ganic phase was separated, washed with H_2O, and then dried over $MgSO_4$. After filtration, solvent was removed *in vacuo* on a steam bath. The residue obtained was chromatographed on neutral alumina with $CHCl_3$ used as the eluent. Solvent was removed from the fraction collected. The free base was either analyzed at this point, purified by recrystallization, or dissolved in butanone and converted to the dihydrochloride salt with ethereal HCl.

Reduction of Bis-Basic Ketones to Bis-Basic Alkanes (Method E). In a typical example, a mixture of 16.8 g (0.033 mol) of **32** and 25 ml of 85% hydrazine hydrate (0.33 mol) in 200 ml of ethylene glycol was heated at 100–120° for 3 hr in an open flask followed by the cautious addition of 18.5 g (0.33 mol) of KOH and allowed to reflux for 16 hr. The cooled reaction mixture was poured into ice water and extracted with $CHCl_3$. The residue obtained after evaporation of the solvent *in vacuo* was dissolved in butanone and made acidic with ethereal HCl to give the crude product. Two recrystallizations from MeOH–butanone gave 6.6 g (36%) of **59** (Table IV).

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Agonist and Antagonist Relationships in 1- and 8-Substituted Analogs of Angiotensin II†

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[1-Pyroglutamic acid,8-alanine]-, [1-D-*allo*-*N*-methylisoleucine,8-isoleucine]-, prolyl[1-sarcosine,8-isoleucine]-, and [1-sarcosine,8-isoleucine]angiotensyl II proline and [8-tryptophan]-, [8-thienylalanine]-, and [1-sarcosine,8-threonine]angiotensin II, synthesized by Merrifield's solid-phase procedure, possess 0.2, 0.5, 0.0, 0.03, 22.2, 26.6, and 0.6% pressor activity of angiotensin II (vagotomized, ganglion-blocked rats) and pA_2 values (rabbit aortic strips) of 7.15, 8.33, 2.49, incalculable, 8.36, 9.36, and 8.79, respectively. The pressor activity of [1-dimethylglycine]angiotensin II was 171.8% of the parent hormone. These results suggest that (a) an increase in the basicity of the N-terminal nitrogen atom enhanced the pressor (or antagonistic) properties of angiotensin II analogs; (b) prolongation of the chain length at the N terminus in [1-sarcosine,8-isoleucine]angiotensin II with a proline residue reduced the antagonistic activity of the compound drastically without any increase in the duration of action (a similar change at the C terminus invoked noncompetitive antagonism); (c) substitution of position 8 with the aromatic groups, *e.g.*, thienylalanine and tryptophan, gave analogs with moderate pressor activity (however, [8-tryptophan]angiotensin II showed competitive type of antagonism to angiotensin II while [8-thienylalanine]angiotensin II, at concentrations over 100 ng/ml, showed noncompetitive antagonism); and (d) substitution of the aliphatic side chain in position 8 with a polar group (threonine) gave good antagonistic activity with the additional advantage that the initial transient pressor activity of [1-sarcosine,8-threonine]angiotensin II was 50% that of [1-sarcosine,8-isoleucine]angiotensin II.

Earlier work from our laboratories indicated that sarcosine in the 1 position of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) enhanced the agonist potency of this hormone by (a) an increased binding affinity for the receptor site and (b) a decreased rate of breakdown due to angiotensinase A, which is the major enzyme responsible for the destruction of angiotensin II in plasma.[1,2] [1-Sarcosine]angiotensin II was found to be 1.5 times as active as angiotensin II as a pressor agent and 8–10 times as active as a myotropic agent.[1,2] To further investigate the factors responsible for protection of these peptides against aminopeptidase, we report the synthesis of [Me_2Gly[1]]angiotensin II.‡ We also modified the antagonists of angiotensin II, [Ala[8]]- and [Ile[8]]angiotensin II, by substituting position 1 in these peptides with pyroglutamic acid and D-*allo*-*N*-methylisoleucine, respectively. Similarly, the chain length of [Sar[1],Ile[8]]angiotensin II was extended with a proline residue either at the C or N terminus.

†Abbreviated designation of amino acid derivatives and peptides is according to the recommendation of IUPAC–IUB Commission (IUPAC Information Bulletin No. 26). In addition, the following abbreviations were used: Me_2Gly = dimethylglycine, Pyr = pyroglutamic acid, *a*MeIle = *allo*-*N*-methylisoleucine, Sar = sarcosine, Tal = thienylalanine.

‡While this manuscript was under preparation Regoli, *et al.*,[3] also reported that [Sar[1]]angiotensin II is more potent and longer acting than angiotensin II on isolated intestinal and vascular smooth muslces but not *in vivo*. Although the *in vitro* results by these authors are qualitatively similar to our findings in isolated smooth muscle, the *in vivo* results (rat pressor assay) are quite different. Regoli, *et al.*,[3] and Rioux, *et al.*,[4] reported that [Sar[1]]- and [Me_2Gly[1]]angiotensin II possess 70 and 88% pressor activity of angiotensin II, respectively; we obtained 150 and 172% pressor activity for these compounds.

Table I. Comparative Biological Properties of 1- and 8-Substituted Analogs of Angiotensin II

Compd no.	Angiotensin II analog	pA_2	Pressor act.[a]
I	[Me_2Gly^1]-		171.75
II	[Pyr^1,Ala^8]-	7.15 ± 0.25[b]	0.18
III	[D-*a*MeIle^1,Ile^8]-	8.33 ± 0.11[c]	0.52
IV	Pro-[Sar^1,Ile^8]-	2.49 ± 0.09	Inactive[d]
V	[Sar^1,Ile^8]-Pro-		0.03
VI	[Trp^8]-	8.36 ± 0.30	22.20
VII	[Tal^8]-	9.36 ± 0.48	26.57
VIII	[Sar^1,Thr^8]-	8.79 ± 0.14	0.60

[a] Relative to [Asp^1,Ile^5]angiotensin II = 100. [b] The pA_2 value of [Ala^8]angiotensin II under similar conditions from our laboratories [R. K. Turker, M. Yamamoto, P. A. Khairallah, and F. M. Bumpus, *Eur. J. Pharmacol.*, 15, 285 (1971)] was reported to be 8.32. [c] The pA_2 value of the corresponding L-MeIle analog[8] was 8.73. [d] Up to a dose level of 10 μg/ml.

We have previously demonstrated that the replacement of the aromatic residue (Phe) in position 8 of angiotensin II by an aliphatic group minimizes the agonist activity and invokes antagonistic properties.[5-11] These results were confirmed by several other investigators.[6,§] The more closely the aliphatic side group in position 8 sterically resembles the aromatic group (Phe) in angiotensin II, the greater the potency of the antagonist. Substitution of phenylalanine in position 8 with aliphatic residues, *e.g.*, isoleucine[5-10] or leucine,[6,10,12,13] produced the most potent antagonists of angiotensin II. A recent addition to this series is [8-*N*-methylphenylalanine] analogs by Pena, *et al.*[14] However, we had already shown that displacement of the aromatic side chain in position 8, *e.g.*, in the 3-amino-2-benzylpropionic acid analog, produced a peptide with inhibitory properties.[10] For a further test of this hypothesis, we attempted to synthesize [8-*N*-methylphenylalanine]angiotensin II but were unsuccessful because of a failure sequence.[15] We also observed that when the aromatic residue was replaced by a saturated alicyclic ring, the analog obtained, [8-cyclohexylalanine]angiotensin II, gave assay results which were difficult to interpret.[10,11] Low initial dose (<100 ng/ml) of this analog caused 25% of the pressor and myotropic response of the parent hormone. Administration of higher doses (1000 ng/ml), however, completely blocked the myotropic effect due to the compound or exogenous angiotensin II. In the *in vitro* assay system (rabbit aortic strips) the log dose–response curves, though shifted to the right with increasing dosage, were not parallel to the control. The response decreased progressively and, therefore, pA_2 value could not be determined. Complete removal of the compound could not be accomplished by washing for 4 hr. It is not known whether this long-lasting inhibition is noncompetitive in nature or whether this antagonistic activity is more related to tachyphylaxis. In order to gain further insight into the effects of size of the side group and polarity in position 8, we report the synthesis of [8-tryptophan]-, [8-thienylalanine]-, and [Sar^1,Thr^8]angiotensin II.

The analogs were synthesized by the solid-phase procedure of Merrifield[16] (*cf.* Experimental Section). Comparative pressor activity of these analogs and their antagonism to contractile activity of angiotensin II is given in Table I.

Biological Results. Table I describes the biological properties of the various antagonists of angiotensin II. Inhibition of contractile activity of angiotensin II was studied on isolated, spirally cut rabbit aortic strips[17] (*cf.* Experimental Section). pA_2 values were calculated according to the method described by Arunlakshna and Schild.[18] The compounds showing agonist properties were tested for their antagonist properties by the following procedure. The analog was administered to the tissue preparation and the initial agonist response was allowed to subside. The antagonistic properties of the analog were then tested by administering varying doses of angiotensin II.

[8-Tryptophan]angiotensin II had approximately 22% pressor response of angiotensin II. Log dose–response curves were parallel and shifted to the right at the concentrations tested (1, 10, 50, and 100 ng/ml). These doses were used to calculate the pA_2 value. The response of the strip returned to normal in 1 hr. At higher doses (1000 ng/ml) the compound demonstrated contractile response. Similarly, pA_2 value (9.36 ± 0.48) reported for [8-thienylalanine]angiotensin II (Table I) is based on low concentrations (1, 10, 50, and 100 ng/ml in the bath solution of the compound). At higher concentrations, *e.g.*, 1000 ng/ml, the analog demonstrated contractile response; the dose–response curves though shifted to the right were not parallel to the control, and the maximum response was also reduced. The response of the aortic strip to angiotensin II did not return to normal level up to 2 hr. For this reason higher doses (100 ng/ml) were not utilized for calculating the pA_2 value. With the nonapeptide, [1-sarcosine,8-isoleucine]angiotensyl II proline, the dose–response curves though shifted to the right were not parallel even at low concentrations (1, 10, 50, and 100 ng/ml) and, therefore, the pA_2 value could not be calculated. However, the response of the aortic strip to angiotensin II returned to normal level in 30 min.

Structure–Activity Relationships. We reported previously that the presence of an α-nitrogen atom at the N terminus and its basicity are both important for maximum agonist properties of angiotensin II and analogs.[7,8] Present results corroborate this finding in that an increase in basicity of the N-terminal nitrogen atom enhanced the pressor activity of angiotensin II analogs. [1-Dimethylglycine]angiotensin II showed higher pressor activity than [1-sarcosine]angiotensin II or the natural angiotensin II.

In the antagonistic series it was previously reported[8] that the presence of sarcosine, dimethylglycine, or guanidineacetic acid in position 1 of [8-isoleucine]angiotensin II enhanced the *in vitro* antagonistic potency of the peptide. Changing the configuration of the amino acid residue in position 1 from L to D, as in [1-D-*allo*-*N*-methylisoleucine,8-isoleucine]angiotensin II, did not increase the duration of action of the compound. Antagonistic activity was reduced when position 1 in [8-alanine]angiotensin II was replaced with the neutral pyrrolidone residue.

Extension of the chain length at the N terminus in [1-sarcosine,8-isoleucine]angiotensin II with a proline residue

§For a detailed review see ref 6.

reduced the antagonistic activity of the compound drastically without any increase in the duration of action. Since the N-terminus part in angiotensin II (Asp-Arg), and its various agonist and antagonist analogs, is characteristically hydrophilic in nature, it is possible that a change to lipophilic character or increase in chain length, or both, may yield a peptide of different conformation. A similar change at the C terminus gave an analog ([1-sarcosine,8-isoleucine]angiotensyl II proline) in which antagonistic properties changed from the competitive to noncompetitive type. These results are difficult to interpret, since it is not known whether this change is due to a changed conformation, a change in the binding characteristics of the compound with the receptor site, or some other factors such as removal of the carboxyl group farther from the residue in the 8 position, etc.

Replacement of position 8 with threonine, as in [1-sarcosine,8-threonine]angiotensin, gave antagonistic activity comparable to [1-sarcosine,8-isoleucine]angiotensin II. This is interesting since a polar group on the aliphatic side chain did not reduce the antagonistic properties. On the contrary, the initial transient pressor activity of this compound was lower than [1-sarcosine,8-isoleucine]angiotensin II.

Substitution of position 8 with thienylalanine or tryptophan gave analogs which possess moderate agonist properties. This was expected since thienylalanine and tryptophan are both aromatic in character. However, [8-thienylalanine]angiotensin II functioned similarly to [8-cyclohexylalanine]angiotensin II in producing tachyphylaxis in rabbit aortic strips. It should also be pointed out that angiotensin II, at the dose levels used for testing these compounds, does not produce tachyphylaxis in the aortic muscle. Further, tachyphylaxis and inhibition may be related phenomena and since all angiotensin II analogs are related in structure, it is difficult to prove that tachyphylaxis is not involved. The mechanism of tachyphylaxis and its relationship to noncompetitive inhibition are not understood.

Experimental Section

Solvents used for ascending tlc were (a) *n*-BuOH–AcOH–H_2O (BAW) (4:1:5); (b) *n*-BuOH–AcOH–H_2O–Pyr (BAWP) (30:6:24:20); (c) *n*-BuOH–AcOEt–AcOH–H_2O (BEAW) (1:1:1:1); (d) *n*-BuOH–Pyr–H_2O (BPW) (10:2:5); (e) *n*-PrOH–H_2O (1:1). Ascending tlc was conducted on cellulose supported on glass plates (Brinkmann celplate-12). Ionophoresis was carried out on filter paper strips on S & S 2043A filter paper strips in Beckman electrophoresis cell (Durrum type) Model R, series D at 400 V, using HCO_2H–AcOH buffer prepared by diluting 60 ml of HCO_2H and 240 ml of AcOH to 2 l. with distilled H_2O (pH 1.9) and Beckman barbiturate buffer B-2 (pH 8.6). Histidine was used as a reference compound and *E*(His) indicates the electrophoretic mobility relative to histidine = 1.00. Detection of the compound on chromatograms was carried out with ninhydrin and/or diazotized sulfanilic acid. The free peptides were hydrolyzed in sealed tubes under N_2 in 6 *N* HCl at 110° for 24 hr in the presence of phenol. Amino acid analyses were performed on Jeolco-5AH amino acid analyzer. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Synthesis and Purification of Analogs. The protocol used for the synthesis was the same as previously described by us.[10] Precautions were taken to avoid racemization by using 1-hydroxybenzotriazole as an additive during the coupling of *tert*-butyloxycarbonylimidazolebenzylhistidine.[19] Instead of using 1-hydroxybenzotriazole, we used the preformed active ester of *tert*-butyloxycarbonyl-N^{im}-benzylhistidine with 1 molar excess of this reagent. (For details see under the synthesis of [Sar^1,Thr^8]angiotensin II.) With this modification, aminolysis was faster than the additive method, and the peptide polymer gave negligible color with ninhydrin after one coupling procedure. A possible explanation may be that *N,N'*-dicyclohexylurea formed during the coupling reaction coats the polymer beads, thus retarding the progress of the coupling reaction. Removal of *N,N'*-dicyclohexylurea by filtration prior to coupling with active ester avoids this difficulty, and it has been suggested that 1-hydroxybenzotriazole accelerates the aminolysis of active esters.[20] In the synthesis of [Trp^8]angiotensin II, the Boc group was removed at each stage with 1 *M* HCl in acetic acid using 2-mercaptoethanol as a scavanger.[21,22] The Boc group during the synthesis of [Tal^8]angiotensin II was removed with 1 *M* HCl in AcOH. In both the above syntheses histidine was protected as *tert*-butyloxycarbonylimidazoletosylhistidine. Cleavage of these peptides from the polymer with HF gave low yields of the crude products. As evidenced by acid hydrolysis of the residual polymer *ca.* 30–40% of the peptides remained uncleaved even after 1 hr of treatment with HF.

During the synthesis of [1-sarcosine,8-isoleucine]angiotensyl II proline, cleavage of the C-terminal dipeptide,[15] as c-(Ile-Pro), was avoided by neutralization of the trifluoroacetate salt of the isoleucylproline polymer ester for a short time (10% NEt_3 in $CHCl_3$ for 3 min with vigorous stirring) followed by quick washing with $CHCl_3$ (three times for 1 min) and coupling with a fivefold excess of *tert*-butyloxycarbonylproline (twice for 10 min).

Coupling of dimethylglycine with DCC appeared difficult due to insolubility of the free amino acid in most organic solvents, including $HCONMe_2$. However, activation with *N*-ethyl-5-phenylisoxazolium 3'-sulfonate[23] in $HCONMe_2$ gave a homogeneous solution and after two couplings the peptide polymer gave a ninhydrin negative test. For the synthesis of [D-*a*MeIle^1,Ile^8]angiotensin II, *tert*-butyloxycarbonyl-D-*allo*-*N*-methylisoleucine was activated as its o-nitrophenyl ester by the general procedure reported by Bodanszky and Funk.[24] The latter was then condensed with the heptapeptide polymer, Arg(NO_2)-Val-Tyr(OBzl)-Ile-His(Bzl)-Pro-Ile-polymer, in $HCONMe_2$.

Purification of the desired peptides was carried out as reported by us in a preceding paper.[7] Except for [Trp^8]- and [Tal^8]angiotensin II which gave low yields (10–15%), the overall yield of the purified peptides, based on initial amino acid attached to the polymer, was 25–40%. No attempt was made to rechromatograph the minor fractions for identification purposes. The homogeneity of the compounds was determined by (a) electrophoresis at pH 8.6 and 1.95, (b) thin-layer chromatography in five solvent systems of different pH, and (c) amino acid analysis.

[1-Dimethylglycine]angiotensin II. The protected heptapeptide polymer [Boc-Arg(NO_2)-Val-Tyr(OBzl)-Ile-His(Bzl)-Pro-Phe-polymer ester, 4 g] was deprotected in the usual way. The trifluoroacetate salt was neutralized (10% NEt_3 in $HCONMe_2$) and the peptide polymer coupled with *N,N*-dimethylglycine activated with *N*-ethyl-5-phenylisoxazolium 3'-sulfonate by the procedure reported in a preceding paper.[8] The analog was purified by the general procedure: tlc (cellulose) R_f 0.50 (BAW), R_f 0.76 (BEAW), R_f 0.27 (BPW), R_f 0.66 (BAWP), R_f 0.35 (PW); *E*(His) 0.82 (pH 1.95), *E*(His) 1.01 (pH 8.6); amino acid ratio in the acid hydrolysate Arg 1.04, Val 0.96, Tyr 1.0, Ile 0.95, His 1.05, Pro 1.10, Phe 1.07.

[1-Pyroglutamic acid,8-alanine]angiotensin II: tlc (cellulose) R_f 0.37 (BAW), R_f 0.66 (BEAW), R_f 0.01 (BPW), R_f 0.53 (BAWP), R_f 0.80 (PW); *E*(His) 0.69 (pH 1.95), *E*(His) 1.10 (pH 8.6); amino acid ratio in the acid hydrolysate Glu 1.04, Arg 0.95, Val 1.00, Tyr 1.01, Ile 1.04, His 0.99, Pro 0.99, Ala 1.00.

***tert*-Butyloxycarbonyl-D-*allo*-*N*-methylisoleucine.** This was prepared by a modified procedure of Olsen.[25] A solution of *tert*-butyloxycarbonylisoleucine (11.6 g) in dry $HCONMe_2$ (250 ml) was magnetically stirred at 80° with Ag_2O (49 g) in a 1-l. flask fitted with a reflux condenser. After 4 hr the mixture was cooled in ice water (0–5°), treated with a solution of CH_3I (10 ml) in $HCONMe_2$ (15 ml), and stirred for 2 hr at 0–5° and 24 hr at room temperature. A second lot of CH_3I (10 ml) in $HCONMe_2$ (15 ml) was added, the mixture stirred for 24 hr, and the process repeated after 24 hr with a third lot of CH_3I (10 ml). At the end of 72 hr of stirring the solvent was evaporated under reduced pressure, keeping the temperature below 30°, and the residue extracted with $CHCl_3$. The extract was washed under ice-cold conditions with H_2O, 5% Na_2CO_3 solution, 5% citric acid solution, H_2O, and saturated NaCl solution. The organic phase was dried (Na_2SO_4) and the solvent removed by evaporation. The residual oil was extracted with anhydrous Et_2O (25 ml), the ethereal layer filtered, and the clear filtrate evaporated under N_2 to give 11.20 g of *tert*-butyloxycarbonyl-D-*allo*-*N*-methylisoleucine methyl ester as a pale yellow oil. The ir spectrum lacked absorption due to amide NH or

carboxyl OH in the region of 3200–3600 cm^{-1}: tlc (silica gel) R_f 0.73 (95:5 $CHCl_3$–AcOH), R_f 0.85 (98:2 Me_2CO–AcOH), R_f 0.92 (85:10:5 $CHCl_3$–MeOH–AcOH), R_f 0.86 (90:10 $CHCl_3$–AcOH). *Anal.* ($C_{13}H_{25}NO_4$) C, H, N. The above ester (11.20 g) was dissolved in EtOH (150 ml) and the solution treated with 46 ml of 1 *N* NaOH. The mixture was stirred at room temperature for 2 hr. EtOH was evaporated *in vacuo* at room temperature, the residual oil taken up in water, and the aqueous layer extracted with AcOEt to remove unsaponified ester. The aqueous phase was cooled, and crushed ice was added. The pH was adjusted to *ca.* 3 with citric acid and the aqueous solution extracted three times with cold AcOEt. The combined extracts were washed with cold H_2O and NaCl solution, dried (Na_2SO_4), and evaporated to give 6.45 g of a clear oil: tlc (silica gel) R_f 0.71 (90:10 $CHCl_3$–AcOH), R_f 0.48 (95:5 $CHCl_3$–AcOH), R_f 0.83 (85:10:5 $CHCl_3$–MeOH–AcOH). *Anal.* ($C_{12}H_{23}NO_4$) C, H, N.

tert-Butyloxycarbonyl-D-*allo*-*N*-methylisoleucine o-Nitrophenyl Ester. The active ester was prepared according to the general procedure reported by Bodanszky and Funk.[24] *tert*-Butyloxycarbonyl-D-*allo*-*N*-methylisoleucine (7.35 g, 30 mmol) and o-nitrophenol (8.35 g, 60 mmol) were dissolved in dry pyridine (75 ml) and the mixture was cooled to an ice–water bath. DCC (6.18 g, 30 mmol) was added to the stirred solution. The mixture was magnetically stirred for 30 min at 0–5°, followed by 3.5 hr at room temperature. The precipitated *N,N'*-dicyclohexylurea was removed by filtration and the filtrate evaporated *in vacuo* on a rotary evaporator. The resulting residue was dissolved in Et_2O and filtered to remove any turbidity. The solvent was evaporated and the residue was dissolved in $CHCl_3$. The solution was washed once with 5% citric acid under ice-cold conditions and several times with 0.1 *N* NaOH until the washings gave a faint orange color. The organic phase was then washed with water and dried ($MgSO_4$) and the solvent evaporated. The residue was dissolved in excess *n*-hexane, the turbidity removed by filtering, and the filtrate evaporated *in vacuo*. The residual oil was found to be homogeneous on tlc (silica gel) R_f 0.80 (9:1 $CHCl_3$–MeOH); R_f 0.93 (85:10:5 $CHCl_3$–MeOH–AcOH). *Anal.* ($C_{18}H_{26}N_2O_6$) N.

[1-D-*allo*-*N*-Methylisoleucine,8-isoleucine]angiotensin II: tlc (cellulose) R_f 0.72 (BAW), R_f 0.78 (BEAW), R_f 0.47 (BPW), R_f 0.88 (BAWP), R_f 0.85 (PW); amino acid ratio in the acid hydrolysate Arg 1.02, Val 1.00, Tyr 0.96, Ile 2.35, His 0.98, Pro 1.01.

Prolyl[1-sarcosine,8-isoleucine]angiotensin II: tlc (cellulose) R_f 0.64 (BAW), R_f 0.72 (BEAW), R_f 0.34 (BPW), R_f 0.73 (BAWP), R_f 0.79 (PW); *E*(His) 0.80 (pH 1.95), *E*(His) 0.96 (pH 8.6); amino acid ratio in the acid hydrolysate Sar 0.98, Pro 2.00, Arg 1.01, Val 1.14, Tyr 1.06, Ile 1.95, His 1.00.

[1-Sarcosine,8-isoleucine]angiotensyl II proline: tlc (cellulose) R_f 0.422 (BAW), R_f 0.76 (BEAW), R_f 0.112 (BPW), R_f 0.73 (BAWP), R_f 0.75 (PW). A minor impurity (<0.5%), R_f 0.31 (BAW), R_f 0.62 (BAWP), could not be removed even after repeated chromatography on Sephadex G-25 (BPW), on silica gel (2:1 *n*-PrOH–H_2O), or gel filtration on Sephadex G-25 (0.1 *M* AcOH). However, the compound was found to be homogeneous in its electrophoretic mobility: *E*(His) 0.81 (pH 1.95), *E*(His) 1.0 (pH 8.6); amino acid ratio in the acid hydrolysate Sar 1.01, Arg 0.94, Val 1.00, Tyr 0.99, Ile 2.04, His 1.09, Pro 2.12.

[8-Tryptophan]angiotensin II: tlc (cellulose) R_f 0.39 (BAW), R_f 0.70 (BEAW), R_f 0.11 (BPW), R_f 0.64 (BAWP), R_f 0.74 (PW); *E*(His) 0.75 (pH 1.95), *E*(His) 1.14 (pH 8.6); amino acid ratio in the acid hydrolysate Asp 1.0, Arg 1.12, Val 1.07, Tyr 1.14, Ile 1.08, His 1.09, Pro 1.10. Alkaline hydrolysis[13] gave an equivalent ratio of tryptophan.

[8-Thienylalanine]angiotensin II: tlc (cellulose) R_f 0.34 (BAW), R_f 0.68 (BEAW), R_f 0.13 (BPW), R_f 0.62 (BAWP), R_f 0.85 (PW); *E*(His) 0.79 (pH 1.95), *E*(His) 1.19 (pH 8.6); amino acid ratio in the acid hydrolysate Asp 0.96, Arg 1.08, Val 0.96, Tyr 1.04, Ile 0.93, His 1.00, Pro 1.00, Tal 1.08.

[1-Sarcosine,8-threonine]angiotensin II. The compound was synthesized by the general procedure except the following modification for coupling histidine. Boc-His(Bzl) (3.45 g, 10 mmol) was dissolved in $HCONMe_2$ (30 ml) by repeated extraction. The clear solution was cooled to −5° and treated with a solution of 1-hydroxybenzotriazole (1.35 g, 10 mmol, freshly dried) in $HCONMe_2$ followed by a solution of DCC (2.06 g, 10 mmol) in CH_2Cl_2 (25 ml). The mixture was stirred at −5° for 2.5 hr. The precipitated *N,N'*-dicyclohexylurea was filtered and the filtrate added to prolylphenylalanine polymer ester. The mixture was mixed for 5 min followed by the addition of another lot of 1-hydroxybenzotriazole (1.35 g) in $HCONMe_2$. After shaking for 5 hr, the solution was filtered and washed (1:1 MeOH–$CHCl_3$, MeOH, $CHCl_3$, $HCONMe_2$, CH_2Cl_2) and the coupling step repeated: tlc (cellulose) R_f 0.09 (BAW), R_f 0.50 (BEAW), R_f 0.04 (BPW), R_f 0.46 (BAW), R_f 0.62 (PW); *E*(His) 0.85 (pH 1.95), *E*(His) 1.02 (pH 8.6); amino acid ratio in the acid hydrolysate Sar 0.97, Arg 1.01, Val 1.01, Tyr 1.01, Ile 0.95, His 0.97, Pro 1.00, Thr 0.97.

[8-Cyclohexylalanine]angiotensin II. This analog was synthesized as reported earlier except the following modification for the synthesis of cyclohexylalanine hydrochloride salt. A solution of L-tyrosine (5 g) in 2 *N* HCl (200 ml) was hydrogenated over PtO_2 (1.5 g) at 50 psi for 48 hr. The catalyst was filtered and the filtrate evaporated *in vacuo*. The residual yellowish crystalline material was triturated with 12 *N* HCl and filtered. The residue was washed several times with 12 *N* HCl and dried *in vacuo* over KOH pellets to give 3.5 g of colorless product: mp 248–250° dec; tlc (silica gel) R_f 0.75 (BAW), R_f 0.76 (BEAW); amino acid analysis gave only one peak with a retention time greater than phenylalanine. *Anal.* ($C_9H_{17}NO_2$·HCl) C, H, N.

Pharmacological Evaluation.[&] The pressor activity was determined by pressor assay in vagotomized, ganglion-blocked rats by the procedure reported by Pickens, *et al.*[26] The results given in Table I represent mean values obtained for three or four pressor responses at different dose levels and for angiotensin II controls and are relative to [Asp[1],Ile[5]]angiotensin II = 100. Sufficient time was given for complete recovery between each injection in order to minimize any tachyphylactic effect which may be present.

Inhibition of contractile activity of angiotensin II was studied on isolated, spirally cut rabbit aortic strips. pA_2 values were calculated according to the method described by Arunlakshna and Schild[18] by the equation: $\log (x - 1) = n \log B + \log K_2$. First a log dose–response curve for angiotensin II was determined on rabbit aortic strips, before and in the presence of the analogs. Next, the dose-ratio of equal response to angiotensin II was calculated in the presence of various analogs at several molar concentrations of antagonist. This was followed by plotting the log dose-ratio minus one [$\log (x - 1)$] against negative logarithm of the molar concentration of antagonist [$-\log B$]. The point on the abscissa that is intercepted by the plotted line is designated as the pA_2 value. The pA_2 values given in Table I represent mean values obtained for five to six separate experiments with each antagonist using [Asp[1],Ile[5]]angiotensin II as the agonist.

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Structure–Activity Relationships in Immunochemistry. 2. Inhibition of Complement by Benzamidines†

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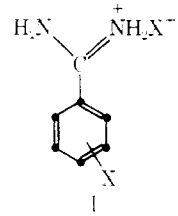
A quantitative structure–activity relationship has been formulated for 108 benzamidine derivatives inhibiting complement. The relationship is expressed as: log $1/C$ = 0.15(MR-1,2) + 1.07(D-1) + 0.52(D-2) + 0.43(D-3) + 2.43 where C is the molar concentration causing 50% inhibition of complement, MR is the molar refractivity of substituents, D-1 is an indicator variable which accounts for activity of the moiety PhX where X represents a variety of bridge units, D-2 is an indicator variable for the presence or absence of a pyridine moiety, and D-3 is an indicator variable for the presence of the structural unit NHCOY attached to the second benzene ring where Y may be a variety of different units. The above equation correlates activity of 108 derivatives with a correlation coefficient of 0.935 and a standard deviation of 0.258. This relationship suggests new approaches to the synthesis of complement inhibitors.

As our understanding of the immune response grows, it becomes more feasible to systematically attempt to influence it by chemical means. It was found in our first study in this area[1] that the inhibition of antibody–antigen interactions by haptens could be formulated in a mathematical relationship. It was shown that the inhibition of the antibody–antigen interaction by haptens of the type X-$C_6H_4COO^-$ and X-$C_6H_4AsO_3H^-$ from the studies of Pauling and Pressman[2] could be described quantitatively by equations such as eq 1. In eq 1, E_s is the Taft steric

$$\log K_{rel} = 0.86E_s^{o} + 0.08E_s^{m} - 0.45E_s^{p} - 0.69 \quad (1)$$
$$n \quad r \quad s$$
$$22 \quad 0.974 \quad 0.177$$

parameter and the superscripts o, m, and p refer to the substituents in the ortho, meta, and para positions of the substituted benzoate haptens. Little is lost in dropping the E_s^m since its coefficient is quite small. In eq 1, n represents the number of data points, r is the correlation coefficient, and s is the standard deviation from the regression line. The positive coefficient with E_s^o in this quantitative structure–activity relationship (QSAR) indicates that large substituents in the ortho position make poor haptens and the negative coefficient with E_s^p indicates that the opposite is true for para substituents. Our success in correlating the structurally demanding hapten–antibody interactions encouraged us to attempt other immunochemical QSAR.

The late B. R. Baker and his students carried out an extensive study of the inhibitory action of derivatives of benzamidine (I) on guinea pig complement. This was part of Baker's generalized search for drugs to inhibit mamma-



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lian proteolytic enzymes. As he pointed out,[3] at least 15 such distinct proteolytic enzymes have been characterized. Since all of these proteolytic enzymes hydrolyze peptide bonds, they must be closely related and their specificity appears to reside mainly in the type of acylated amino acid amide preferred for complexing.[4] Hence, designing an inhibitor for one such enzyme is not an easy problem and requires the study of inhibitors on a variety of proteolytic enzymes. Baker's group was particularly concerned with trypsin, chymotrypsin, and complement. Complement consists of 11 distinct proteins[5,6] which are required for cell lysis brought about *via* antibodies and complement. The function of the antibodies is to identify the invading cell as a foreign organism and activate complement attack which results in cell lysis by means of the proteolytic enzymes. When complement is activated by an antibody, it of course could attack the host's own cells. The antibody circumvents this problem by fixing complement on the surface of the foreign cell.

Thus it is apparent that there are several routes open for the inhibition of the rejection of tissue or organ transplants. One might inhibit antibody formation, control formed antibodies with haptens, or inhibit the functioning of the complement system. Baker chose to study complement inhibitors.

Coats[7] has studied a subset of 25 of Baker's benzamidine complement inhibitors. Although he obtained a good

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