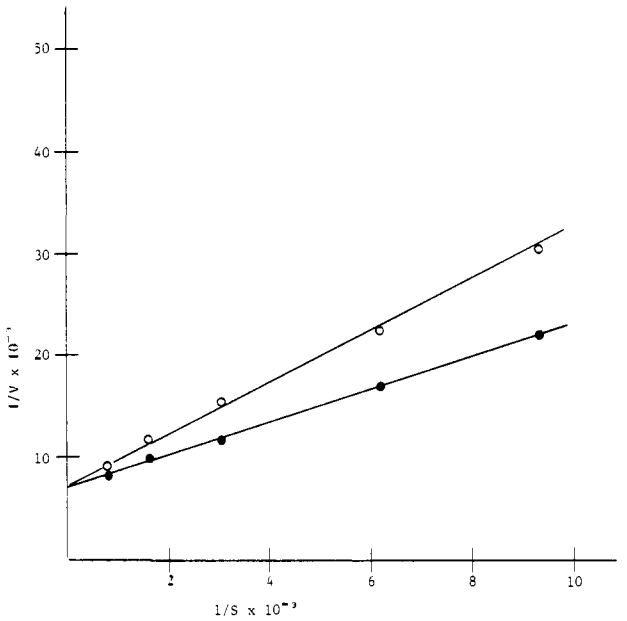

Figure 3. Double-reciprocal plots of the effect of **20** on porcine plasma converting enzyme activity using angiotensin I as substrate: (●–●) enzyme activity without inhibitor; (○–○) enzyme activity in the presence of 4.2×10^{-7} M **20**. S = substrate concentration; V = reaction velocity.

source and assay procedures. A plot of the kinetic data according to the method of Lineweaver and Burk[21] showed **1** to be a competitive inhibitor as previously reported.[11] Compound **20** (Figure 2), on the other hand, had noncompetitive enzyme kinetics.

Examination of enzyme kinetic studies on the various venom peptide converting enzyme inhibitors[22] showed that, whereas most of these peptides, including pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, were competitive inhibitors with the synthetic substrate Hip-His-Leu, the pentapeptide pGlu-Lys-Trp-Ala-Pro was a noncompetitive inhibitor with this substrate. In addition, it was reported that when angiotensin I was used as the substrate for the kinetic studies all the venom peptides tested, including pGlu-Lys-Trp-Ala-Pro, were competitive inhibitors.

Kinetic studies of **20** with angiotensin I as substrate (Figure 3) showed **20** to be a competitive inhibitor of this substrate with $K_i = 8.7 \times 10^{-7}$. Since **20** was modeled indirectly after pGlu-Lys-Trp-Ala-Pro, it is not surprising that it shows the same mixed type of enzyme kinetics. This indicates that both pGlu-Lys-Trp-Ala-Pro and **20** bind at the same region of the angiotensin converting enzyme active site. This region of the active site may differ somewhat from the binding position of either Hip-His-Leu, **1**, or pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro.

If on the other hand one looks at the hypothetical active site of angiotensin converting enzyme (Figure 4) as proposed by Ondetti et al.,[3,10] compound **20** would be expected to fit this site quite well. Since the synthetic converting enzyme substrate Hip-His-Leu presumably binds to this same active site, it is hard to explain why **20** and Hip-His-Leu do not compete for binding to the enzyme.

Compounds **20** and **1** were also compared for their ability to inhibit the angiotensin I induced contractions of guinea pig ileum. Table II shows that in this assay **20** had one-tenth the activity of **1**. The discrepancy between the enzyme and guinea pig ileum results for **20** and **1** may

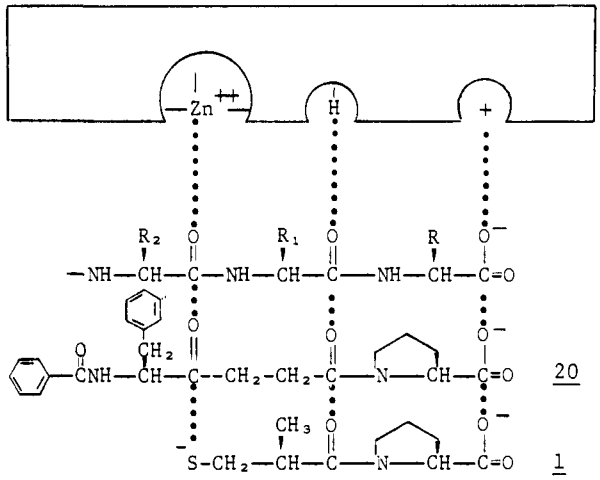

Figure 4. Schematic representation of the proposed[3,10] active site of angiotensin converting enzyme.

Table II. Inhibition of Guinea Pig Ileum Contractions Caused by 25 ng/mL Angiotensin I

inhibitor	IC_{50},[a] ng/mL
1	50
20	500

[a] These values are the average of results obtained in three experiments.

indicate that **20** does not pass through cell membranes as readily as **1**.

Further biological studies on **20** and its chemical derivatives are planned. Special emphasis will be placed on in vivo experiments to investigate the oral activity of these compounds.

Experimental Section

General Methods. All optically active amino acids used were of the L configuration. Standard abbreviations were used for the peptide derivatives, i.e., Cbz = benzyloxycarbonyl, Bzl = benzyl, Bz = benzoyl, Ts = *p*-toluenesulfonyl, Phe = phenylalanine, Gly = glycine, Pro = proline. Melting points were determined on a Thomas-Hoover Uni-melt and are uncorrected. Optical rotations were measured using a Perkin-Elmer 141 automatic polarimeter. Mass spectrums were taken on an LKB 9000 GC–MS spectrometer. ^{1}H NMR spectra were taken using a Varian EM390 spectrometer. ^{13}C NMR spectra were taken on a Varian XL-100 FT (25.17 MHz) spectrometer. Thin-layer chromatography was carried out on Uniplates from Analtech coated with 250 μm of silica gel GF. Evaporations were performed at 40 °C under house vacuum on a Büchi Rotavapor unless otherwise stated. Elemental analyses were conducted by Eric Meier, Stanford University, Palo Alto, Calif. Preparative high-performance LC was performed using the Waters Prep LC/System 500 using silica gel cartridges. The existence of solvents of crystallization was confirmed by ^{1}H NMR whenever possible.

Cbz-Phe-Ala-Pro-OBzl (2). A mixture of L-proline benzyl ester (0.133 g, 0.648 mmol), *N*-(benzyloxycarbonyl)-L-phenylalanyl-L-alanine (0.240 g, 0.648 mmol), and *N*-hydroxysuccimide (74.6 mg, 0.648 mmol) were dissolved and stirred in dry DMF (6.0 mL) at ice-bath temperature. A solution of dicyclohexylcarbodiimide (0.134 g, 0.648 mmol) in dry DMF (2.0 mL) was added. The resulting mixture was stirred for 1 h at 5 °C and 16 h at ambient temperature. The mixture was then cooled to 5 °C and filtered. Evaporation of the filtrate under vacuum (1 mmHg) at 40 °C yielded a yellow residue. This residue was mixed with EtOAc (5 mL), cooled to 5 °C, and filtered. The filtrate was diluted to 20 mL with EtOAc and washed successively with ice-cold 2 N HCl (15 mL), 0.1 N NaOH (15 mL), and H_2O (15 mL). The EtOAc layer was dried (Drierite) and evaporated to a yellow gum: yield 0.317 g. This gum was purified by preparative silica gel TLC on 20 × 40 cm plates. The plates were developed once in $CHCl_3$ and then with 3% MeOH in $CHCl_3$. The major UV-

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(22) H. S. Cheung and D. W. Cushman, *Biochim. Biophys. Acta*, **293**, 451 (1973).

absorbing band was extracted with $CHCl_3$ and concentrated to an off-white hygroscopic solid foam, **2**: yield 0.264 g (73.1%); TLC R_f 0.30 ($CHCl_3$–Et_2O, 2:1); ^1H NMR ($CDCl_3$) δ 1.30 (d, 3, J = 6.0 Hz, Ala CH_3), 2.03 (m, 4, Pro CH_2's), 3.08 (d, 2, J = 6.0, Phe CH_2), 3.63 (m, 2, Pro NCH_2), 5.03 (s, 2, Bzl or Cbz CH_2), 5.10 (d, 2, J = 2.0, Bzl or Cbz CH_2), 7.17, 7.23, 7.28 (3 s, 15, 3 phenyl rings). Anal. ($C_{32}H_{35}N_3O_6 \cdot 0.5H_2O$) C, H, N.

Phe-Ala-Pro (3). A mixture of **2** (0.239 g, 0.428 mmol), glacial acetic acid (17 mL), and 10% palladium on carbon (240 mg) was stirred under 1 atmosphere of hydrogen for 4 h. The mixture was filtered through Celite and evaporated to a light yellow syrup. This syrup was stirred vigorously with EtOAc (20 mL) and H_2O (15 mL) for 15 min. The H_2O layer was separated and evaporated to **2** as a white solid foam: yield 0.135 g (80.3%); MS ($2Me_4Si - M^+$, 477). Anal. ($C_{17}H_{23}N_3O_4 \cdot {}^1/_3CH_3COOH \cdot 2H_2O$) C, H, N.

Cbz-Phe-Gly-OEt (4). A mixture of glycine ethyl ester (6.69 g, 64.9 mmol), *N*-hydroxysuccinimide (7.47 g, 64.9 mmol), and *N*-Cbz-L-Phe (19.4 g, 64.9 mmol) was stirred in dry DMF (200 mL) in an ice bath. Dicyclohexylcarbodiimide (13.4 g, 64.9 mmol) was dissolved in dry DMF (60 mL) and added to the reaction mixture. The reaction was stirred at ice-bath temperature for 30 min and at ambient temperature for 17 h. The mixture was filtered and the filtrate was evaporated at 45 °C at 1 mmHg pressure. The resulting yellow residue was dissolved in $CHCl_3$ (350 mL) and washed successively with 2 N HCl (350 mL), 1 N NaOH (350 mL), and H_2O (350 mL). The $CHCl_3$ layer was dried (Drierite) and evaporated to a white solid. This solid was dissolved in DMF–H_2O (3:1, 200 mL) and filtered to remove some remaining dicyclohexylurea. The filtrate was evaporated at 45 °C at 1 mmHg pressure to a white solid. This solid was dissolved in $CHCl_3$ (150 mL) and washed with H_2O (200 mL). The $CHCl_3$ layer was dried (Drierite) and evaporated to a white solid: yield 24.1 g. This solid was crystallized from EtOAc–petroleum ether (bp 30–60 °C) to give white needles of **4**: yield 19.6 g; mp 110–111 °C. Further crystallization of the mother liquor yielded more **4**: yield 1.07 g (total yield, 83.0%); mp 110–111 °C (lit.[23] mp 108–110 °C). Anal. ($C_{21}H_{24}N_2O_5$) C, H, N.

Ts-Phe-Gly-OEt (5). To a solution of **4** (34.5 g, 89.7 mmol) in absolute ethanol (1 L) was added 10% palladium on carbon, 17.2 g, and cyclohexene, 69 mL. This mixture was refluxed for 15 min, cooled to room temperature, and filtered through Celite. The filtrate was evaporated to the amino intermediate as a yellow syrup. The syrup was dissolved in dry pyridine (860 mL), cooled in an ice bath with stirring, and *p*-toluenesulfonyl chloride (18.8 g, 98.6 mmol) was added. The mixture was stirred at ice bath temperature for 30 min and at room temperature for 42 h. The orange solution was then evaporated at 45 °C (azeotroping with toluene) to an orange syrup. This syrup was dissolved in $CHCl_3$ (1 L) and washed successively with ice-cold 2 N HCl (1 L), saturated $NaHCO_3$ solution (1 L), and H_2O (1 L). The $CHCl_3$ layer was dried (Drierite) and evaporated to a pale orange syrup. This syrup was dissolved in absolute EtOH (350 mL), treated with activated charcoal, and evaporated to a solid. This solid was mixed with ether and reevaporated to an off-white solid. This solid was slurried in ether and collected by filtration and dried as **5**: yield 25.0 g (68.7%); mp 104–106 °C (lit.[24] mp 105–107 °C). Anal. ($C_{20}H_{24}N_2O_5S$) C, H, N, S.

Ts-Phe-Gly (6). A solution of **5** (8.00 g, 19.8 mmol) in acetone (70 mL) was stirred at room temperature while 1 N NaOH solution (20.8 mL, 20.8 mequiv) was added. After stirring for 24 h, the mixture was acidified with 2 N HCl. The mixture was washed into a separatory funnel with H_2O (110 mL) and $CHCl_3$ (300 mL). After shaking the solution, the $CHCl_3$ layer was separated and the H_2O layer was further extracted with $CHCl_3$ (50 mL). The two $CHCl_3$ extracts were combined, dried (Na_2SO_4), and evaporated to an off-white solid foam. This foam was crystallized from $CHCl_3$–ether to white crystalline **6**: yield 6.48 g (87.0%); mp 148–149 °C; ^1H NMR ($CDCl_3$–CD_3OD, 3:1) δ 2.40 (s, 1, tosyl CH_3). Anal. ($C_{18}H_{20}N_2O_5S$) C, H, N, S.

Ts-Phe-Gly-Pro-OBzl (7). A mixture of **6** (0.606 g, 1.61 mmol), L-proline benzyl ester (0.330 g, 1.61 mmol), and *N*-hydroxysuccinimide (0.185 g, 1.61 mmol) was stirred in dry DMF (8 mL), and dicyclohexylcarbodiimide (0.332 g, 1.61 mmol) in dry DMF (4 mL) was added. After stirring at room temperature for 16 h, the mixture was cooled in an ice bath and filtered. The filtrate was evaporated at 45 °C under 1 mmHg vacuum to a yellow solid. This solid was dissolved in $CHCl_3$ (30 mL) and washed successively with 2 N HCl (30 mL), 1 N NaOH (30 mL), saturated NaCl solution (30 mL), and H_2O (30 mL). The $CHCl_3$ layer was dried (Drierite) and evaporated to a white solid. This solid was slurried in refluxing EtOAc (15 mL) and cooled, and the white solid **7** was collected by filtration: yield 0.671 g (74.0%); mp 173–174 °C; ^1H NMR ($CDCl_3$) δ 2.37 (s, 1, tosyl CH_3), 5.17 (s, 1, benzyl ester CH_2). Anal. ($C_{30}H_{33}N_3O_6S$) C, H, N, S.

Ts-Phe-Gly-Pro (8). A mixture of **7** (1.50 g, 2.66 mmol), 10% palladium on carbon (1.50 g), cyclohexene (2.70 mL, 26.6 mmol), and MeOH (70 mL) was refluxed for 20 min. The mixture was cooled to room temperature and filtered through Celite. The filtrate was evaporated to a white gummy foam. This foam was crystallized from $CHCl_3$–ether to white solid **8**, mp softens 112–125 °C, melts 128–130 °C. Anal. ($C_{23}H_{27}N_3O_6S$) C, H, N, S.

Cbz-Phe-Gly (9). A solution of **4** (1.00 g, 2.60 mmol) in acetone (10 mL) was stirred and 1 N NaOH (2.73 mL, 2.73 mequiv) was added. After the solution was stirred at room temperature for 1 h, H_2O (12 mL) was added and the clear mixture was accidified with 2 N HCl. The resulting solution was cooled to 5 °C and the white needles of **9** which formed were collected by filtration and dried: yield 0.617 g; mp 150 °C. The mother liquor yielded additional crystalline **9**; yield 0.197 g (total yield 87.8%); mp 150 °C. Anal. ($C_{19}H_{20}N_2O_5$) C, H, N.

Cbz-Phe-Gly-Pro-OBzl (10). A mixture of **9** (0.521 g, 1.46 mmol), L-proline benzyl ester (0.300 g, 1.46 mmol), *N*-hydroxysuccinimide (0.168 g, 1.46 mmol), and dry DMF (6 mL) was cooled with stirring in an ice bath and dicyclohexylcarbodiimide (0.301 g, 1.46 mmol) in DMF (4 mL) was added. The mixture was stirred at ice-bath temperature for 15 min and at room temperature for 16 h. After cooling in an ice bath, the mixture was filtered, and the filtrate was evaporated under 1 mmHg vacuum to a yellow residue. This residue was mixed with EtOAc (5 mL), cooled in an ice bath, and filtered. The filtrate was diluted to 25 mL with EtOAc and washed successively with 2 N HCl (25 mL), 1 N NaOH (25 mL), and 20% NaCl solution (50 mL). The EtOAc layer was dried (Drierite) and evaporated to a white solid foam. This foam was crystallized from EtOAc to white solid **10**: yield 0.645 g (81.2%); mp 122–124 °C; ^1H NMR ($CDCl_3$) δ 5.03, 5.13 (2 s, 2 × 2, benzyloxy CH_2's), 7.17, 7.27, 7.30 (3 s, 15, 3 phenyls). Anal. ($C_{31}H_{33}N_3O_6$) C, H, N.

Phe-Gly-Pro (11). A mixture of **10** (0.400 g, 0.736 mmol), 10% palladium on carbon, 0.400 g, and glacial acetic acid (30 mL) was stirred under 1 atmosphere of hydrogen for 17 h. The mixture was filtered through Celite and evaporated (azeotroping with EtOH) to a white solid. This solid was slurried in absolute EtOH, collected by filtration, and dried to a white hygroscopic powder, **11**: yield 0.203 g (86.4%); mp 213 °C dec. Anal. ($C_{16}H_{21}N_3O_4 \cdot 0.5H_2O$) C, H, N.

Bz-Phe-Gly-Pro (12). A small sample of **11** (76.0 mg, 0.238 mmol) was mixed with H_2O (0.8 mL) and 10 N NaOH (0.076 mL, 0.76 mequiv). The resulting clear solution was cooled in an ice bath and benzoyl chloride (0.027 mL, 0.233 mmol) was added. The reaction mixture was stirred at 5 °C for 5 min and at room temperature for 40 min. The mixture was then acidified with 9 N HCl. A gummy white precipitate formed and the mixture was diluted with H_2O (7 mL) and extracted with $CHCl_3$ (10 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to a white foamy film. This film was dissolved in EtOH (1.0 mL) and added to stirring ether (15 mL). A white precipitate formed immediately and on cooling at −20 °C overnight white globular crystals also formed. The white solid that was present was collected by filtration. More solid was obtained by crystallization of the filtrate from EtOH–ether. These two portions of crude solid product were combined and crystallized from $CHCl_3$ to a white cottony solid, **12**: yield 21.7 mg (21.8%); mp 205–206 °C. Anal. ($C_{23}H_{25}N_3O_5$) C, H, N.

2-(3-Oxo-5-phenyl-(4*S*)-4-phthalimidopentyl)-1,3-dioxolane (13). In a nitrogen atmosphere a solution of 2-(2-bromoethyl)-1,3-dioxolane (from Tridom Chemical; 92.3 g, 0.510 mol) in dry THF (660 mL) was added over 1.5 h to a stirred mixture

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of magnesium turnings (16.5 g, 0.679 mol) in dry THF (25 mL) at 30–35 °C. The mixture was then stirred for 1 h more at 30–35 °C and transferred with filtering to a dry addition funnel. In a nitrogen atmosphere one-third of this solution was added over 15 min to a stirred solution of *N*-phthaloyl-L-phenylalanine-2-mercaptopyridyl thioester[18] (66.0 g, 0.170 mol) in dry THF (660 mL) with external cooling to maintain a 30–35 °C reaction temperature. After the addition the mixture was stirred at ambient temperature for 2 h. The remainder of the Grignard solution was added in two equal portions at 30–35 °C, followed by 2 h ambient temperature stirring as described above. Stirring was then continued at ambient temperature overnight (16 h). The next day saturated NH_4Cl solution was added until the cloudy mixture became clear (~85 mL). After standing for 4 h the mixture was filtered and the filtrate was evaporated to an orange residue. The residue was mixed with EtOAc (1 L) and was extracted with 0.1 N NaOH (2 × 1 L) and saturated NaCl solution (1 L). The EtOAc solution was dried (Drierite) and evaporated to an orange syrup: yield 91.0 g. This syrup was eluted through a silica gel (ICN no. 404526, Activity III, 910 g) column with CH_2Cl_2. After a 400-mL forerun the next 2750 mL of effluent was collected and evaporated to a light yellow residue: yield 32.5 g. This residue was further purified by preparative high-performance LC on a silica gel cartridge using ether–petroleum ether (bp 30–60 °C), 1:1, as the eluting solvent. The fractions corresponding to product R_f 0.64 ($CHCl_3$–Et_2O, 2:1) were combined and evaporated to a pale yellow residue. This residue was crystallized from ether–petroleum ether (bp 30–60 °C) to white crystalline **13**: yield 22.3 g (35%); mp 96–97 °C; $[\alpha]^{21}_D$ −154° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.00 (m, 2, CH_2), 2.60 (t, 2, *J* = 7.0 Hz, CH_2), 3.50 (2 d, 2, *J* = 6 and 11 Hz, CH_2), 3.82 (m, 4, acetal CH_2's), 4.83 (t, 1, J = 4, acetal CH), 5.04 (dd, 1, *J* = 6 and 11 Hz, CH), 7.00 (s, 5, phenyl), 7.67 (m, 4, phthalimide). Anal. ($C_{22}H_{21}NO_5$) C, N, N.

2-[2-(1,3-Dioxolan-2-yl)ethyl]-2-[(1*S*)-1-phthalimido-2-phenylethyl]-1,3-dioxolane (14). A mixture of **13** (1.00 g, 2.63 mmol), dry toluene (50 mL), ethylene glycol (9 mL), and *p*-toluenesulfonyl chloride (40 mg) was stirred and slowly distilled. Each time 10 mL of distillate had accumulated an additional 10 mL of dry toluene was added to the reaction mixture. After 11 h of this procedure, the reaction mixture was allowed to cool and the ethylene glycol was separated from the toluene in a separatory funnel. The toluene layer was evaporated to a yellow syrup. This syrup was dissolved in $CHCl_3$ (50 mL) and washed successively with 0.1 N NaOH (50 mL) and H_2O (50 mL). The $CHCl_3$ layer was dried (Drierite) and evaporated to a pale yellow gum: yield 1.11 g. This gum was crystallized from ether–petroleum ether (bp 30–60 °C) to white needles of **14**: yield 0.644 g (58.0%); mp 105–108 °C, $[\alpha]^{20}_D$ −111° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.77 (m, 2, CH_2), 2.10 (m, 2, CH_2), 3.90 (m, 4, acetal CH_2's), 4.04 (m, 4, ketal CH_2's), 4.90 (t, 1, *J* = 4.5 Hz, acetal CH), 7.13 (s, 5, phenyl), 7.63 (m, 4, phthalimide). Anal. ($C_{24}H_{25}NO_6$) C, H, N.

2-[2-(1,3-Dioxolan-2-yl)ethyl]-2-[(1*S*)-1-benzamido-2-phenylethyl]-1,3-dioxolane (16). A mixture of **14** (2.00 g, 4.72 mmol), absolute ethanol (120 mL), and 97% hydrazine (1.05 mL, 33.0 mmol) was stirred at reflux for 15 h. The mixture was then evaporated to a semisolid residue. This residue was washed into a separatory funnel with $CHCl_3$ (150 mL) and 0.3 N NaOH (200 mL). After shaking the solution, the $CHCl_3$ layer was separated, washed with H_2O (200 mL), dried (Drierite), and evaporated to the amine **15** as a clear syrup: yield 1.30 g. The amine was dissolved in pyridine (75 mL) and cooled in an ice bath with stirring while benzoyl chloride (0.620 mL, 5.35 mmol) was added. The mixture was stirred at ice-bath temperature for 30 min and at room temperature for 3 days. Then the yellow solution was evaporated at 50 °C to a yellow residue. This residue was azeotroped twice with toluene (2 × 100 mL) to an off-white partially solid residue. The residue was dissolved in $CHCl_3$ (200 mL) and washed successively with ice-cold 0.1 N HCl (200 mL), 0.1 N NaOH (200 mL), and H_2O (200 mL). The $CHCl_3$ layer was dried (Drierite) and evaporated to a yellow solid: yield 1.69 g. Solidification of this solid from $CHCl_3$–Et_2O yielded white powder **16**: yield 1.37 g (72.9% based on **14**); mp 146–147 °C; $[\alpha]^{21}_D$ −54° (*c* 1.0, $CHCl_3$); MS (M^+ 397). Anal. ($C_{23}H_{27}NO_5$) C, H, N.

(5*S*)-5-Benzamido-4-oxo-6-phenylhexanoic Acid (18). A solution of **16** (1.30 g, 3.27 mmol) in acetone (325 mL) was cooled in an ice bath with stirring and CrO_3 (3.27 g, 32.7 mmol) in 35% H_2SO_4 (100 mL) was added. The resulting dark orange reaction mixture was stirred at room temperature for 1 h. The green solution was then poured into a separatory funnel containing $CHCl_3$ (650 mL) and H_2O (650 mL). After shaking the solution, the $CHCl_3$ layer was separated. The H_2O layer was reextracted with $CHCl_3$ (130 mL). The two $CHCl_3$ extracts were combined, washed with H_2O (650 mL), and dried (Na_2SO_4). Evaporation of the $CHCl_3$ yielded crude **17** as a white solid foam: yield 1.17 g. [Crude **17** could be crystallized from $CHCl_3$–Et_2O to a 59% yield of a white solid, mp softens 100–120 °C, melts 143–165 °C. Anal. ($C_{21}H_{23}NO_5 \cdot 0.025CHCl_3$) C, H, N.] This solid foam was stirred at room temperature in 9:1 trifluoroacetic acid-water (10 mL) for 16 h. The mixture was evaporated at 50 °C [azeotroping twice with absolute EtOH (2 × 50 mL)] to a white solid. This solid was dissolved in a small amount of $CHCl_3$–MeOH, 10:1, and diluted with $CHCl_3$ (120 mL). This $CHCl_3$ solution was washed with H_2O (120 mL), dried (Na_2SO_4), and evaporated to a white solid foam: yield 1.18 g. This solid foam was crystallized from $CHCl_3$–ether to white solid **18**: yield 0.535 g; mp 141.5–142.5 °C. The mother liquor was evaporated and then retreated with 9:1 trifluoroacetic acid–water (3.0 mL) for 16 h. After the same workup and crystallization procedure as above, more white solid **18** was obtained: yield 0.251 g (total yield from **15**, 74%); mp 140.5–141.5 °C; $[\alpha]^{21}_D$ +38.9° (*c* 0.98, $CHCl_3$); ^{13}C NMR ($CDCl_3$–CD_3OD, 10:1) δ (peak height, mm) 27.9 (38, CH_2-phenyl), 35.2, 36.7 (62 and 60, CH_2-COOH, $COCH_2$), 59.9 (56, CH), 207.8 (7, ketone); MS (Me_4Si – M^+, 397). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

(5*S*)-5-Benzamido-4-oxo-6-phenylhexanoyl-L-proline Benzyl Ester (19). A mixture of **18** (5.90 g, 18.1 mmol), L-proline benzyl ester (4.38 g, 18.1 mmol), 1-hydroxybenzotriazole hydrate (2.77 g, 18.1 mmol), and CH_2Cl_2 (270 mL) was stirred and triethylamine (2.51 mL, 18.1 mmol) was added. The nearly homogeneous mixture was cooled in an ice bath and dicyclohexylcarbodiimide (3.74 g, 18.1 mmol) in CH_2Cl_2 (25 mL) was added. The mixture was kept at ice-bath temperature for 30 min and at room temperature for 3 days. After cooling again in an ice bath, the mixture was filtered (remove DCU) and the filtrate was diluted to 400 mL with CH_2Cl_2. This CH_2Cl_2 solution was washed with ice-cold 2 N HCl (300 mL), 0.3 N NaOH (300 mL), and H_2O (300 mL) successively. The CH_2Cl_2 layer was dried (Drierite) and evaporated to a foam. The foam was mixed with EtOAc (25 mL), cooled in an ice bath, and filtered (remove DCU). The filtrate was evaporated to a foam: yield 8.3 g. This foam was purified by preparative high-performance LC using $CHCl_3$–ether, 2:1, as the eluting solvent. The first 700 mL of effluent was discarded and the next 1600 mL was evaporated to an off-white gummy foam, **19**: yield 6.6 g (71%); TLC R_f 0.3 ($CHCl_3$–ether, 2:1); ^1H NMR ($CDCl_3$) δ 5.13 (s, 2, benzyl CH_2); ^{13}C NMR ($CDCl_3$) δ (peak height, mm) 22.5 (7), 24.6 (33, proline CH_2), 28.3 (29, CH_2-phenyl), 28.6 (10), 29.1 (34, proline CH_2), 31.3 (7), 34.4 (9), 34.6, 36.6 (30 and 35, CH_2CON, $COCH_2$), 46.5 (9), 46.9 (34, CH_2-N), 58.9 (37, CH), 59.5 (10), 59.9 (38, CH), 66.7 (36, benzyl ester CH_2), 67.1 (9), 167.1, 170.3 and 172.0 (13, 17, and 14, respectively, amide and ester CO's), 170.5 (6), 207.6 (20, ketone CO), 207.9 (8). Anal. ($C_{31}H_{32}N_2O_5 \cdot {}^1/_{15}CHCl_3$) C, H, N.

(5*S*)-5-Benzamido-4-oxo-6-phenylhexanoyl-L-proline (20). In glacial acetic acid (125 mL) **19** (5.24 g, 10.2 mmol) and 10% Pd/C (5.24 g) were stirred at room temperature under 1 atmosphere of hydrogen for 15 h. The mixture was then filtered through Celite and evaporated at 50 °C (azeotroping with EtOH) to a pale yellow syrup. This syrup was mixed with EtOAc (300 mL) and acetone (25 mL) and extracted with H_2O (200 mL). The H_2O layer was back extracted with EtOAc (2 × 100 mL). The three EtOAc extracts were combined, dried (Na_2SO_4), and evaporated to a white solid: yield 3.53 g. This solid was slurried in hot EtOAc (35 mL) and collected by filtration as **20**: yield 2.79 g; mp 156–160 °C. Crystallization of the mother liquor from EtOAc yielded more **20**: yield 0.311 g (total yield 71.8%); mp 151–153 °C; $[\alpha]^{21}_D$ −83.2° (*c* 0.98, $CHCl_3$); MS (M^+, 422); ^{13}C NMR ($CDCl_3$–CD_3OD, 3:1) δ (peak height, mm) 22.4 (22), 24.4 (69, proline CH_2), 28.2 (52, CH_2-phenyl), 29.0 (58, proline CH_2), 31.0 (17), 34.0, 36.0 (63 and 53, CH_2CON, $COCH_2$), 46.4 (19), 47.0 (42, CH_2-N), 58.5 (46, CH), 59.2 (22), 60.2 (59, CH), 167.9, 171.2, 173.5 (65, 99, and 58, respectively, amide and acid CO's), 171.5 (39), 173.3 (32), 207.6 (95, ketone CO), 207.9 (29). Anal ($C_{24}H_{26}N_2O_5$) C, H, N.

Fluorometric Assay of Angiotensin Converting Enzyme. Materials. Porcine plasma converting enzyme was purchased from Miles Laboratories, IL. Hippurylhistidylleucine, histidylleucine, and *o*-phthaldehyde was obtained from Sigma Chemical Co., St, Louis, MO. Angiotensin I was supplied by Bachem Fine Chemicals, Marina Del Rey, CA. All chemicals used were reagent grade.

Enzyme Assay.[25] The enzyme activity was measured by the fluorometric determination of histidylleucine, a product of the enzyme reaction. The substrate used was either hippurylhistidylleucine or angiotensin I. The concentration ranges were 2.6×10^{-4} to 2.04×10^{-3} M and 1.1×10^{-4} to 1.4×10^{-3} M for hippurylhistidylleucine and angiotensin I, respectively. The assay was carried out by mixing 10 μL of phosphate buffer (0.1 M, pH 7.6, containing 0.3 M NaCl) containing the testing inhibitor with substrate dissolved in 10 μL of phosphate buffer. Then, 1 mg of porcine plasma converting enzyme in 50 μL of buffer was added. The mixture was incubated at 37 °C for 90 min with constant shaking, and the reaction was stopped by adding 50 μL of 10% TCA. The samples were then diluted with 0.7 mL of water, followed by 0.4 mL of 2 N NaOH. To the alkalized mixture, 0.1 mL of 1% (w/v) *o*-phthaldehyde in methanol was added. After exactly 4 min, 0.2 mL of 6 N HCl was added. The contents of all tubes were thoroughly mixed after each addition. The samples were then centrifuged at 10000*g* for 10 min and the fluorescence of the supernatant was measured with exitation at 365 nm and emission at 495 nm on an Aminco Bowman fluorometer. The fluorescent product of histidylleucine with *o*-phthaldehyde is not stable in alkaline solution but is stabilized upon acidification. The fluorescence of the acidified solution is stable up to 1 h, so all readings should be made within 1 h.[26] Each sample was run in duplicate and an average of the two readings obtained was calculated.

A standard curve of histidylleucine was always prepared with each assay by mixing various amounts of histidylleucine with 1 mg of enzyme in 70 μL of phosphate buffer. The tubes containing the standards were treated exactly as those containing the samples.

A reagent blank containing all reagents but no substrate was also run for each assay.

Test for the Effect of Inhibitors. For testing the effect of inhibitors on angiotensin converting enzyme, two assays were run in parallel. One contained the substrate (1–2 mM), enzyme (1 mg), and various concentrations (1 nM–10 mM) of an inhibitor; the other contained only the substrate and enzyme. The assay conditions were the same as described above. The product formed with an inhibitor relative to that without an inhibitor was calculated to give the percent of inhibition. By plotting the percent of inhibition vs. various concentrations of an inhibitor, the I_{50} was obtained.

For determination of the K_i of an inhibitor, the enzyme assay was carried out as described above using various concentrations of substrate with and without an inhibitor. When hippurylhistidylleucine was used as substrate, the concentrations were 2×10^{-4} to 2.1×10^{-3} M. When angiotensin I was used as substrate, the concentrations were 1.1×10^{-4} to 1.4×10^{-3} M. The K_i determinations for 1 and **20** were performed using Hip-His-Leu as substrate at two different inhibitor concentrations (0.52 and 0.39 μM for 1 and 0.14 and 0.08 μM for **20**). By using the Michaelis–Menten equation[27] and double-reciprocal plot,[28] the amount of product formed at each substrate concentration with and without the inhibitor can be graphed and the K_m and K_i can be calculated.

Guinea Pig Ileum Assay. Male guinea pigs weighing 200 g were sacrificed. The ileum was isolated from each animal and a 10-cm segment nearest to the caecum junction was discarded. A 2–3 cm segment was suspended in a tissue bath filled with a modified Krebs solution at 37 °C and bubbled with 95% O_2–5% CO_2. The contractions were monitored with a transducer (Grass FT 0.03) in the presence of a 1-g load. A polygraph (Grass Model 7D) was used to record the contractions.

After equilibration, uniform contractions were observed at 10-min intervals after adding angiotensin I (25 ng/mL). In order to test the inhibitory activity (IC_{50}) of a drug, the method described by Rubin[12,29] was used. Each drug was kept in the bath 2 min before adding angiotensin I. After each drug test, the ileum was washed three times with Krebs solution, allowed to rest 8 min, and then tested again.

The Krebs solution had the following composition, mM: NaCl, 118; KCl, 4.75; $NaHCO_3$, 25; KH_2PO_4, 1.19; $MgSO_4$, 1.20; $CaCl_2$, 2.54; glucose, 11.

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Antihypertensive 5,6-Diarylpyridazin-3-ones[1]

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The synthesis of a series of 5,6-diarylpyridazinones is described. Some members of this series display an antihypertensive effect in both the spontaneously hypertensive rat (SHR) model and the deoxycorticosteroid (DOCA) model of hypertension. The most potent compounds in the series have halogen substituents on the 5,6-diphenyl rings, a β-substituted alkyl group at the 2 position of the ring, and an acetyl or cyano substituent at the 4 position.

There have been previous reports of antihypertensive agents which contain a pyridazinone ring system. These compounds can be classified into three groups. One series, hydrazino-substituted pyridazines, is probably related to

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