Table I. Chromatographic Properties of Cycloserine and Several Derivatives

Compd	R_f Solvent A	R_f Solvent B	Color formed 0.4% $FeCl_3$	Color formed 4% Nitroprusside
1	0.7	0.7	Brown	Blue
3	0.8	0.8		
5	0.3	0.15	Brown	Blue
9	0.7	0.7	Pink	Blue

ments (at 10 a.m. and 4 p.m.) of 120 mg/kg (total dosage 240 mg/kg/day).

For the injection of **3**, a fresh solution containing 180 mg/ml in 7% ethanol was prepared and a total dosage of 600 mg/kg/day was injected through the tail vein in increments of 300 mg/kg twice per day, at 10 a.m. and 4 p.m.

Chromatographic Analysis of Rat Urine Samples. 1. Paper Chromatography. Samples were spotted 2.5 cm away from each other and 3 cm from the bottom of a 28.5 × 42.0 cm sheet of Whatman No. 1 filter paper which was developed ascendingly with either 1-propanol–water (5:1, v/v, solvent A) or 1-butanol–acetic acid–water (4:1:1, v/v, solvent B). The two most useful detection reagents were 4% aqueous sodium nitroprusside[14] and 0.4% aqueous ferric chloride. Iodine vapor and uv light were used to detect **3**. The R_f values of **1** and **9** were so similar that their relative migratory rates often reversed from experiment to experiment. This did not present a major problem because standards were always used and **1** and **9** gave different colored spots with $FeCl_3$. The chromatographic properties of **1**, **3**, **5**, and **9** are presented in Table I.

After it had been determined by the use of $FeCl_3$ that **1** was being excreted in the urine of rats which had been administered **3**, nitroprusside was used to obtain a semiquantitative value.

Semiquantitative Estimation. The percentages of administered compounds which were excreted as **1** or **5** could be estimated by comparison of the size and intensity of spots resulting from the chromatography of known amounts of **1** and **5** with spots derived from urine samples. The total amounts of **1** and **5** excreted in the 24-hr urine sample were then calculated from the total volume of urine excreted in this time. Since it was found that 5 μg of **5** and 10 μg of **1** could be detected by sodium nitroprusside, it can be calculated that less than 12% of the administered **5** was excreted as **1**, and less than 10% of **1** was excreted as **5**. There is no evidence that any interconversion of this type occurred; these calculations merely show that a small amount of **5** → **1** and **1** → **5** could occur without detection.

2. Gas–Liquid Chromatography. Although the results of paper chromatography indicated that the rat transformed **3** to **1** rather than to **9**, more evidence that **9** was not being formed was desirable. This was obtained by treatment of rat urine samples (after preliminary column chromatography using Amberlite GC-400 resin to remove interfering substances) with diazomethane to convert any **9** which was present to the methyl ester **11**. This was then subjected to glc. Control experiments in which **9** was carried through the same procedure indicated that 1 mg could easily be detected. When 50-ml urine samples from rats treated with **1**, **3**, or **5** were analyzed by this technique, no evidence of **11** was found.

References

(1) P. H. Hidy, E. B. Bridge, V. V. Young, R. L. Harned, G. A. Brewer, W. F. Phillips, W. F. Runge, H. E. Staveley, A. Pohland, H. Boaz, and H. R. Sullivan, *J. Amer. Chem. Soc.*, **77**, 2345 (1955).
(2) F. A. Kuehl, F. J. Wolf, N. R. Trenner, R. L. Peak, R. Howe, B. D. Hunnewell, G. Downing, E. Newstead, R. P. Buhs, I. Putter, R. Ormoud, J. E. Lyons, L. Chalet, and K. Folkers, *J. Amer. Chem. Soc.*, **77**, 2344 (1955).
(3) C. H. Stammer in "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins," Vol. I, B. Weinstein, Ed., Marcel Dekker, New York, N. Y., 1971, p 23.
(4) F. C. Neuhaus in "Antibiotics, Mechanism of Action," Vol. I, D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, New York, N. Y., 1967, p 40.
(5) T. Viswanatha, *Proc. Nat. Acad. Sci. U. S.*, **50**, 967 (1963).
(6) W. J. D. Whish and T. Viswanatha, *Can. J. Biochem.*, **48**, 218 (1970).
(7) G. W. Milne and L. A. Cohen, *Tetrahedron*, **23**, 65 (1967).
(8) J. C. Howard, *J. Org. Chem.*, **36**, 1073 (1971).
(9) G. M. Conzelman, Jr., *Amer. Rev. Tuberc. Pulm. Dis.*, **74**, 739 (1956).
(10) H. Boyd, I. C. Calder, S. J. Leach, and B. Milligan, *Int. J. Peptide Protein Res.*, **4**, 109, 117 (1972).
(11) F. A. Kuehl, U. S. Patent 2,845,432 (1957); *Chem. Abstr.*, **52**, 20198*d* (1958).
(12) C. H. Stammer, *J. Org. Chem.*, **27**, 2957 (1962).
(13) F. Arndt, "Organic Syntheses," Collect. Vol. II, Wiley, New York, N. Y., 1943, p 165.
(14) L. R. Jones, *Anal. Chem.*, **28**, 39 (1956).

Angiotensin-Like and Antagonistic Activities of N-Terminal Modified [8-Leucine]angiotensin II Peptides†

Therezinha B. Paiva,* Gilberto Goissis, Luiz Juliano, Maria E. Miyamoto, and Antonio C. M. Paiva

Department of Biophysics and Physiology, Escola Paulista de Medicina, 04023 São Paulo, S.P., Brazil. *Received May 22, 1973*

Two series of peptides related to [Ile^5]angiotensin II (AII) were synthesized by the solid-phase method: a Phe^8 series, composed of AII, [Suc^1]AII, des-Asp^1-AII, and des-Asp^1,Arg^2-AII; and a Leu^8 series, composed of [Leu^8]AII, [Suc^1,Leu^8]AII, des-Asp^1-[Leu^8]AII, and des-Asp^1,Arg^2-[Leu^8]AII. The AII-like and AII-antagonistic activities of these peptides were studied on the isolated guinea-pig ileum, rat uterus, and rabbit aorta and on the rat blood pressure. Comparison of pA_2, pD_2, and intrinsic activities of the two series of peptides led to the conclusion that [Leu^8]AII, [Suc^1,Leu^8]AII, and des-Asp^1-[Leu^8]AII antagonized AII by binding on the same receptor site, but it is suggested that a different site is involved in the agonistic activities of these peptides. Conclusions were also drawn on the importance of the N-terminal end for binding and for the intrinsic activity at the two postulated receptor sites.

Several angiotensin II analogs with aliphatic amino acids in position 8 of the peptide chain have been synthesized and shown to be specific competitive angiotensin antagonists.[2] Among the most potent of these inhibitors is [Leu^8]angiotensin II,[2b,d] which, however, possesses small angiotensin-like agonistic activities. It was thought of interest to study structure–activity relationships of 8-leucine angiotensin peptides, both as agonists and antagonists, and to compare these relationships with those observed

†This investigation was supported by grants from the Fundacão de Amparo a Pesquisa do Estado de São Paulo (Projeto Bioq/FAPESP), the Brazilian National Research Council (CNPq), and FINEP. Abbreviations used are: Boc = *tert*-butyloxycarbonyl; Suc = succinyl. The amino acids are of the L configuration and are abbreviated as recommended by the IUPAC Commission on nomenclature.[1]

Table I. N-Terminal Modified Analogs of Angiotensin II and [Leu^8]angiotensin II

Compd	Amino acid sequence
1	Asp·Arg·Val·Tyr·Ile·His·Pro·Phe
2	Suc·Arg·Val·Tyr·Ile·His·Pro·Phe
3	Arg·Val·Tyr·Ile·His·Pro·Phe
4	Val·Tyr·Ile·His·Pro·Phe
5	Asp·Arg·Val·Tyr·Ile·His·Pro·Leu
6	Suc·Arg·Val·Tyr·Ile·His·Pro·Leu
7	Arg·Val·Tyr·Ile·His·Pro·Leu
8	Val·Tyr·Ile·His·Pro·Leu

for angiotensin II. N-Terminal modified analogs and homologs were chosen for this study because the Asp^1 and Arg^2 residues of angiotensin are important but not essential requisites for biological activity.[3]

Two series of peptides were synthesized (Table I): a Phe^8 series, composed of peptides 1–4, and a Leu^8 series, with peptides 5–8. These compounds were studied both as agonists (1–8) and antagonists (5–8) in the rat blood pressure, the isolated guinea-pig ileum, the isolated rat uterus, and the isolated rabbit aorta.

Results and Discussion

The relative biological activities of compounds 1–4 are shown in Table II. The pressor and oxytocic activities of these peptides have been studied previously by several authors,[3-8] but it is difficult to compare results obtained in different laboratories.[3a] One of the difficulties is that it is not always clear whether the reported activities refer to weight or to moles of the peptides or their salts. For this reason we shall use the values of Table II, where the activities are expressed on a molar basis, taking into account the peptide content. There are two notable discrepancies between the values of Table II and previously published results: (a) the relative pressor activity of 2 was found to be about twice those reported earlier;[4,7] (b) the oxytocic activity of 3 was found to be about three times greater than previously reported values.[5,6]

Table II shows that the large loss of activity observed upon removal of the two N-terminal residues of angiotensin II (4) is very similar in the four biological preparations. It can also be seen that, in spite of the significant differences between the reactivities of the three smooth muscle preparations, there is a similarity in the relative activities of peptides 1–4 for each preparation: the absence of the amino group of angiotensin II resulted in a significantly larger loss of activity (2) than the lack of the N-terminal aspartyl residue (3). The same cannot be said of the *in vivo* pressor activity (Table II) where 2 is as active as 1. This may be because 2 is more resistant to the blood "angiotensinase" activity, since the absence of an N-terminal amino group makes it immune to attack by aminopeptidases.

The peptides of the Leu^8 series also showed angiotensin-like activities toward the preparations studied (Table III), with the exception of the rabbit aortic strips, where they were inactive in concentrations up to 10^{-4} *M*. The activities in the other smooth muscles and *in vivo* were three to four orders of magnitude lower than those of angiotensin II. It is not probable that these activities could be due to impurities in the peptides because, from what is known of the structure–activity relationships in the angiotensins,[3a] none of the possible contaminants would be expected to have high enough activity for that.

It is interesting to compare the relative activities of compounds 5–8 (Table III) with those of the corresponding peptides with phenylalanine in position 8 (Table II). In the Leu^8 series, the absence of the amino group (6) induced a significant increase of the pressor activity, and the loss of myotropic activity was much smaller than in the corresponding Phe^8 analog 2. Removal of the aspartyl residue resulted in further decrease of the myotropic activities in the Leu^8 series (7), in contrast to the raise observed with the Phe^8 analog 3. Also, the relative activities of the hexapeptide of the Leu^8 series (8) were much greater than those of the Phe^8 analog 4.

The dissimilarities in the agonistic activities of the two series of analogs are further evidenced in Table IV, where intrinsic activities and pD_2 values for the agonistic myotropic activities of all the compounds are shown. Table IV also shows the pA_2 values for peptides 5, 6, and 7, which showed competitive inhibitory activity toward angiotensin II. Compound 8 did not behave as an antagonist in concentrations up to 10^{-4} *M*. Peptides 5–7 also antagonized the pressor action of angiotensin II on the rat blood pressure, but pA_2 values cannot be calculated in this case.

The pA_2 values for peptides 5, 6, and 7 are similar to the corresponding pD_2 values for 1, 2, and 3, both in their order of magnitude and in their relative values. In each of the three smooth muscle preparations, both the pD_2 values for the Phe^8 series and the pA_2 values for the Leu^8 series decreased significantly in the order: full octapeptide, des-Asp^1 heptapeptide, and des-amino octapeptide. This indicates that the same receptor site is involved in both series of peptides, as should be expected by the competitive nature of the inhibition of angiotensin II activity by the Leu^8 analogs. It is also apparent that the receptors

Table II. Relative Biological Activities[a] of Angiotensin II Analogs Modified at the N Terminus

Compd	Guinea-pig ileum	Rat uterus	Rabbit aorta	Rat blood pressure
1	100	100	100	100
2	11 ± 2	47 ± 3	12 ± 2	101 ± 3
3	34 ± 1	66 ± 2	22 ± 2	47 ± 4
4	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.02	0.14 ± 0.02

[a]The activities ± one standard deviation are expressed in percentage of the activity of 1. Each value is the average of at least four independent assays.

Table III. Relative Biological Activities[a] of [Leu^8]angiotensin II Analogs Modified at the N Terminus

Compd	Guinea-pig ileum	Rat uterus	Rat blood pressure
5[b]	100 (0.016 ± 0.001)	100 (0.15 ± 0.02)	100 (0.086 ± 0.016)
6	81 ± 5	73 ± 4	139 ± 5
7	42 ± 3	36 ± 3	20 ± 2
8	4.4 ± 0.5	2.9 ± 0.3	10 ± 1

[a]The activities ± one standard deviation are expressed in percentage of the activity of 5. Each value is the average of at least five independent assays. [b]The activities of 5, in percentage of the activity of 1, are shown inside parentheses.

Table IV. Agonistic and Antagonistic Properties of Angiotensin II and Analogs

Compd	Guinea-pig ileum			Rat uterus			Rabbit aorta		
	Intrinsic activity[a]	pD_2	pA_2	Intrinsic activity[b]	pD_2	pA_2	Intrinsic activity[c]	pD_2	pA_2
1	1.00 ± 0.02	8.80 ± 0.06		0.97 ± 0.01	8.74 ± 0.09		1.00 ± 0.02	8.72 ± 0.07	
2	1.00 ± 0.03	7.83 ± 0.09		0.99 ± 0.01	8.41 ± 0.05		0.99 ± 0.01	7.79 ± 0.05	
3	0.99 ± 0.02	8.41 ± 0.07		0.99 ± 0.02	8.56 ± 0.06		0.91 ± 0.01	8.07 ± 0.09	
4	0.92 ± 0.02	5.84 ± 0.06		0.94 ± 0.01	5.75 ± 0.05		0.85 ± 0.01	5.73 ± 0.06	
5	0.48 ± 0.01	5.00 ± 0.05	8.92 ± 0.05	0.69 ± 0.01	5.92 ± 0.05	8.33 ± 0.09			9.15 ± 0.08
6	0.50 ± 0.02	4.91 ± 0.05	7.24 ± 0.08	0.77 ± 0.02	5.79 ± 0.08	7.51 ± 0.06			8.05 ± 0.07
7	0.63 ± 0.03	4.62 ± 0.06	8.46 ± 0.12	1.02 ± 0.01	5.48 ± 0.05	8.14 ± 0.10			8.41 ± 0.09
8	0.59 ± 0.03	3.65 ± 0.08		1.00 ± 0.02	4.25 ± 0.06				

[a] Relative to histamine. [b] Relative to oxytocin. [c] Relative to epinephrine.

of the three tissues are similar, since they respond in a comparable manner to the octapeptide, the des-amino octapeptide, and the heptapeptide.

The pD_2 values for the agonistic activities of peptides **5–8** in the guinea-pig ileum and rat uterus were smaller than those of peptides **1–4**, respectively, by two or four orders of magnitude. Furthermore, in the Leu[8] series, the decrease in pD_2 by removal of the amino group (**6**) was smaller than that observed with the des-Asp[1] analog **7**. In the case of the guinea-pig ileum, however, there was no significant difference between the values for peptides **5** and **6**.

Another striking contrast between the agonistic activities of the two series of peptides resides in the effect of the N-terminal modifications on the intrinsic activities (Table IV). In the Phe[8] series, **1** and **2** are full agonists for the three tissues; **3** is a full agonist for the ileum and uterus, but its intrinsic activity in the rabbit aorta is significatively smaller than unity. Peptide **5** was not a full agonist toward any of the three tissues. In the Leu[8] series, no agonistic activity was found toward the rabbit aorta and peptides **5** and **6** had low intrinsic activities toward the other two tissues. However, peptides **7** and **8** had higher intrinsic activities than **5** and **6**, and they were full agonists in the rat uterus. This is in marked contrast to what was observed in the Phe[8] series.

Our data strongly indicate that the agonistic activities of the Leu[8] angiotensin analogs toward the guinea-pig ileum and the rat uterus are due to interaction with receptor sites that are different from those involved in the response to angiotensin II. These receptors were not found in rabbit aorta and are characterized by a relatively low affinity for the peptides. They also differ from angiotensin receptors by their relative affinity for the amino group and the N-terminal aspartyl residue and, most strikingly, by the increase of the intrinsic activity observed with the des-Asp[1] and des-Asp[1],Arg[2] analogs.

Experimental Section

Syntheses. The peptides were synthesized by the solid-phase method.[9,10] The C-terminal *tert*-butyloxycarbonylamino acid was esterified to 2% cross-linked chloromethyl polymer, and chain elongation was performed on an automatic peptide synthesizer.[11] $CHCl_3$ was used as solvent for all reagents and amino acids, with the exception of Boc-Arg(NO_2), which was dissolved in $CHCl_3$–DMF (2:1). The Boc groups were removed with 25% (v/v) CF_3COOH in $CHCl_3$ for 30 min. The coupling steps were carried out with 2.5 equiv of *tert*-butyloxycarbonylamino acid and DCI. The amino acids with reactive side chains were used in the form of the following derivatives: Boc-Asp(Bzl), Boc-Arg(NO_2), Boc-Tyr(Bzl), and Boc-His(Tos). After the last coupling, the peptide was cleaved from the resin and deprotected by stirring for 60 min at 0° in anhydrous HF containing 5% (v/v) anisole. After removal of HF and anisole, by vacuum distillation and washing with EtOAc, the peptides were extracted with glacial HOAc and lyophilized.

Purification of Peptides. The crude peptides were submitted to 800–1200 transfers of countercurrent distribution in *n*-BuOH–HOAc–H_2O (4:1:5), and the distribution coefficient (K) observed for each product is given in Table V. This was followed by ion-exchange chromatography on a 7 × 1.5 cm column of Dowex-1 resin, with an elution gradient from 0.01 M NH_4OAc (pH 8) to 0.1 M NH_4OAc (pH 6). When necessary, the peptide was further purified by chromatography on a 9 × 1.7 cm carboxymethylcellulose column with a linear gradient between 0.1 M NH_4OAc (pH 4.7) and 3.5 M HOAc and by chromatography on a 8 × 2 cm column of Dowex-50 resin with an NH_4OAc gradient from 0.01 (pH 5.5) to 0.1 M (pH 8.0). All peptides were lyophilized until constant weight to remove NH_4OAc. Each peptide was purified until the following criteria for purity were met. (a) The amino acid analysis of the acid hydrolyzates, performed on a Beckman Model 120C amino acid analyzer, yielded a molar ratio within 3% of the theoretical value for all the amino acid residues (with two exceptions:

Table V. Physical Properties of Compounds 1–8

Compd	Yield,[a] %	% peptide content[b]	Mp, °C	$[\alpha]^{25}D$[c]	K^d	R_f on tlc[a] A	B	C	Electrophoretic migration[a] pH 2.8 (R_{His})	pH 4.9 (R_{Arg})	pH 9.9 (R_{Picr})
1	11	86	254–258	−74.4[e]	0.22	0.25	0.55	0.88	0.56	0.43	0.15
2	10	90	219–220	−66.7	0.82	0.60	0.70	0.82	0.54	0.25	0.24
3	25	80	210–213	−70.0	0.19	0.36	0.68	0.85	0.73	0.76	−0.06
4	15	93	185–190	−70.6	0.57	0.46	0.67	0.85	0.65	0.52	0.49
5	13	87	245 dec	−90.5[f]	0.18	0.27	0.63	0.83	0.55	0.46	0.20
6	16	93	225 dec	−86.3[f]	0.79	0.45	0.70	0.85	0.55	0.24	0.21
7	18	79	210 dec	−88.4	0.12	0.26	0.58	0.88	0.84	0.75	0.10
8	13	86	175–179	−79.4	0.63	0.44	0.67	0.85	0.73	0.52	0.25

[a] See Experimental Section. [b] Obtained from amino acid analysis. [c] c 0.1, 1 *N* HCl. [d] CCD with *n*-BuOH–HOAc–H_2O (4:1:5). [e] c 0.3, 0.1 *N* HCl. [f] c 0.5, 1 *N* HCl.

7, Tyr 0.96; 8, His 0.96). (b) The peptide content, obtained from the amino acid analysis, agreed within 1% with that obtained from the absorbance of aqueous solutions at 275 nm, taking the value of 1375 for the molar extinction coefficient. (c) Only one spot was detected with Pauly, ninhydrin, and Sakaguchi reagents after tlc on silica gel (Eastman chromagram plates, 0.1 mm) with the following solvent systems: (A) *n*-BuOH-HOAc-H_2O (5:1:1), (B) *n*-BuOH-EtOAc-HOAc-H_2O (1:1:1:1), (C) pyridine-HOAc-H_2O (50:30:15). (d) Only one component, with the expected mobility, could be detected after paper electrophoresis at 1000 V for 60 min in three different buffers: 1 *M* HOAc (pH 2.8), 0.1 *M* pyridine acetate (pH 4.9), and 0.2 *M* sodium carbonate-bicarbonate (pH 9.9). The electrophoretic behavior of the peptide is reported here by the ratio of its migration to that of a simultaneously run amino acid standard. At pH 2.8 the migrations are relative to histidine (R_{His}), at pH 4.9 to arginine (R_{Arg}), and at pH 9.9 to picric acid (R_{Picr}), and they were given a negative sign when peptide and standard had opposite charges.

Yields of the syntheses were calculated from the peptide content of the final products, related to the amount of *tert*-butyloxycarbonylamino acids initially esterified to the resin. Melting points were determined with a Leitz Wetzlar hot stage apparatus and are reported uncorrected. The optical rotations were measured with a Bellingham & Stanley "Pepol 60" photopolarimeter with a precision of 0.002°. No tests for optical purity were made, but 1 was found to have an activity of 103%, with 95% fiducial limits of ±3%, when assayed against the [Val^5]angiotensin II standard proposed by the Division of Biological Standards of the National Institute for Medical Research, Mill Hill, London. The physical properties of the peptides are shown on Table V.

Bioassays. The isolated guinea-pig ileum,[12] rat uterus,[13] and rabbit aorta[14] preparations were done as described earlier. The bath volume was 5 ml, the media were aerated with a stream of air, and the isotonic contractions were recorded under a 1-g load with sixfold magnification. Intrinsic activities and pD_2 values[15] were determined from isometric contractions recorded with the aid of a Sanborn force transducer and a Hewlett-Packard Model 211A amplifier. For the intrinsic activities, the maximum responses to the peptides were compared to those to histamine on the guinea-pig ileum, to oxytocin on the rat uterus, and to epinephrine on the rabbit aorta. The pA_2 values were estimated from the regression equation relating log $(x - 1)$ to −log (inhibitor concentration) (pA_x), where x is the ratio of the agonist's concentrations that produced the same response in the presence and in the absence of the inhibitor.[16] The regression equations were calculated by the method of least squares, from at least eight data points obtained from at least four different preparations. The standard error of the pA_2 values was estimated from the standard error of the estimate and of the slope of the regression equation. The rat blood pressure assays were performed on urethane-anesthetized animals. The blood pressure was recorded from the carotid artery and the two femoral veins were canulated to permit simultaneous infusion and single injections.

Acknowledgments. We are indebted to Mr. Clovis Nakaie and Mr. Luiz F. C. La Regina for their technical contributions to this work.

References

(1) *J. Biol. Chem.*, **247**, 977 (1972).
(2) (a) P. A. Khairallah, A. Toth, and F. M. Bumpus, *J. Med. Chem.*, **13**, 181 (1970); (b) D. Regoli, W. K. Park, F. Rioux, and C. S. Chan, *Rev. Can. Biol.*, **30**, 319 (1971); (c) M. Yamamoto, R. K. Turker, P. A. Khairallah, and F. M. Bumpus, *Eur. J. Pharmacol.*, **18**, 316 (1972); (d) M. C. Khosla, R. A. Leese, W. L. Maloy, A. T. Ferreira, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, **15**, 792 (1972); (e) D. C. Fessler, F. Sipos, G. S. Denning, Jr., D. T. Pals, and F. D. Masucci, *ibid.*, **15**, 1015 (1972); (f) E. C. Jorgensen, G. C. Windridge, K. H. Hsieh, and T. C. Lee, "Chemistry and Biology of Peptides," J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 513.
(3) (a) E. Schröder, and K. Lübke, "The Peptides," Vol. 2, Academic Press, New York, N. Y., 1966, Chapter I; (b) E. C. Jorgensen, G. C. Windridge, and T. C. Lee, *J. Med. Chem.*, **13**, 352 (1970); (c) E. C. Jorgensen, G. C. Windridge, and T. C. Lee, *ibid.*, **14**, 631 (1971).
(4) R. Schwyzer and H. Turrian, *Vitam. Horm. (New York)*, **18**, 273 (1960).
(5) A. C. M. Paiva and T. B. Paiva, *Biochem. Pharmacol.*, **5**, 187 (1966).
(6) F. M. Bumpus, P. A. Khairallah, K. Arakawa, I. H. Page, and R. R. Smeby, *Biochim. Biophys. Acta*, **46**, 38 (1961).
(7) K. Arakawa, R. R. Smeby, and F. M. Bumpus, *J. Amer. Chem. Soc.*, **84**, 1424 (1962).
(8) E. Havinga, C. Shattenkerk, G. H. Visser, and K. E. T. Kerling, *Recl. Trav. Chim. Pays-Bas*, **83**, 672 (1964).
(9) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963).
(10) J. M. Stewart and J. Young, "Solid Phase Peptide Synthesis," W. H. Freeman, San Francisco, Calif., 1969.
(11) R. B. Merrifield, J. M. Stewart, and N. Jernberg, *Anal. Chem.*, **38**, 1905 (1966).
(12) T. B. Paiva and A. C. M. Paiva, *Brit. J. Pharmacol. Chemother.*, **15**, 557 (1960).
(13) T. B. Paiva and A. C. M. Paiva, *J. Pharmacol. Exp. Ther.*, **130**, 177 (1960).
(14) R. F. Furchgott and S. Bhadrakom, *ibid.*, **108**, 129 (1953).
(15) E. J. Ariëns, "Molecular Pharmacology," Vol. 1, Academic Press, New York, N. Y., 1964.
(16) H. O. Schild, *Brit. J. Pharmacol. Chemother.*, **4**, 277 (1949).