as no reaction, i.e. zero measurement. The mean of each wheal was calculated by multiplying two measurements (in two perpendicular directions for each wheal) together and taking the square root of the product.

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Registry No. 4, 638-07-3; 10, 4949-44-4; 11, 36187-69-6; 12, 103-71-9; 13, 115320-88-2; 14, 115320-89-3; 15, 115320-90-6; 16, 51287-60-6; 17, 115320-91-7; 18, 115320-92-8; 19, 115320-93-9; 20, 96286-63-4; 21, 115320-94-0; 22, 23976-42-3; 26, 58337-19-2; 27, 115320-95-1; 28, 36717-55-2; 29, 58337-16-9; 30, 58337-23-8; 31, 115320-96-2; 32, 106212-55-9; 33, 115320-97-3; 34, 106212-56-0; 35, 115320-98-4; 36, 106212-54-8; 37, 58337-17-0; 38, 115320-99-5; 39, 106212-58-2; 40, 115321-00-1; 41, 115321-01-2; 42, 115321-02-3; 43, 58337-18-1; 44, 115321-03-4; 45, 115321-04-5; 46, 115321-05-6; 47, 115321-06-7; 48, 115321-07-8; 49, 115321-08-9; 50, 115321-09-0; 51, 103921-11-5; 52, 103921-12-6; 53, 103921-15-9; 54, 103921-14-8; 55, 103921-16-0; 56, 103921-17-1; MeNCO, 624-83-9; *i*-PrNCO, 1795-48-8; o-$OCNC_6H_4OMe$, 700-87-8; m-$F_3CC_6H_4NCO$, 329-01-1; m-$O_2NC_6H_4NCOp$, 3320-87-4; p-ClC_6G_4NCO, 104-12-1; p-BrC_6H_4NCO, 2493-02-9; p-$OCNC_6H_4CO_2Et$, 30806-83-8; M-MeC_6H_4NCO, 621-29-4; p-$MeOC_6H_4NCO$, 5416-93-3; o-MeC_6H_4NCO, 614-68-6; ethyl isobutyrylacetate, 7152-15-0; 1-naphthyl isocyanate, 86-84-0; acetoacetanilide, 102-01-2; 4-bromoacetoacetanilide, 1205-74-9; 4'-chloroacetoacetanilide, 101-92-8; 4-bromo-4'-chloroacetoacetanilide, 19359-22-9; 4'-methoxyacetoacetanilide, 5437-98-9; 4-bromo-4'-methoxyacetoacetanilide, 20335-27-7; 2'-methoxyacetoacetanilide, 52405-54-6; 4-bromo-2'-methoxyacetoacetanilide, 52405-54-6; 2'-methylacetoacetanilide, 93-68-5; 4-bromo-2'-methylacetoacetanilide, 23976-47-8; 2,2,6-trimethyl-4*H*-1,3-dioxen-4-one, 5394-63-8; *p*-toluidine, 106-49-0; *N*-(4-methylphenyl)-3-oxobutamide, 2415-85-2.

Renin Inhibitors Containing Hydrophilic Groups. Tetrapeptides with Enhanced Aqueous Solubility and Nanomolar Potency

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Nineteen tetrapeptides containing statine (Sta) and 4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA) were prepared. Solubility measurements of these compounds were carried out in H_2O and in pH 7.4 phosphate buffer solution, and their partition coefficients were determined in a 1:1 1-octanol/sodium phosphate-citric acid buffer system. The tetrapeptides were tested in vitro for their ability to inhibit porcine, canine, and human plasma renins. Four compounds, **6**, **12**, **14**, and **20**, were potent inhibitors against all renins tested ($IC_{50} = 10^{-9}$ M). Compound **12** was administered orally to dogs and substantially inhibited plasma renin activity for up to 5 h. The addition of polar groups to the C-terminus of Sta- and ACHPA-containing tetrapeptides renders them soluble in aqueous milieu and provides a valuable tool with which to examine the role of the renin-angiotensin system in physiological and pathological circumstances.

A potential alternative to angiotensin converting enzyme (ACE) inhibitors in the treatment of various hypertensive states is an agent that blocks the preceding step in the enzymatic sequence, the renin reaction. Over the past decade, several different categories of renin inhibitors have appeared[1] with one of the most promising types, the renin substrate analogues,[2] achieving potency levels in the nanomolar range. In fact, one of these compounds has been tested in humans and found to lower blood pressure.[3] However, despite encouraging results regarding potency and specificity, important questions remain to be answered before medicinally useful renin inhibitors result that rival the therapeutic profile of ACE inhibitors.

In our analysis of these problems, we concluded that a key issue concerning the efficacy of renin inhibitors relates to their solubility in physiological media. The therapeutic potential of many peptidal renin inhibitors is limited because of their hydrophobic nature and low aqueous solubility. In the past, this shortcoming was remedied by the addition of hydrophilic amino acids and oligopeptides to either terminus of the peptide renin inhibitors.[4] Alternative solubilizing tactics such as employing various aqueous vehicles[5] or derivatization with polysaccharides[6] have also been explored. Nevertheless, even in the most favorable cases, only moderate success has been achieved.

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In a separate paper[7] we established the practicability of incorporating nonpeptidal segments at the carboxy terminus of the pentapeptide renin inhibitor Boc-Phe-His-Sta-Leu-Phe-NH_2, 1. As these end groups were hydrophobic in nature, the corresponding renin inhibitor peptides also displayed low aqueous solubility. These observations led us to propose that further modifications at the carboxy terminus of the peptide renin inhibitor 1 could provide a solution to the above mentioned liabilities. Accordingly, the tetrapeptide Boc-Phe-His-(3*S*,4*S*)-Sta-Leu-$NHCH_2C_6H_5$, **3**, was established as our benchmark (vis-a-vis solubility and renin inhibitor potency) against which subsequent analogues in this study were to be judged. We disclose herein the results of our work in which common, readily available amines were systematically incorporated at the C-terminus of a series of tetrapeptides containing (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (Sta) and (3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA)[8] with the principle objectives of increasing aqueous solubility and refining further the structural requirements that maximize potency versus human plasma renin.[9]

Results and Discussion

Chemistry. The tetrapeptides Boc-L-Phe-L-His-(3*S*,4*S*)-Sta-L-Leu-OCH_3 and Boc-L-Phe-L-His-(3*S*,4*S*)-ACHPA-L-Leu-OCH_3 were synthesized according to previously described procedures[7,8] and converted to the amines **2–22** listed in Table I by using the azide coupling method. In the cases of the preparation of compounds **9**, **11**, and **12**, a 15-fold excess of the symmetrical diamines, 1,2-diaminoethane and *m*-xylylenediamine, was used in the corresponding coupling reaction to minimize formation of the respective dimers. The requisite amines (i.e. polar end groups) were obtained commercially or made available in the following ways: 2-(1,2-diaminoethyl)pyridine (for **8**) was synthesized in a two-step procedure with use of 2-pyridinecarboxaldehyde, ammonia, and diethyl phosphorocyanidate followed by reduction with borane THF; [3-(aminomethyl)phenoxy]acetic acid (for **19** and **20**) was prepared according to the method of Loeffler.[10]

The pyridyl *N*-oxide analogue **7** was obtained without incident by peracid oxidation of 4-pyridylmethyl amide **5**. No competing N-oxidation of the imidazole ring in **5** was detected. Aminoguanidines **10**, **13**, and **14** were prepared from the corresponding amines **9**, **11**, and **12** by using 3,5-dimethylpyrazole-1-carboxamidine nitrate as the reagent of choice.[11] *S*-Ethylisothiourea hydrogen bromide failed to give readily isolable products in this case.

Solubility. The solubilities of compounds **1–12** were measured in doubly distilled H_2O and in a phosphate buffer solution (pH 7.4) by using the protocol described in the experimental section. The partition coefficients of these compounds in 1-octanol/citric acid–phosphate buffer were also determined with the results tabulated in Table I.

A perusal of the data indicates that relative to our standard, tetrapeptide **3**, we were successful in increasing the solubility of this potent renin inhibitor over a range of 100 to more than 1000-fold. Importantly, this was accomplished without significant increase in molecular weight and/or peptide chain length. While the list of polar end groups in Table I is not exhaustive, an attempt was made to include specimens representative of a wide pK_a range. The group, therefore, includes acidic, basic, and zwitterionic moieties.

The two groups with the most dramatic enhancement of aqueous solubility were the (aminoalkyl)guanidine **10** and aminosulfonic acid (taurine) **21** moieties. Not surprisingly, these polar end groups had the largest effect on the partition coefficients as well.

Biology. The compounds in this study were tested in vitro in three enzymatic assays as described in the experimental section. The results of these experiments are collated in Table II.

Earlier studies confirmed the viability of replacing the C-terminal Phe-NH_2 in 1 with benzylamine to give **3**.[7] As the data in Table II show, further modifications at the C-terminus of 1 with acidic, basic, and zwitterionic functional groups yielded good results in some instances.

A considerable range of activities was observed among the compounds tested in the three assays. However, the renin inhibitors, **4–22**, containing polar end groups, were uniformly more effective in inhibiting human renin than porcine renin. They displayed equally high human versus canine specificity.

The variation in primarily hydrophobic C-termini which was previously incorporated in the renin inhibitor 1[7] indicated that human and porcine renins are highly selective but subject to several variables. Subtle structural and electronic changes at the C-terminus of these renin inhibitors were correspondingly reflected in inhibitor potency and specificity. These results were obtained in the present study as well. For example, substituting benzylamine **3** with (2-pyridylmethyl)amine **4** resulted in a 1 order of magnitude decrease in porcine renin inhibitor potency, whereas human renin inhibitor potency was only modestly affected. The trend in the 4-pyridyl analogue **5** was similar, but N-oxidation of the latter compound to give **7**, while enhancing aqueous solubility, was not beneficial for inhibitor potency.

The potency exhibited by analogues **9**, **10**, **21**, and **22** in the human renin assay indicates that certain aliphatic-substituted amines are tolerated and that an aromatic

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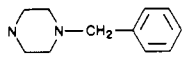
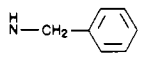
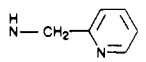
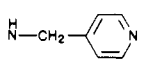
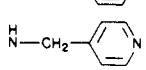
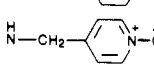
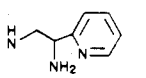
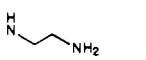
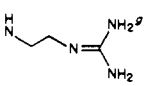
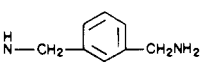
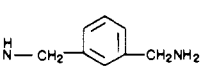
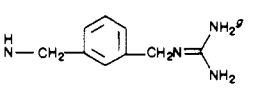
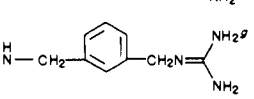
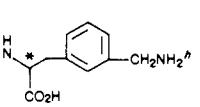
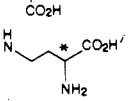
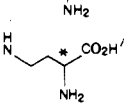
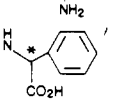
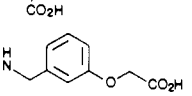
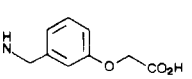
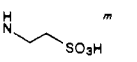
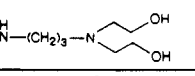
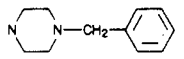
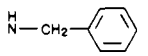
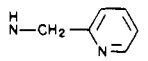
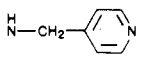
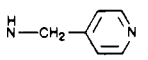
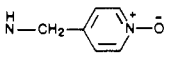
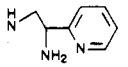
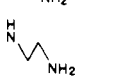
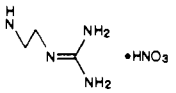
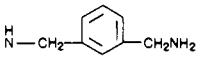
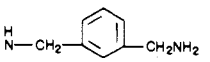
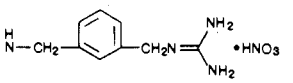
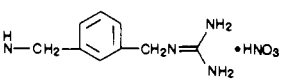
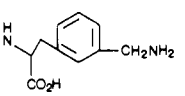
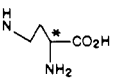
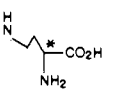
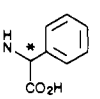
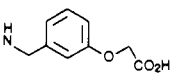
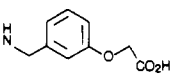
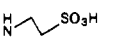
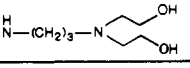
Table I. Physical Properties of Tetrapeptide Substrate Analogues

Boc-L-Phe-L-His-X-L-Leu-R

compd	X	R	chromatography solvent system[a]	HPLC purity[b]	formula[c]	solubility, μM: pH 7.4 buffer	solubility, μM: H_2O[d]	PC[e]
1	Sta	Phe-NH_2	A	94.0	$C_{43}H_{62}N_8O_8$	<20	<20	>100
2	Sta		B	90.1	$C_{45}H_{66}N_8O_7$	<10	<10	>100
3	Sta		A	97.8	$C_{41}H_{59}N_7O_7$	<10	<10	>100
4	Sta		B	99.6	$C_{40}H_{58}N_8O_7$	180	380	>100
5	Sta		B	97.0	$C_{40}H_{58}N_8O_7$	340	440	>100
6	ACHPA		C	98.8	$C_{43}H_{62}N_8O_7$	<10	ND[f]	>100
7	Sta		D	95.5	$C_{40}H_{58}N_8O_8$	4200	6500	ND
8	Sta		D	90.9	$C_{41}H_{61}N_9O_7$	400	890	>100
9	Sta		D	98.9	$C_{36}H_{58}N_8O_7$	4900	2600	8.9
10	Sta		E	99.3	$C_{37}H_{61}N_{11}O_{10}$	11000	24000	2.1
11	Sta		D	94.6	$C_{42}H_{62}N_8O_7$	1400	880	77
12	ACHPA		F	98.0	$C_{45}H_{66}N_8O_7$	20	20	>100
13	Sta		E	92.1	$C_{43}H_{65}N_{11}O_{10}$	930	3000	10.6
14	ACHPA		D	98.0	$C_{46}H_{69}N_{11}O_{10}$	40	>250	>100
15	Sta		G	88.5	$C_{44}H_{64}N_8O_9$	1500	390	68.2
16	Sta		H	95.9	$C_{38}H_{60}N_8O_9$	6100	2300	12.1
17	Sta		H	89.6[j]	$C_{38}H_{60}N_8O_9$[k]	1400	3400	10.7
18	Sta		I	91.2	$C_{42}H_{59}N_7O_9$	2200	740	16.5
19	Sta		I	88.8	$C_{43}H_{61}N_7O_{10}$	1800	270	>100
20	ACHPA		E	87.0	$C_{46}H_{65}N_7O_{10}$	210	30	>100
21	Sta		I	82.4	$C_{36}H_{57}N_7O_{10}S$	>11000	>11000	4.3
22	Sta		D	97.9	$C_{41}H_{68}N_8O_9$	4300	960	25.4

[a] All compounds were purified chromatographically on silica gel with the indicated solvent systems: (A) $CHCl_3$–MeOH–NH_4OH (concentrated), 80:10:1; (B) $CHCl_3$–EtOH–NH_4OH (concentrated), 80:10:1; (C) $CHCl_3$–MeOH–H_2O, 144:10:1; (D) $CHCl_3$–EtOH–NH_4OH (concentrated), 80:20:2; (E) $CHCl_3$–EtOH–NH_4OH (concentrated), 70:30:3; (F) $CHCl_3$–MeOH–H_2O, 100:15:1.5; (G) EtOAc–pyridine–HOAc–H_2O, 15:5:1:2; (H) EtOAc–pyridine–HOAc–H_2O, 20:5:1:1; (I) EtOAc–pyridine–HOAc–H_2O, 10:5:1:2. [b] Waters C-18, 3.9 mm × 30 cm column; acetonitrile–trimethylamine phosphate buffer pH 3.2. [c] Amino acid analyses were within 5% of the theoretical value. Values for statine were uniformly low (5–10%). [d] Doubly distilled in a glass apparatus, pH 7.2. [e] PC = partition coefficient. [f] ND = not determined. [g] Isolated and characterized as the nitrate salt. [h] Mixture of diastereomers. [i] Configuration at α-carbon not established. [j] Contaminated with 6.7% of compound 16. [k] Anal. (Phe-His-Sta-Leu) Phe, Leu; His: found, 93.3%. [l] D-Phenylglycine. [m] One component by TLC; major impurity (15.95%) may be D–Leu diastereomer.

Table II. Inhibition of Renins by Tetrapeptide Substrate Analogues Boc-L-Phe-L-His-X-L-Leu-R

compd	X	R	IC_{50},[a] nM: human plasma	dog kidney	porcine kidney
1	Sta	Phe-NH_2	140	290	63
2	Sta		127[b]	310	280
3	Sta		26	16	25
4	Sta		50	92	240
5	Sta		51	20	210
6	ACHPA		1.2[b]	0.99	6.7
7	Sta		120	120	29% (10^{-7})
8	Sta		100	1000	940
9	Sta		140	32% (10^{-6})	31% (10^{-6})
10	Sta		64	32% (10^{-6})	710
11	Sta		9.0	110	54
12	ACHPA		1.0[b]	3.3	8.3
13	Sta		9.0	44	20
14	ACHPA		1.4[b]	0.59	9.0
15	Sta		880	18% (10^{-6})	0 (10^{-7})
16	Sta		25% (10^{-6})	15% (10^{-6})	0 (10^{-7})
17	Sta		8% (10^{-6})	22% (10^{-6})	0 (10^{-6})
18	Sta		44% (10^{-6})	45% (10^{-6})	0 (10^{-7})
19	Sta		6.8	14	43
20	ACHPA		1.4[b]	1.3	ND[c]
21	Sta		92	350	0 (10^{-7})
22	Sta		170	ND[c]	900

[a] Values were found to be reproducible within ±20% and were determined according to ref 7. [b] Revised value (cf. ref 9). [c] ND = not determined.

C-terminus is not a prerequisite for inhibitor potency for this class of renin inhibitors. It appears that this feature is more critical in inhibiting porcine kidney renin.[7] However, the potency-enhancing effect of ACHPA[8] was uniform. Substituting ACHPA for Sta resulted in improved inhibition against all renins tested (cf. **6** vs **5**, **12** vs **11**, **14** vs **13**, and **20** vs **19**), with compounds **6**, **12**, **14**, and **20** being the most potent compounds examined.

The [*m*-(aminomethyl)benzyl]amino group (AMBA) emerged from our study as the best C-terminal group. Substitution of this amine for benzylamine (cf. **3**) afforded **11**; further potency optimization with ACHPA yielded the

Table III. Percent Inhibition of Plasma Renin Activity in Dogs after Oral Administration[a] of Compound 12

time, min	%I PRA dog 1[b]	dog 2[c]	dog 3[c]
10	98	97	97
15	95	100	95
30	95	99	94
45	90	96	94
60	87	94	96
75	83	92	94
90	82	97	93
120	73	96	82
150	67	96	71
180	53	93	84
240	43	91	68
300	43	82	42

[a] Administered by gavage. [b] Compound 12 (24 mg/kg) was administered in 0.5% acetic acid–5% dextrose solution. [c] Compound 12 (30 mg/kg) was administered in olive oil containing 20 mg/mL palmitoyl carnitine chloride.

Table IV. Change in Mean Blood Pressure[a] in Dogs after Oral Administration[b] of Compound 12

time, min	dog 1	dog 2	dog 3
10	−12	−10	−1
15	−18	−6	1
30	−2	−3	−6
45	−6	−4	−7
60	−4	0	−5
75	−7	1	−9
90	1	−5	−2
120	−2	−4	1
150	−1	−3	−2
180	−13	−2	−5
240	1	−1	−3
300	−10	−1	−3

[a] Millimeters of mercury. [b] Administered by gavage.

tetrapeptide **12**, an inhibitor that merits further study. This tetrapeptide displayed excellent inhibitor potency vs human, canine, and porcine renins. Its solubility and that of the corresponding Sta analogue **11** was sufficient in aqueous medium to permit in vivo evaluation. Thus, when administered intravenously to sodium deficient dogs[8] at a dose of 2.1 mg/kg for **11** and 2.2 mg/kg for **12**, plasma renin activity was suppressed for 6.9 and 5.7 h,[12] respectively.

Compound **12** was also evaluated orally in three dogs. It was administered in olive oil and as a suspension in acetic acid dextrose solution. As there were no obvious differences among the dogs relative to the vehicle, the resultant plasma renin activity (PRA) data were combined and are summarized in Table III. Within 10 min of oral dosing, PRA was virtually completely inhibited in all three dogs. Inhibition was maximal at the earliest time points monitored and declined slowly toward control levels throughout the course of the experiment. Plasma renin activity remained substantially inhibited even 300 min after treatment. The mean arterial blood pressure was monitored continuously, and these data are collected in Table IV. As is evident, mean arterial blood pressure was reduced during the first 10–15 min after dosing in two of the dogs receiving compound **12** and appeared not to be reduced in the third. (We have used an arbitrary figure of 10-mmHg decrease in pressure as a biologically significant change. This criterion is consistent with the variability seen in conscious dog blood pressure measurement and is conservative.) Blood pressure had returned essentially to control levels by 30 min after treatment. The variable fall in pressure and its recovery despite continued inhibition of PRA is consistent with our previous observation that PRA must be inhibited completely before additional renin inhibitor will lower arterial blood pressure.[13]

In summary, the addition of selected polar groups to the C-terminus of Sta- and ACHPA-containing tetrapeptides resulted in an increase in solubility of these compounds at physiological pH. This tactic serves as one device to retain the inhibitor potency of shortened peptides versus human plasma renin.[14] Application of this strategy has led to the development of potent renin inhibitors, one of which, Boc-Phe-His-ACHPA-Leu-AMBA (**12**), was demonstrated to suppress PRA in vivo. It also showed good duration of action. However, the high oral dose needed to achieve inhibition is indicative of low oral availability.

Experimental Section

^{1}H NMR spectra were recorded on a Varian EM 390 or Nicolet NT-360 spectrometer. Amino acid analyses were carried out on a 121 MB Beckman amino acid analyzer by using Type AA-10 resin and a standard four buffer program. HPLC was performed on a Hewlett-Packard 1084B instrument; Waters C-18 column, pH 3.2 phosphate buffer–acetonitrile gradient; UV (210 nm) detector. Flash chromatography was performed on silica gel (E. Merck, 0.04–0.063 mm). Thin-layer chromatography (TLC) and preparative thick-layer chromatography (PTLC) were carried out on E. Merck 60F-254 precoated silica gel plates (0.25, 0.5, and 2.0 mm). Visualization was done with UV light, iodine vapor, ninhydrin, phosphomolybdic acid, Sanger stain, and/or *tert*-butyl hyopchlorite–starch–KI.

General Synthetic Procedure for Peptides in Table I. The tetrapeptides Boc-Phe-His-(3*S*,4*S*)-Sta-Leu-OCH_3 and Boc-Phe-His-(3*S*,4*S*)-ACHPA-Leu-OCH_3 were prepared according to previously described procedures and converted to the corresponding amides (Table I) via the azide coupling method.[7,8] All compounds were isolated, purified chromatographically, and characterized (Table I). Compounds **5**, **9**, **11**, and **12** were further elaborated to give **7**, **10**, **13**, and **14**, respectively.

Boc-L-Phe-L-His-(3*S*,4*S*)-Sta-L-Leu 4-Pyridylmethylamide *N*-Oxide (7). The pyridylmethyl amide **5** (300 mg, 0.39 mmol) was dissolved in 20 mL of chloroform. The resulting solution was protected from moisture, cooled to 0 °C, and treated with *m*-chloroperoxybenzoic acid, 80–85% (85 mg, 1 equiv). The homogeneous reaction mixture was allowed to warm to room temperature and then to stand for 5 days. The solvent was removed under reduced pressure, and the residual material was directly applied to a silica gel column and flash chromatographed to yield 104 mg of the analytical product as a white solid.

Boc-L-Phe-L-His-(3*S*,4*S*)-Sta-L-Leu *m*-Guanidylmethylbenzylamide (13). The [*m*-(aminomethyl)benzyl]amide **11** (150 mg, 0.189 mmol), 3,5-dimethylpyrazole-1-carboxyamidine nitrate (400 mg, 1.98 mmol), and triethylamine (0.28 mL, 2.0 mmol) were combined in 3 mL of DMF at room temperature. The resulting mixture was protected from moisture and stirred overnight. Concentration of the reaction mixture under reduced pressure afforded a semisolid, which was applied directly to a silica gel column and flash chromatographed. In this way, 125 mg of the analytical material was obtained as an off-white solid after trituration with ethyl acetate–ether.

(12) Indicates time for recovery to control levels.

(13) Blaine, E. H.; Schorn, T. W.; Boger, J. *Hypertension* **1984**, *6*(Suppl. 1), I-111.

(14) Since the completion of these studies reports of renin inhibitors containing polar and/or charged groups have appeared; cf. (a) Thaisrivongs, S.; Pals, D. T.; Harris, D. W.; Kati, W. M.; Turner, S. R. *J. Med. Chem.* **1986**, *29*, 2088. (b) Pals, D. T.; Thaisrivongs, S.; Lawson, J. A.; Kati, W. M.; Turner, S. R.; DeGraaf, G. L.; Harris, D. W.; Johnson, G. A. *Hypertension* **1986**, *8*, 1105. (c) Nisato, D.; Lacour, C.; Roccon, A.; Gayraud, R.; Cazaubon, C.; Carlet, C.; Tonnerre, B.; Wagnon, J.; Gagnol, J. P. *Abstracts of Papers*, 3rd European Meeting on Hypertension, Milan, Italy, June 1987; No. 405. (d) Kempf, D. J.; deLara, E.; Plattner, J. J.; Stein, H.; Cohen, J.; Kleinert, H. D. *Abstracts*, 10th American Peptide Symposium, St. Louis, MO, May 23–28, 1987; p 130.

Solubility Measurements. Saturated solutions of compounds 1–22 in H_2O and pH 7.4 phosphate buffer were prepared by magnetically stirring the initial suspensions for 3 h at 25 °C. The resulting solutions were clarified by passage through an MF Millipore HA 45 μm filter. Concentrations were measured by HPLC on a Waters C-18 column (H_2O–acetonitrile 5–99% gradient, 210 nm detection) and were calibrated versus a water–acetonitrile solution (1:1) of known concentration.

Partition coefficients were determined by agitating the test compounds at 25 °C with a 1:1 mixture of 1-octanol/sodium phosphate–citric acid buffer, pH 7.4, on a flatbed shaker for 45 min. The resulting suspensions were clarified by centrifugation at 2000 rpm for 5 min. Concentrations of aliquots from both phases were measured by HPLC (vide supra).

Biological Methods. Human and dog plasma renin assays were performed by radioimmunoassay for angiotensin I as described by Haber et al.[15] with use of a commercial kit (Clinical Assays), at pH 7.4 (phosphate buffer), 37 °C. Pooled plasma samples were collected on ice in EDTA from mongrel dogs. Lyophilized human plasma was obtained from Ortho Diagnostics (low renin) or Clinical Assays (high renin). IC_{50} values were obtained as previously described.[16]

Porcine kidney renin was assayed as described by Rich et al.[17] by using [^{14}C]Leu-labeled decapeptide substrate, except the pH was raised to 7.3 by use of 0.05 M citrate–phosphate buffer, 30 °C. IC_{50} values were determined by linear regression of logit vs log concentration over a 15–90% inhibition range, with use of three to six concentrations, and were found to be reproducible within ±20%.

The oral dog studies were carried out as follows: Conditioned female mongrel dogs (14–17 kg) were prepared with chronically indwelling catheters in the femoral artery. The catheters were tunneled subcutaneously to exit between the shoulders. When not in use, the catheters were filled with heparinized (40 units/mL) 5% dextrose solution. The dogs were maintained on a low sodium diet (10 mmol/day) and received furosemide (5 mg/kg, po) twice during the first week of low sodium intake and another 5 mg/kg po the evening prior to an experiment. Water was provided ad libitum except during the experiment, but food was removed 18 h prior to any measurements being made. The arterial catheter was used to monitor mean pressure and for collection of 3-mL blood samples for determination of plasma renin activity (PRA). Blood samples were collected in chilled tubes containing EDTA (final concentration 4 mM), centrifuged at 4 °C, and stored frozen until assayed for PRA by radioimmunoassay of generated angiotensin 1.

The conscious dogs were trained to stand quietly in a modified Pavlov sling during the course of the experiment. Compound **12** was administered by gavage in olive oil or as a suspension in 5% acetic acid, 5% dextrose solution. Blood pressure was recorded continuously except during collection of blood samples.

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Registry No. 1, 87063-27-2; 2, 105192-85-6; 3, 109585-11-7; 4, 87691-49-4; 5, 87691-50-7; 6, 105192-86-7; 7, 100902-03-2; 8, 100901-99-3; 9, 105192-87-8; 10, 105192-89-0; 10 (free base), 105192-88-9; 11, 100901-98-2; 12, 100902-07-6; 13, 105228-98-6; 13 (free base), 98858-48-1; 14, 105228-99-7; 14 (free base), 100902-08-7; 15 (diastereomer 1), 115511-03-0; 15 (diastereomer 2), 115511-04-1; 16, 115511-05-2; 18, 115511-06-3; 19, 100902-05-4; 20, 100902-09-8; 21, 100902-06-5; 22, 87691-52-9; AMBA, 1477-55-0; BOC-Phe-His-(3*S*,4*S*)-Sta-Leu-OCH_3, 87700-33-2; BOC-Phe-His-(3*S*,4*S*)-ACHPA-Leu-OCH_3, 115464-34-1; $PhCH_2NH_2$, 100-46-9; $H_2N(CH_2)_2NH_2$, 107-15-3; (±)-$H_2NCH(CO_2H)$-$CH_2C_6H_4CH_2NH_2$, 115511-07-4; $H_2N(CH_2)_2CH(NH_2)CO_2H$, 305-62-4; *m*-$H_2NCH_2C_6H_4OCH_2CO_2H$, 115464-36-3; $H_2N(CH_2)_2SO_3H$, 107-35-7; $H_2N(CH_2)_3N(CH_2CH_2OH)_2$, 4985-85-7; renin, 9015-94-5; 2-pyridinecarboxaldehyde, 1121-60-4; diethyl phosphorocyanidate, 2942-58-7; 3,5-dimethylpyrazole-1-carboxyamidine nitrate, 38184-47-3; D-phenylglycine, 875-74-1; benzylpiperazine, 2759-28-6; 2-(aminomethyl)pyridine, 3731-51-9; 4-(aminomethyl)pyridine, 3731-53-1; 2-(1,2-diaminoethyl)pyridine, 115464-35-2.

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(16) Poe, M.; Wu, J. K.; Florance, J. R.; Rodkey, J. A.; Bennett, C. D.; Hoogsteen, K. J. *J. Biol. Chem.* **1983**, *258*, 2209.
(17) Rich, D. H.; Sun, E. T. O.; Ulm, E. H. *J. Med. Chem.* **1980**, *23*, 27.

A Comprehensive Method for Determining Hydrophobicity Constants by Reversed-Phase High-Performance Liquid Chromatography

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The development of a method for determining hydrophobicity constants for small, organic molecules by reversed-phase liquid chromatography (RPLC) is presented. The method uses capacity ratios measured at a number of different compositions of methanol to obtain derived values, denoted log k'_w, upon which a new scale of hydrophobicity constants can be developed. This scale eliminates potential problems such as peak inversion that hamper RPLC methods using isocratic data to estimate hydrophobicity. The differential hydrogen bond effect observed in most correlations of RPLC data with logarithms of octanol–water partition coefficients (log $P_{o/w}$) for compounds of opposite net hydrogen bonding capabilities (noncongeners) was minimized by adding trace quantities of *n*-decylamine and 1-octanol to the eluent and using an octyl-modified silica gel stationary phase. Values of log k'_w are shown to be largely column-independent as long as the hydrophobic properties of columns are similar. The correlation of log k'_w values with the logarithms of bovine serum albumin binding constants (log $1/C$) is shown to be statistically indistinguishable from the correlation of log $1/C$ with log $P_{o/w}$, indicating that this data models log $1/C$ as well as log $P_{o/w}$ for these compounds. Additionally, the chromatographic system is automatable and thus capable of higher sample throughput than measurements of log $P_{o/w}$ by the shake-flask method.

Reversed-phase liquid chromatography (RPLC) has been touted widely in recent years as a rapid, precise means for estimating physicochemical properties of organic molecules, particularly octanol–water partition coefficients (log $P_{o/w}$), which are used extensively in correlations with other properties such as biological activity (log $1/C$). Many of the early RPLC methods used capacity ratios measured by monocratic methods to estimate log $P_{o/w}$. According

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