Synthesis of Angiotensin II Antagonists Containing N- and O-Methylated and Other Amino Acid Residues[1]

M. C. Khosla,* H. Mũnoz-Ramírez, M. M. Hall, R. R. Smeby, P. A. Khairallah, F. M. Bumpus,*

Research Division, The Cleveland Clinic Foundation, Cleveland, Ohio 44106

and M. J. Peach

Department of Pharmacology, University of Virginia, Charlottesville, Virginia 22903. *Received July 17, 1975*

[1-*N*-Methylisoasparagine,8-isoleucine]- (I), [1-sarcosine,4-*N*-methyltyrosine,8-isoleucine]- (II), [1-sarcosine,5-*N*-methylisoleucine,8-isoleucine]- (III), [1-sarcosine,8-*N*-methylisoleucine]- (IV), [1-sarcosine,5-*N*-methylisoleucine,8-*N*-methylisoleucine]- (V), [1-sarcosine,8-*O*-methylthreonine]- (VI), [1-sarcosine,8-methionine]- (VII), and [1-sarcosine,8-serine]angiotensin II (VIII), synthesized by Merrifield's solid-phase procedure, possess respectively 0.8, 0.3, 0.5, 1.0, 0.0, 0.5, 3.7, and 0.7% pressor activity of angiotensin II (vagotomized, ganglion-blocked rats). They caused an initial rise in blood pressure (30 min of infusion, 250 ng/kg/min in vagotomized, ganglion-blocked rats) of 16.57, 9.80, 22.80, 32.00, 7.00, 15.06, 32.50, and 11.42 mmHg and showed secretory activity (isolated cat adrenal medulla) of 1.0, 0.1, 0.01, 0.1, <0.01, 0.1, <0.01, and 0.05% of angiotensin II. On isolated organs pA_2 values (rabbit aortic strips) of 8.74, 7.44, 7.64, 7.85, 7.89, 8.76, 8.63, and 8.08, and pA_2 values (cat adrenal medulla) of 8.16, 9.16, 9.31, 8.00, 8.00, 7.00, 9.16, and 9.33 were obtained. Dose ratios (ratio of ED_{20} of angiotensin II during infusion of the antagonist and before infusion of the antagonist) in vagotomized, ganglion-blocked rats, infused at 250 ng/kg/min, were 33.43, 2.14, 3.26, 2.99, 0.62, 62.52, incalculable, and 11.15, respectively. The results obtained suggest that (a) analogs I and VI are potent antagonists of the pressor response of angiotensin II in normal rat, VI being the most potent antagonist thus far synthesized; (b) replacement of position 4 (Tyr) with MeTyr or position 5 and/or 8 (Ile) with MeIle in [1-sarcosine,8-isoleucine]angiotensin II reduced the antagonist activity of this peptide (rabbit aortic strips and rats), indicating that steric hindrance imposed due to N-methylation in positions 4, 5, or 8 was not favorable in eliminating the initial pressor activity or prolonging the duration of action of [Sar[1],Ile[8]]angiotensin II without reducing its antagonistic properties; (c) except II, none of the analogs showed any enhanced duration of action, suggesting that N-methylation in positions 5 or 8 did not afford protection against proteolytic enzymes; and (d) perfusion studies in cat adrenals indicated that all of these analogs are only very weak secretagogues. With the exception of [Sar[1],Thr(O$^{\beta}$Me)[8]]angiotensin II, which gave lower antagonistic properties, all other analogs had either similar antagonistic properties or were better antagonists in adrenal medulla than in smooth muscle.

Studies of the structure–activity relationships of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) analogs from ou laboratories have indicated that [1-sarcosine,-8-isoleucine]angiotensin II is the most potent antagonist of the pressor and myotropic response to angiotensin II.[3,4] However, this and other similar antagonistic peptides suffer from two limitations: all these compounds possess an initial agonist activity which is equal to 1–2% of that of the parent hormone and all have a short in vivo half-life.[5] Intravenous infusion of these analogs in anesthetized, ganglion-blocked, vagotomized rats[6] causes an initial rise in blood pressure which, depending upon the nature of the peptide and the time of infusion (3–30 min), varies between 7 and 22 mmHg at a dose level of 250 ng/kg/min. A partial blockade of this rise in pressure is obtained with phentolamine, thus suggesting that part of this pressor response is due to the release of catecholamines.[6] The present study has been undertaken in an attempt to eliminate these limitations and to find potent antagonists of the pressor and myotropic response to angiotensin II.

A study of degradation of angiotensin II in plasma indicates that "angiotensinases" A_2, B, and C split the bonds between Asp-Arg, Tyr-Ile, and Pro-Phe, respectively.[7] In view of this pattern of degradation we replaced positions 1, 4, 5, and 8 with N-methylated amino acids to determine if these substituents would prolong the in vivo half-life of these peptides without reducing their potency. We first attempted to synthesize [MePhe[8]]angiotensin II but were unsuccessful because of a failure sequence.[8] Recently, it has been shown that [1-sarcosine,8-(*N*-methylphenylalanine)]angiotensin II is an inhibitor of the pressor action of angiotensin II in the rat but it also retains high pressor activity.[9] Synthesis of various analogs of [8-isoleucine]angiotensin II with variations in position 1 indicated that, except sarcosine, no other N-methylated amino acid in this position enhanced the antagonistic activity of this peptide.[6,10,11] To further evaluate the effect of N-methylated amino acids we have now replaced position 1 (aspartic acid) in [8-isoleucine]angiotensin II with *N*-methylisoasparagine, position 4 (tyrosine) with *N*-methyltyrosine, and position 5 and/or 8 (isoleucine) with *N*-methylisoleucine residues.

We have previously shown that substitution of the aliphatic side chain in position 8 of [1-sarcosine,8-isoleucine]angiotensin II with a polar group (e.g., in threonine) did not change the antagonistic activity of this compound.[11] Thus, [1-sarcosine,8-isoleucine]- and [1-sarcosine,8-threonine]angiotensin II were found to be equipotent as antagonists of the myotropic[11] (rabbit aortic strips) and the pressor response (rats) of angiotensin II.[12,13] [1-Sarcosine,8-threonine]angiotensin II had an additional advantage in that the initial pressor activity of this compound was ~50% that of [Sar[1],Ile[8]]angiotensin II.[11-13] In order to get information on the role of the hydroxyl group in position 8, it was replaced with the *O*-methyl function, as it is in [Sar[1],Thr(O$^{\beta}$Me)[8]]angiotensin II. For studying the effect of chain length and branching in the side chain of position 8 [Sar[1],Ser[8]]- and [Sar[1],Met[8]]angiotensin II were synthesized.

All the analogs reported in this paper were synthesized by the solid-phase procedure.[14] The protocol used for the synthesis and purification was similar to the one previously described by Khosla et al.[8,15,16] Determinations of the pressor activity[17] of the analogs as compared to angiotensin II (expressed as percent) and of the comparative antagonistic activity[6] (expressed as dose ratio) were carried out on vagotomized ganglion-blocked rats, as were the infusion studies[6] to determine the initial pressor activity (expressed as mmHg). Inhibition of contractile activity of angiotensin II was studied on isolated spirally cut rabbit aortic strips (expressed as pA_2 values). The pA_2 value has been defined as the negative logarithm of the molar concentration of a competitive antagonist that reduces the effect of a double

Table I. Comparative Agonist and Antagonist Effects of Analogs of Angiotensin II

Compd no.	Angiotensin II analog	Agonist[a]		Antagonist, pA_2	
		Pressor act.[b]	Secretory act., isolated cat adrenal medulla	Rabbit aortic strips	Isolated cat adrenal medulla
I	[MeAsp-NH_2[1],Ile[8]]-	0.76	1.01	8.74 ± 0.14 (6)	8.16 ± 0.12 (6)
II	[Sar[1],MeTyr[4],Ile[8]]-	0.34	0.11	7.44 ± 0.22 (11)	9.16 ± 0.15 (8)
III	[Sar[1],MeIle[5],Ile[8]]-	0.46	0.01	7.64 ± 0.21 (5)	9.31 ± 0.22 (6)
IV	[Sar[1],MeIle[8]]-	1.06	0.10	7.85 ± 0.13 (5)	8.00 ± 0.27 (6)
V	[Sar[1],MeIle[5],MeIle[8]]-	0.00[c]	<0.01	7.89 ± 0.21 (5)	8.00 ± 0.16 (8)
VI	[Sar[1],Thr(O$^{\beta}$Me)[8]]-	0.48	0.10	8.76 ± 0.08 (6)	7.00 ± 0.20 (6)
VII	[Sar[1],Met[8]]-	3.74	<0.01	8.63 ± 0.36 (6)	9.16 ± 0.23 (8)
VIII	[Sar[1],Ser[8]]-	0.66	0.05	8.08 ± 0.23 (6)	9.33 ± 0.18 (8)
	[Sar[1] Ile[8]][d]	1.00	3.00	9.17 ± 0.04	9.50
	[Sar[1],Thr[8]][e]	0.60	0.00	8.79 ± 0.14	9.00

[a] Relative to [Asp[1],Ile[5]]angiotensin II = 100. [b] In ganglion-blocked vagotomized rats. [c] Up to a dose level of 10 μg/ml. [d] See ref 10 and 20. [e] See ref 11 and 35.

concentration of agonist to that of a single one.)[11,18,19] The percent secretory activity as compared to angiotensin II or secreto-inhibitory activity (expressed as pA_2 values) in adrenals was determined in isolated retrogradely perfused cat adrenal medulla.[20]

Results

All of the analogs, except V, showed low pressor activity (0.34–3.74% of angiotensin II) (Table I). Similarly an initial rise in blood pressure (7–33 mmHg) was observed (Table II) when these analogs were infused in rats. Substitution of isoleucine in positions 5 or 8 with *N*-methylisoleucine enhanced the initial pressor activity of [Sar[1],Ile[8]]angiotensin II. This effect was not diminished when [Sar[1],MeIle[8]]angiotensin II was tested in adrenalectomized rats, thus suggesting that the pressor effect with this analog was not due to the release of catecholamines from adrenal medulla. On the other hand, when both isoleucine residues in positions 5 and 8 were replaced with *N*-methylisoleucine simultaneously, the initial pressor activity was diminished drastically. Similarly, replacement of tyrosine with *N*-methyltyrosine in position 4 (II) reduced the initial pressor response of [Sar[1],Ile[8]]angiotensin II. Replacement of position 8 with methionine (VII) gave agonist properties similar to [Sar[1],MeIle[8]]angiotensin II but the blood pressure did not return to the normal level at the end of a 30-min infusion period (250 ng/kg/min). It is due to this reason that its antagonistic activity could not be determined. Preliminary experiments in adrenalectomized rats indicated no alteration in the pressor effect of this analog. Studies in adrenal chromaffin tissue indicated (Table I) that all these analogs were very weak secretagogues with less than 1% or less secretory activity as compared to that of angiotensin II.

When tested for antagonistic properties on rabbit aortic strips, these analogs were found to be weak to moderately potent inhibitors of the myotropic response to angiotensin II (Table I) compared to [Sar[1],Ile[8]]angiotensin II. The log dose–response curves were parallel and shifted to the right with increasing doses, thus suggesting competitive inhibition. However, with [Sar[1],MeIle[5],MeIle[8]]angiotensin II at higher dose levels (100 ng/ml or 1 μg/ml) the log dose–response curves were not parallel to the control and the maximum response was reduced with increasing concentration of angiotensin II. In general, the duration of action of these analogs was similar to [Sar[1],Ile[8]]angiotensin II. However, with [Sar[1],MeTyr[4],Ile[8]]angiotensin II, complete recovery of the aortic strip could not be effected by washing for more than 3 hr. The average response to varying doses of angiotensin II after 3 hr was 44% of the expected value.

The results obtained in isolated cat adrenal medulla indicated that, with the exception of [Sar[1],Thr(O$^{\beta}$Me)[8]]angiotensin II which gave lower antagonistic activity, all other analogs had either comparable antagonistic properties or were better antagonists in adrenal medulla than in smooth muscle (Table I).

The results obtained in the in vivo studies (Tables II and III) suggest the following. (a) [MeAsp-NH_2[1],Ile[8]]angiotensin II is equipotent to [Sar[1],Ile[8]]angiotensin II. (b) Substitution of Tyr (position 4), Ile (position 5), and/or Ile (position 8) in [Sar[1],Ile[8]]angiotensin II with the corresponding N-methylated amino acids drastically reduced the antagonistic activity of this peptide. Because of the high pressor response during infusion, the antagonistic potency of [Sar[1],Met[8]]angiotensin II could not be determined. The dose–ratio of [Sar[1],MeIle[8]]angiotensin II could be obtained only in four out of ten rats. [Sar[1],MeIle[5],MeIle[8]]angiotensin II did not show any antagonistic effect up to a dose of 6 μg/kg/min. (c) Replacement of position 8 with serine (VIII) gave a less potent antagonist than [Sar[1],Ile[8]]-, or [Sar[1],Thr[8]]angiotensin II. (d) [Sar[1],Thr(O$^{\beta}$Me)[8]]angiotensin II was found to be the most potent antagonist in the normal rat. However, this activity was somewhat diminished in the adrenalectomized rats, but in spite of this, the in vivo antagonistic activity of this compound was higher than any other competitive antagonist of angiotensin II thus far synthesized.

Discussion

Results obtained (rabbit aortic strips and rats) indicate that replacement of position 4 (Tyr) with MeTyr or position 5 and/or 8 (Ile) with MeIle in [Sar[1],Ile[8]]angiotensin II reduced the antagonistic activity of this peptide. Although [Sar[1],MeIle[5],MeIle[8]]angiotensin II (V) has a pA_2 value of 7.89 in the rabbit aortic strip and 8.0 in the cat adrenal medulla, when given intravenously it showed no antagonistic activity; if anything, it had slight potentiating activity up to a dose level of 6 μg/kg/min ($0.05 < p < 0.2$) (Table II). These results suggest that steric hindrance imposed due to N-methylation in positions 4, 5, or 8 was not favorable in eliminating the limitations of [Sar[1],Ile[8]]angiotensin II without reducing its antagonistic activity. However, a similar change in position 1, as in [MeAsp-NH_2[1],Ile[8]] angiotensin II, gave an analog which was equipotent to [Sar[1],Ile[8]]angiotensin II.

The inhibitory action of [Sar[1],MeTyr[4],Ile[8]]angiotensin II on rabbit aortic strips lasted longer than that of [Sar[1],Ile[8]]angiotensin II. However, the low in vivo antagonistic activity suggests that this analog may not be useful clinically as an antagonist. Since no other N-methylated analog showed any marked increase in the

Table II. Comparative Agonist and Antagonist Effects of Analogs of Angiotensin II. Infusion into Ganglion-Blocked Vagotomized Rats

Compd no.	Angiotensin II analog	Dose, ng/kg/min	Rise in blood pressure during analog infusion, mmHg ± SEM[a] 3 min	10 min	30 min	Angiotensin II ED_{20}, g × 10^{-9} ± SEM: Before infusion of the analog	During infusion of the analog	Dose ratio[b]	p^c
I	[MeAsp-NH_2[1],Ile[8]]-	250	10.14 ± 1.03	16.57 ± 1.36	16.57 ± 1.88	1.65 ± 0.17	54.25 ± 12.49	33.43 ± 8.22 (6)[d]	<0.01
II	[Sar[1],MeTyr[4],Ile[8]]-	250	8.00 ± 2.42	10.00 ± 2.64	9.80 ± 1.74	1.79 ± 0.03	3.83 ± 0.65	2.14 ± 0.38 (5)	<0.02
		6000	39.20 ± 1.49	21.60 ± 3.37	14.40 ± 3.96	1.32 ± 0.14	14.98 ± 2.27	12.09 ± 2.78 (4)	<0.001
III	[Sar[1],MeIle[5],Ile[8]]-	250	15.66 ± 1.68	23.00 ± 2.67	22.80 ± 3.87	1.60 ± 0.28	4.93 ± 0.74	3.26 ± 0.37 (6)	<0.01
IV	[Sar[1],MeIle[8]]-	250	25.10 ± 2.15	36.00 ± 2.75	32.00 ± 3.40	2.24 ± 0.34	6.32 ± 0.91	2.99 ± 0.55 (4)	<0.01
V	[Sar[1],MeIle[5],MeIle[8]]-	250	1.00 ± 0.77	3.20 ± 1.01	7.00 ± 2.36	1.78 ± 0.29	1.013 ± 0.21	0.62 ± 0.06 (5)	>0.05
		6000*	2.50 ± 0.76	3.16 ± 0.79	7.33 ± 2.29	1.49 ± 0.18	1.26 ± 0.17	0.82 ± 0.09 (6)	>0.05
VI	[Sar[1],Thr(O$^{\beta}$Me)[8]]-	250	13.82 ± 0.98	15.88 ± 0.85	15.06 ± 1.63	1.55 ± 0.10	95.26 ± 23.83	62.52 ± 14.93 (17)	<0.001
VII	[Sar[1],Met[8]]-	250	36.75 ± 2.86	36.75 ± 3.59	32.50 ± 4.34				
VIII	[Sar[1],Ser[8]]-	250	9.57 ± 1.84	9.57 ± 2.28	11.42 ± 1.78	1.65 ± 0.14	19.57 ± 3.87	11.15 ± 1.74 (7)	<0.001
	[Sar[1],Ile[8]]-[e]	250	17.81 ± 0.99	18.27 ± 1.72	14.81 ± 2.16	2.19 ± 0.15	59.18 ± 15.03	27.79 ± 6.25 (8)	<0.001
	[Sar[1],Thr[8]][f]	250	9.46 ± 0.79	9.66 ± 2.00	9.20 ± 4.26	1.71 ± 0.26	44.55 ± 8.20	26.9 ± 3.30 (10)	<0.001

[a] Unless stated, the level of significance (ED_{20} values) between pressor response to analog and saline infusion was $p < 0.001$, $^{\dagger}p < 0.01$, $^{*}p < 0.05$. [b] ED_{20} of angiotensin II was determined before infusion of the analog and during infusion of the analog, and the dose-ratio was calculated as ED_{20} of angiotensin II during infusion of the analog divided by ED_{20} of angiotensin II determined before infusion of the analog. [c] Level of significance between ED_{20} values. [d] The number in parentheses is the number of rats used. [e] See ref 6. [f] See ref 13.

Table III. Comparative Pressor and Antagonistic Properties of Analogs of Angiotensin II. Infusion Studies in Normal or Adrenalectomized Rats (Ganaglion-Blocked Vagotomized) with or without Treatment with Propranolol

Compd no.	Angiotensin II analog	Dose, ng/kg/min	Rise in blood pressure during analog infusion, mmHg ± SEM[a] 3 min	30 min	Angiotensin II ED_{20}, g × 10^{-9} ± SEM: Before infusion of the analog	During infusion of the analog	Dose ratio[b]	p^c
	Control (saline) (14)[d]	0.0206[j]	0.78 ± 0.26	3.66 ± 0.74				
IV	[Sar[1],MeIle[8]]- (10)	250[i]	25.10 ± 2.15	32.00 ± 3.40	2.24 ± 0.34	6.32 ± 0.91	2.99 ± 0.55 (4)[d]	<0.01
		250[f]	24.66 ± 3.44	30.33 ± 3.48	1.62 ± 0.08	4.11 ± 1.02	2.53 ± 0.61 (6)	<0.05
	IV + Ppl	250[f]	21.00 ± 3.18	17.71† ± 5.76	1.38 ± 0.14	3.33 ± 0.51	2.56 ± 0.52 (7)	<0.01
VI	[Sar[1],Thr(O$^{\beta}$Me)[8]]-	125[i]	5.25 ± 1.26	8.75* ± 2.67	1.45 ± 0.13	17.13 ± 5.48	11.65 ± 3.01 (8)	<0.02
		250[i]	13.82 ± 0.98	15.63 ± 1.63	1.55 ± 0.10	95.26 ± 23.83	62.52 ± 14.93 (17)	<0.001
		250[e]	19.25 ± 3.30	11.00 ± 3.87	1.40 ± 0.30	57.25 ± 5.25	45.86 ± 9.34 (4)	<0.001
		250[f]	7.75 ± 1.37	7.62 ± 2.80	1.70 ± 0.11	39.75 ± 7.79	22.96 ± 3.59 (8)	<0.001
		250[g]	3.00 ± 0.39	−0.4 ± 1.80	4.38 ± 0.43	49.00 ± 7.33	11.61 ± 2.05 (5)	<0.001
		250[h]	5.00 ± 3.02	2.25* ± 4.26	5.90 ± 0.79	71.63 ± 13.84	12.06 ± 1.69 (4)	<0.01
	VI + Ppl	250[i]	11.50 ± 0.92	−1.25 ± 4.70	1.63 ± 0.15	79.25 ± 19.98	54.16 ± 18.57 (8)	<0.01
	[Sar[1],Ile[8]][k]	250[i]	17.81 ± 0.99	14.81 ± 2.16	2.19 ± 0.15	59.18 ± 15.03	27.79 ± 6.25 (8)	<0.001
		250[f]	8.90 ± 1.50	1.80 ± 0.87	2.35 ± 0.21	13.90 ± 2.51	6.04 ± 1.45 (10)	<0.001
	[Sar[1],Thr[8]][l]	250[i]	9.46 ± 0.79	9.20 ± 4.26	1.71 ± 0.26	44.55 ± 8.20	26.91 ± 3.30 (10)	<0.001
		250[f]	7.57 ± 1.06	4.57 ± 1.54	2.37 ± 0.26	12.07 ± 2.10	5.52 ± 1.15 (7)	<0.001

[a-d] See corresponding footnotes in Table II. Ppl = propranolol, injected (1 mg/kg) before infusion of the analog. [e] Sham operated rats. [f] Acute adrenalectomized rats infusion within 4 hr of adrenalectomy. [g] Chronic adrenalectomized rats maintained 6 days after adrenalectomy. [h] Chronic adrenalectomized rats without pentolinium. [i] Normal rats. [j] Dose in milliliters. [k] See ref 6. [l] See ref 13.

duration of action, it appears that N-methylated amino acid residues in positions 5 or 8 do not afford protection against proteolytic enzymes.

Although the pA_2 value of [Sar[1],Thr(O$^\beta$Me)[8]]angiotensin II (8.76) is comparable to that of [Sar[1],Thr[8]]- (8.79) and less than [Sar[1],Ile[8]]angiotensin II (9.17) in rabbit aortic strips, this compound is the most potent antagonist of the pressor response to angiotensin II in rats. There are a number of possible factors which are likely to contribute to enhancing the antagonistic activity when the aliphatic hydroxyl side chain is replaced with the corresponding OMe ether linkage. First, space-filling models indicate that the size of this ether side chain is similar to that of isoleucine. Second, the *O*-methyl side chain is more lipophilic than the hydroxyl group containing side chain. Since the *O*-methyl analog is more potent than the corresponding isoleucine derivative, size and lipophilicity do not appear to be the only factors involved in this enhancement of activity. The *O*-methyl group can act only as a proton acceptor while the hydroxyl group can act both as a proton acceptor and as a proton donor. These may be important factors for invoking the biologically active conformation or binding on the receptor site. However, pA_2 values for both these compounds, which are competitive antagonists, are similar thus indicating that their binding characteristics should also be similar. Size, lipophilicity, resistance to carboxypeptidases, proton-accepting ability, and nonaromatic nature are some of the requirements of the substituent in position 8 to invoke maximum antagonistic activity.

We have previously demonstrated that infusion of several angiotensin II antagonists[6] in vagotomized ganglion-blocked rats elicited a pressor response (~17 mmHg at 250 ng/kg/min) whether a branched or a nonbranched aliphatic residue was substituted for phenylalanine in position 8. As is evidenced after adrenalectomy or after blockade with phentolamine and phenoxybenzamine, the initial pressor activity observed with all the antagonistic peptides is partially due to release of catecholamines by adrenal gland and partially due to the direct myotropic effect. Compounds listed in Table I show varying degrees of pressor activity. The substituent in position 8 has an effect on the initial pressor effect. As compared to [Sar[1],Ile[8]]angiotensin II, the pressor activity decreased when the side chain contained a hydroxyl group (Thr) while it increased when the side chain was substituted with -SMe functions as in methionine. *N*-Methylisoleucine residue either in position 5 or position 8 invoked agonist activity higher than the corresponding isoleucine analogs.

The pressor effect of [Sar[1],Thr(O$^\beta$Me)[8]]angiotensin II was reduced in adrenalectomized rats (Table III), which suggests a partial involvement of adrenal catecholamines. In contrast, the pressor effect of [Sar[1],MeIle[8]]angiotensin II was not reduced in acute adrenalectomized rats.

Following the administration of propranolol the pressor effect of [Sar[1],Thr(O$^\beta$Me)[8]]- and [Sar[1],MeIle[8]]angiotensin II was reduced at the 30-min infusion period suggesting activation of cardiac β-receptors.

Perfusion studies in cat adrenals indicated that these analogs are only very weak secretagogues. This corroborates the earlier findings of Peach and Ober[20] that sarcosine in the 1 position reduced the agonist activity. Present data suggest that the same is true for *N*-methylisoasparagine in the 1 position. While analogs having position 8 substituted with aliphatic residues (Ala, Ile, Leu, Val, etc.) have 10–30% of the agonist activities of angiotensin II in the adrenal medulla, removal of aspartic acid in position 1 from [Ile[8]]angiotensin II yields the peptide, [des-Asp[1],Ile[8]]angiotensin II, which was devoid of any agonist activity[10,21] up to a dose level of 10^{-5} *M*.

Experimental Section

Boc-Thr(O$^\beta$Me), Boc-Ser(Bzl), Boc-Met, and Boc-His(Dnp) were purchased from Bachem, Inc.

Ascending TLC of *tert*-butyloxycarbonylamino acid derivatives was conducted on silica gel supported on glass plates (Brinkmann Silplate 52). The solvent systems used were (a) $CHCl_3$–MeOH (CM) 1:3; (b) $CHCl_3$–AcOH (CA) 95:5, (c) $CHCl_3$–MeOH–AcOH (CMA) 85:10:5, (d) Me_2CO–MeOH (AcM) 98:2. TLC of angiotensin analogs was conducted on cellulose supported on glass plates (Brinkmann Celplate-12). The solvent systems (upper phase) used were (e) *n*-BuOH–AcOH–H_2O (BAW) 4:1:5; (f) *n*-BuOH–AcOH–H_2O–Prd (BAWP) 30:6:24:20; (g) *n*-BuOH–AcOEt–AcOH–H_2O (BEAW) 1:1:1:1; (h) *n*-BuOH–Prd–H_2O (BPW) 10:2:5; (i) *n*-BuOH–Prd–H_2O (BPW) 65:35:65; (j) *n*-PrOH–H_2O (PW) 1:1. Ionophoresis was carried out on filter paper strips on S & S 2043A filter paper strips in a 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 1.95) and Beckman barbiturate buffer B-2 (pH 8.6). Histidine was run simultaneously as a reference compound and *E*(His) indicates the electrophoretic mobility relative to histidine = 1.00. Location of compounds on chromatograms was carried out with ninhydrin reagent and/or diazotized sulfanilic acid. For locating *tert*-butyloxycarbonylamino acid derivatives, the plates were first stored in a closed tank saturated with HCl vapors, followed by spraying with ninhydrin. Unless stated otherwise, single symmetrical spots were observed for purified compounds.

Free peptides were hydrolyzed in sealed tubes under N_2 in the presence of phenol or mercaptoethanol in 6 *N* HCl at 110° for 24 hr or in a 1:1 mixture of 12 *N* HCl–propionic acid; peptide polymer esters were invariably hydrolyzed in the latter solvent system. Amino acid analyses were performed on Jeolco-5AH amino acid analyzer. Optical rotations were determined on a Perkin-Elmer Polarimeter Model 141, equipped with a digital readout. 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 these elements or functions were within ±0.4% of the theoretical values.

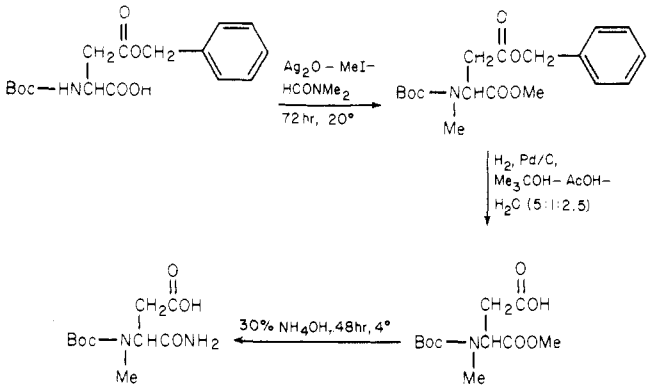
Synthesis and Purification of Angiotensin II Analogs. C-Terminal *tert*-butyloxycarbonylamino acid was attached to 2% cross-linked chloromethyl polymer and chain elongation was performed on a manual, nitrogen stirred apparatus.[15] Unless stated, the protocol used for the synthesis was the same as previously described.[8,15,16] 1-Hydroxybenzotriazole was used as an additive to avoid racemization of the histidine residue during coupling with Boc-His(Bzl).[22]

Amino acid analysis of the crude hydrogenolyzed products was carried out routinely to detect the presence of (a) His(Bzl) or (b) β-cyclohexylalanine, which has been reported to be formed during hydrogenation of aromatic amino acid residues in 70% AcOH using 10% palladium on charcoal.[23] We have observed that β-cyclohexylalanine is not formed by hydrogenating the peptides in a mixture of MeOH–AcOH–H_2O (5:1:1) under 2 atm of H_2 for 36–48 hr in a Parr hydrogenation apparatus.[8] The catalyst used was palladium black or 5% palladium on $BaSO_4$.

tert-Butyloxycarbonyl-*N*-methylamino acids showed resistance to coupling on the amino group with DCC. However, this difficulty was avoided by coupling through activation with *N*-ethyl-5-phenylisoxazolium 3-sulfonate.[24] This reagent was also found useful in coupling *tert*-butyloxycarbonyl-*N*-methylisoasparagine since asparagine or glutamine type amino acids do not give side products with this reagent. Once Woodward's reagent K was used for coupling a residue, the polymer gave a light pink color with ninhydrin which was occasionally deceptive in judging the completion of the coupling reaction. In such a case the peptide polymer was submitted to amino acid analysis after hydrolysis.

Purification of the desired peptides was carried out as previously reported.[16] Fractions in the column chromatography were cut with emphasis on purity rather than on yield and no attempt was made to rechromatograph the minor fractions for identification

Scheme I. Synthesis of *tert*-Butyloxycarbonyl-*N*-methylisoasparagine



purposes. The homogeneity of the compounds was determined by (a) thin-layer chromatography in five solvent systems of different pH, (b) electrophoresis at pH 1.95 and 8.6, and (c) amino acid analysis.

Boc-MeAsp-NH_2. This was synthesized by following the sequence shown in Scheme I. For N-methylation[11,25] a solution of Boc-Asp(OBzl) (13.1 g, 53 mmol) in dry $HCONMe_2$ (250 ml) was magnetically stirred at 80° with Ag_2O (49 g, 212 mmol). After 4 hr the mixture was cooled to 5°, treated with a solution of CH_3I (10 ml) in $HCONMe_2$ (15 ml), and stirred for 2 hr at 5° and 24 hr at room temperature. A second portion 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 portion of CH_3I (10 ml). At the end of 72 hr, the solvent was evaporated in vacuo at 25° and the residue extracted with $CHCl_3$. The extract was washed successively with H_2O, 10% citric acid, 10% Na_2CO_3, H_2O, and saturated NaCl solution. The $CHCl_3$ solution was dried and evaporated to give 15 g of Boc-MeAsp(OBzl)-OMe: TLC (silica gel) R_f 0.72 (AcM), R_f 0.88 (CMA), comparative R_f values of Boc-Asp(OBzl) in these solvents were 0.52 and 0.70, respectively. Anal. ($C_{18}H_{25}NO_6$) N. The ir spectrum lacked absorption due to amide NH or carboxyl OH on the region of 3200–3600 cm^{-1}. A solution of Boc-MeAsp(OBzl)-OMe (6 g) in *t*-BuOH–H_2O–AcOH (50:25:10)[26] was hydrogenated (24 hr) over 5% palladium on charcoal (5 g) at 30 psi. The catalyst was removed by filtration and the filtrate evaporated in vacuo at 20°. The residue was dissolved in 10% Na_2CO_3 to pH 8 and extracted with AcOEt to remove the diester. The aqueous layer was treated at 0° with citric acid to pH 4 and extracted with AcOEt, and the organic phase was washed (10% citric acid, H_2O, and NaCl solution) and dried (Na_2SO_4). The solvent was removed in vacuo to give Boc-MeAsp-OMe (3.93 g): TLC (silica gel) R_f 0.57 (MeOH), R_f 0.45 (CA), R_f 0.84 (CMA); $[\alpha]^{23}D$ –56.70° (c 1, MeOH). Anal. ($C_{11}H_{19}NO_6$) C, H, N. A solution of Boc-MeAsp-OMe (1.3 g) in concentrated NH_4OH (30%, 7 ml) in a stoppered flask was shaken (48 hr) at 4°. Excess ammonia and solvent were removed in vacuo, the residual aqueous solution was acidified to pH 4 with citric acid and extracted with $CHCl_3$, and the extract was dried over Na_2SO_4. TLC (silica gel, CMA) showed one major spot (R_f 0.47) and two minor spots (R_f 0.4 and 0.72). The $CHCl_3$ extract was chromatographed on a column of silica gel (2.5 × 100 cm). The column was first eluted with $CHCl_3$ (to remove a yellow band), followed by a mixture of $CHCl_3$–MeOH (95:5). Fractions 251–280 (12 ml each) with the latter solvent yielded Boc-MeAsp-NH_2 (880 mg): TLC (silica gel) R_f 0.12 (CA), R_f 0.47 (CMA). A sample was recrystallized from AcOEt for elemental analysis: mp 177–178°; $[\alpha]^{23}D$ –1.1° (c 1, MeOH). Anal. ($C_{10}H_{18}N_2O_5$) C, H, N.

Boc-L-Tyr(2,6-Cl_2Bzl). The procedure used for the synthesis of *O*-(2,6-dichlorobenzyl)-L-tyrosine was similar to the one used by Morley[27] for the synthesis of Tyr(Bzl) with modifications gaining in yield and simplicity of procedure.

A solution of L-tyrosine (18.1 g, 0.1 mol) in 2 *N* NaOH (100 ml) was treated with $CuSO_4 \cdot 5H_2O$ (12.48 g, 0.05 mol) in H_2O (50 ml) in a 1-l. round-bottom flask fitted with a medium porosity sintered disk (10 cm in diameter) and a 3-mm Teflon stopcock at the bottom for filtration. The reaction and the washings were carried out in this vessel. The mixture was shaken on a wrist shaker for 10 min; MeOH (300 ml) was added followed by the addition of 2,6-$Cl_2C_6H_3CH_2Br$ (25.19 g, 0.10 mol). The flask was stoppered and the mixture shaken for 20 hr at room temperature. The copper complex was filtered and washed with H_2O (three 50-ml portions) and MeOH (two 50-ml portions). The semi-air-dried product was treated with preheated (40°) 1 *N* HCl (200 ml) when the copper chelate went into solution. It was transferred to a beaker, magnetically stirred at 40° for 20 min, cooled in an ice–water bath, and filtered. The precipitate was again transferred to a beaker, preheated 1 *N* HCl (40°) (200 ml) was added, and the precipitate was stirred (20 min), cooled, and filtered. The process was repeated until the filtrate was colorless. The residue was again transferred to a beaker and stirred with a solution of AcONa (16.4%, 100 ml) at 80–90° for 15 min. The colorless precipitate was filtered, washed with hot H_2O, and dried in vacuo over P_2O_5 (25.5 g, 74.5%). L-Tyr(2,6-Cl_2Bzl) thus obtained was homogeneous and identical with an authentic sample obtained by deblocking a commercial sample of Boc-Tyr(2,6-Cl_2Bzl) (Bachem, Inc.): TLC (silica gel) R_f 0.54 (BAW), R_f 0.56 (BAWP), R_f 0.49 (BPW, 65:35:65). It was derivatized into the corresponding Boc derivative which cochromatographed with an authentic sample (Bachem, Inc.): TLC (silica gel) R_f 0.59 (EtOH), R_f 0.77 ($CHCl_3$–MeOH–AcOH, 85:10:5), R_f 0.56 ($CHCl_3$–MeOH, 1:3).

Boc-MeTyr(2,6-Cl_2Bzl)-OMe. Boc-Tyr(2,6-Cl_2Bzl) (4.69 g) was treated with Ag_2O–MeI to give 4.50 g of the N-methylated ester as an oil: TLC (silica gel) R_f 0.70 (AcM), R_f 0.87 (CMA). Comparative R_f values of Boc-Tyr(2,6-Cl_2Bzl) in these solvents were 0.64 and 0.71, respectively. Anal. ($C_{23}H_{28}NO_5Cl_2$) C, H, N, Cl.

Boc-MeTyr(2,6-Cl_2Bzl). This was obtained by the saponification[25] of the above methyl ester. Crystallization from *n*-heptane or EtOH gave mp 84–85°: TLC (silica gel) R_f 0.46 (EtOH), R_f 0.51 (AcM), R_f 0.85 (CMA), R_f 0.67 (CM); $[\alpha]^{23}D$ –45.9° (c 1, MeOH). Anal. ($C_{22}H_{26}NO_5Cl_2$) C, H, N.

[MeAsp-NH_2[1],Ile[8]]angiotensin II (I). The protected heptapeptide polymer [Boc-Arg(NO_2)-Val-Tyr(OBzl)-Ile-His(Bzl)-Pro-Ile-polymer ester] (4 g) was deprotected at the N terminus in the usual way. The trifluoroacetate salt was neutralized (NEt_3–$HCONMe_2$) and the peptide polymer coupled with Boc-MeAsp-NH_2 activated with Woodward's reagent K[24] as follows. A solution of Boc-MeAsp-NH_2 (738 mg, 3 mmol) in CH_2Cl_2 (35 ml) was cooled to 0° under magnetic stirring. NEt_3 (0.42 ml, 3 mmol) was added and after 5 min of stirring Woodward's reagent K (759 mg, 3 mmol) was added in small portions over a period of 10 min. The mixture was stirred at 0° for 2 hr at which time all of the reagent went into solution. The solution was filtered and the filtrate added to the resin. After stirring overnight, it was filtered and washed ($HCONMe_2$) and the coupling step repeated. At the end (when the resin did not give color with ninhydrin) the resin was washed with $HCONMe_2$ and 10% NEt_3 in $HCONMe_2$ to remove the brownish color from the resin. The analog was cleaved from the resin, deprotected, and purified by the usual procedure: TLC (cellulose) R_f 0.51 (BAW), R_f 0.75 (BEAW), R_f 0.21 (BPW 10:1:5), R_f 0.60 (BPW 65:35:65); R_f 0.64 (BAWP); *E*(His) 0.80 (pH 1.9); *E*(His) 0.95 (pH 8.6). A minute trace of an impurity was detected by TLC with BAW (R_f 0.53) and in BPW (65:35:65, R_f 0.49) solvents and by ionophoresis in pH 1.9 buffer [*E*(His) 0.68] and this could not be removed in spite of repeated column chromatography. Amino acid ratio in the acid hydrolysate: Arg 1.00, Val 1.05, Tyr 0.89, Ile 2.00, His 0.94, Pro 1.08. The peak for *N*-methylaspartic acid appeared just before the peaks for aspartic acid or proline peaks (retention time 40 min); since the color intensity of this amino acid with ninhydrin was very low, only a qualitative estimation could be carried out: $[\alpha]^{21}D$ –79.1° (c 0.53, 5 *N* AcOH).

[Sar[1],MeTyr[4],Ile[8]]angiotensin II (II). Boc-MeTyr(2,6-Cl_2Bzl) (1.4 g, 3 mmol) was activated by treatment with NEt_3 (0.43 ml, 3 mmol) and Woodward's reagent K[24] (759 mg, 3 mmol) in CH_2Cl_2 and the solution added to the tetrapeptide polymer [Ile-His(Bzl)-Pro-Ile-polymer ester, 5 g containing 3 mmol of the peptide]. The coupling reaction was carried to completion and the pentapeptide polymer was coupled with Boc-Val, Boc-Arg(NO_2), and Boc-Sar, respectively. The analog was worked up and purified by the general procedure.[16] TLC (cellulose) R_f 0.48 (BAW), R_f 0.72 (BEAW), R_f 0.37 (BPW 10:2:5), R_f 0.59 (BPW 65:35:65), R_f 0.71 (BAWP); *E*(His) 0.83 (pH 1.95), *E*(His) 0.94 (pH

8.6); $[\alpha]^{21}D$ -75.7° (c 1, 5 *N* AcOH). Amino acid ratio in the acid hydrolysate: Sar 1.00, Arg 1.00, Val 1.00, Ile 2.02, His 1.01, Pro 1.00.

[Sar¹,MeIle⁵,Ile⁸]angiotensin II (III). Boc-MeIle (1.96 g), prepared as reported earlier,[10] was activated by treatment with NEt_3 (1.15 ml) and Woodward's reagent K (2.02 g) in $HCONMe_2$. The solution was divided into two parts to carry out two successive couplings with the tripeptide polymer [His(Bzl)-Pro-Ile-polymer ester, 4.6 g containing 1.93 mmol of the peptide]. The peptide polymer was hydrolyzed at the tetrapeptide stage to ascertain coupling with Boc-MeIle. This was necessary before coupling with Boc-Val, since the peak for *N*-methylisoleucine merged into that of valine (retention time 86 min) and the color intensity of this amino acid was 150-fold less as compared to valine. The analog was worked up by the general procedure:[16] TLC (cellulose) R_f 0.47 (BAW), R_f 0.81 (BEAW), R_f 0.24 (BPW 10:1:5), R_f 0.70 (BAWP), R_f 0.80 (PW); *E*(His) 0.86 (pH 1.95), *E*(His) 1.15 (pH 8.6); $[\alpha]^{21}D$ -80.0° (*c* 1, 5 *N* AcOH). Amino acid ratio in the acid hydrolysate: Sar 1.00, Arg 1.11, Val 1.15, Tyr 1.01, His 1.01, Pro 1.04, Ile 1.00.

[Sar¹,MeIle⁸]angiotensin II (IV). Cleavage of the C-terminal dipeptide[8,28] as c-(Pro-MeIle) was avoided by neutralization of the trifluoroacetate salt of the prolyl-*N*-methylisoleucyl-polymer ester for a short time (10% NEt_3 in $CHCl_3$ for 3 min with vigorous shaking at -10°) followed by quick washing with $CHCl_3$ (three times for 1 min at -10° until the filtrate had neutral pH); coupling with excess of Boc-His(Bzl) was carried out at -5 to -10° in the reverse order[29] by adding DCI before the addition of Boc-His(Bzl). 1-Hydroxybenzotriazole (1 equiv) was used as an additive to minimize racemization of histidine during the coupling reaction.[22] The apparatus used was of the manual type[30] and was found to be convenient for carrying out synthesis at low temperature. The coupling step was repeated twice until the ninhydrin test was negative. No attempt was made to detect diketopiperazine formation. However, amino acid analysis indicated that histidine and proline were present in equal ratio and that the molarity of proline per gram of the polymer before and after coupling with Boc-His(Bzl) was within the expected range. Similar results were obtained when coupling was carried out (-5° in $HCONMe_2$) with the *p*-nitrophenyl ester of Boc-His(Bzl) using 1 equiv of 1-hydroxybenzotriazole:[31] TLC (cellulose) R_f 0.57 (BAW), R_f 0.71 (BEAW), R_f 0.24 (BPW 10:2:5), R_f 0.76 (BAWP), R_f 0.74 (PW); *E*(His) 0.91 (pH 1.95), *E*(His) 0.93 (pH 8.6); $[\alpha]^{21}D$ -97.7° (c 1, 5 *N* AcOH). Amino acid ratio in the acid hydrolysate: Sar 1.00, Arg 0.94, Val 1.03, Tyr 1.00, Ile 1.00, His 0.97, Pro 1.17.

[Sar¹,MeIle⁵,MeIle⁸]angiotensin II (V). The procedure for this synthesis was the same as described for compounds III and IV, except that coupling of Boc-Tyr (2,6-Cl_2Bzl) was carried out first with DCI followed by activation with Woodward's reagent K in $HCONMe_2$. Amino acid ratio in the acid hydrolysate at the tetrapeptide stage: Tyr 1.00, His(Bzl) 1.00, Pro 1.00, MeIle ~2.0. Subsequent coupling with Boc-Val, Boc-Arg(NO_2), and Boc-Sar was carried out by the general procedure: TLC (cellulose) R_f 0.39 (BAW), R_f 0.73 (BEAW), R_f 0.14 (BPW 10:2:5), R_f 0.58 (BAWP), R_f 0.73 (PW); *E*(His) 0.88 (pH 1.95), *E*(His) 1.00 (pH 8.6); $[\alpha]^{21}D$ -83.9° (c 1, 5 *N* AcOH). Amino acid ratio in the acid hydrolysate: Sar 1.00, Arg 1.00, Val 1.00, Tyr 0.92, His 1.00, Pro 1.1.

[Sar¹,Thr(O$^{\beta}$Me)⁸]angiotensin II (VI). TLC (cellulose) gave R_f 0.33 (BAW), R_f 0.67 (BEAW), R_f 0.13 (BPW 10:2:5), R_f 0.37 (BPW 65:35:65), R_f 0.58 (BAWP), R_f 0.77 (PW); *E*(His) 0.87 (pH 1.95), *E*(His) 1.1 (pH 8.6); $[\alpha]^{21}D$ -69.5° (c 1, 5 *N* AcOH). Amino acid ratio in the acid hydrolysate: Sar 1.00, Arg 1.08, Val 1.01, Tyr 1.05, Ile 1.04, His 1.00, Pro 1.01, Thr 1.0.

[Sar¹,Met⁸]angiotensin II (VII). In this synthesis *tert*-butyloxycarbonylmethionine was esterified onto hydroxymethyl polymer using *N,N'*-carbonyldiimidazole, the remaining hydroxyl groups being protected by formation of their acetates.[32,33] Boc-His(Dnp) was used as the protected histidine residue. At the end of the synthesis, the peptide was removed from the polymer with HF–anisole and the Dnp-protecting group removed with $HSCH_2CH_2OH$–$HCONMe_2$ (24 hr, 20°).[34] For amino acid analysis, hydrolysis was carried out in evacuated tubes in the presence of $HSCH_2CH_2OH$: TLC (cellulose) R_f 0.62 (BAW), R_f 0.73 (BEAW), R_f 0.43 (BPW 65:35:65), R_f 0.64 (BAWP), R_f 0.70 (PW); *E*(His) 0.9 (pH 1.95), *E*(His) 1.0 (pH 8.6); $[\alpha]^{20}D$ -14.4° (c 0.2, 6 *N* AcOH). Amino acid ratio in the acid hydrolysate: Sar 1.00, Arg 1.02, Val 1.12, Tyr 1.00, Ile 1.07, His 1.01, Pro 1.0, Met 0.95.

[Sar¹,Ser⁸]angiotensin II (VIII). TLC (cellulose) gave R_f 0.25 (BAW), R_f 0.62 (BEAW), R_f 0.26 (BPW 65:35:35), R_f 0.56 (BAWP), R_f 0.60 (PW); *E*(His) 0.85 (pH 1.95), *E*(His) 1.15 (pH 8.6); $[\alpha]^{20}D$ -14.7° (c 0.2, 6 *N* AcOH). Amino acid ratio in the acid hydrolysate: Sar 1.00, Arg 1.07, Val 1.08, Tyr 1.17, Ile 1.08, His 1.00, Pro 1.00, Ser 1.0.

Acknowledgment. This investigation was supported in part by Grant No. HL-6835, General Research Support Grant FR-5674, and Abbott Laboratories Research Grant. The authors wish to thank Drs. J. Seeley and M. Thomas of Abbott Laboratories for HF treatment of the methionine analog. We also wish to express our appreciation to Messrs. Eugene Bacznsky, Andrew Strobel, John Fraser, and Milo Milovanovic for their excellent technical assistance.

References and Notes

(1) Presented in part by M. C. Khosla, R. R. Smeby, and F. M. Bumpus at the 169th National Meeting of the American Chemical Society, Philadelphia, Pa., April 6–11, 1975,[2a] and at the 4th American Peptide Symposium, New York, N.Y., June 1–6, 1975.[2b] Abbreviated designation of amino acid derivatives and peptides is according to the recommendation of the IUPAC–IUB Commission (IUPAC Information Bulletin No. 26). In addition, the following abbreviations were used: Prd = pyridine, Boc-MeAsp-OMe = *tert*-butyloxycarbonyl-*N*-methylaspartic acid α-methyl ester, MeAsp-NH_2 = *N*-methylisoasparagine, MeTyr = *N*-methyltyrosine, MeIle = *N*-methylisoleucine, Thr(O$^{\beta}$Me) = *O*-methylthreonine.

(2) (a) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, Abstracts of the 169th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1975, MEDI-57. (b) "Chemistry, Biophysics and Biology of Peptides", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., in press.

(3) F. M. Bumpus, S. Sen, R. R. Smeby, C. Sweet, C. M. Ferrario, and M. C. Khosla, *Circ. Res., Suppl. 1*, **32, 33**, I-150 (1973).

(4) For a detailed review, see M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *Handb. Exp. Pharmacol.*, **37**, 126–161 (1974).

(5) W. A. Pettinger and K. Keeton, *Circulation, Suppl. 3*, **49, 50**, III-104 (1974) (abstract).

(6) H. Munoz-Ramirez, M. C. Khosla, F. M. Bumpus, and P. A. Khairallah, *Eur. J. Pharmacol.*, **31**, 122 (1975).

(7) J. G. Ledingham and W. P. Leary, *Handb. Exp. Pharmacol.* **37**, 111–122 (1974).

(8) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *J. Am. Chem. Soc.*, **94**, 4721 (1972).

(9) C. Pena, J. M. Stewart, and T. C. Goodfriend, *Life Sci.*, **14**, 1331 (1974).

(10) M. C. Khosla, M. M. Hall, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, **17**, 431 (1974).

(11) M. C. Khosla, M. M. Hall, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, **17**, 1156 (1974).

(12) F. M. Bumpus and M. C. Khosla, *Clin. Sci. Mol. Med., Suppl. II*, **48**, 15S (1975).

(13) H. Munoz-Ramirez, M. C. Khosla, and P. A. Khairallah, FASEB Proceedings 1975, Abstr. No. 3113.

(14) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).

(15) 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).

(16) M. C. Khosla, M. M. Hall, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, **16**, 829 (1973).

(17) P. T. Pickens, F. M. Bumpus, A. M. Lloyd, R. R. Smeby, and I. H. Page, *Circ. Res.*, **17**, 438 (1965).

(18) R. F. Furchgott and S. Bhadrakom, *J. Pharmacol. Exp. Ther.*, **103**, 129 (1953).

(19) O. Arunlakshna and H. O. Schild, *Br. J. Pharmacol.*, **14**, 48 (1959).

(20) M. J. Peach and M. Ober, *J. Pharmacol. Exp. Ther.*, **190**, 49 (1974).

(21) E. L. Bravo, M. C. Khosla, and F. M. Bumpus, *J. Clin. Endocrinol. Metab.*, **40**, 530 (1975).
(22) W. Koenig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(23) S. Visser, J. Raap, K. E. T. Kerling, and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, **89**, 865 (1970).
(24) R. B. Woodward, R. A. Olofson, and H. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).
(25) R. K. Olsen, *J. Org. Chem.*, **35**, 1912 (1970).
(26) V. J. Hruby, F. Muscio, C. M. Groginsky, P. M. Gitu, and D. Saba, *J. Med. Chem.*, **16**, 624 (1973).
(27) J. S. Morley, *J. Chem. Soc. C*, 2410 (1967).
(28) M. C. Khosla, R. R. Smeby, and F. M. Bumpus in "Chemistry and Biology of Peptides", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 227.
(29) B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, **94**, 3102 (1972).
(30) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *Science*, **156**, 253 (1967).
(31) W. Koenig, R. Geiger, and H. Wissman, German Offen 2202613 (1973); *Chem. Abstr.*, **79**, 137510 (1973).
(32) H. C. Beyerman and R. A. int'l Veld, *Recl. Trav. Chim. Pays-Bas*, **88**, 1019 (1969).
(33) B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, **94**, 6165 (1972).
(34) J. M. Stewart and J. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969.
(35) M. J. Peach, Symposium on the Pharmacology of Angiotensin Antagonists, FASEB Proceedings, Atlantic City, N.J., March 1975, in press.

Synthesis and Some Pharmacological Properties of Five Analogs of Oxytocin Having L-Homocysteine in Position 6[1]

Clark W. Smith*[2] and Martha F. Ferger

Department of Chemistry, Cornell University, Ithaca, New York 14853. *Received June 20, 1975*

Five analogs of oxytocin have been synthesized with a homocysteine residue in position 6 and 2-, 3-, or 4-carbon residues in position 1. The compounds, which contain 20-, 21-, and 22-membered disulfide rings, respectively, were [1-α-mercaptoacetic acid,6-homocysteine]oxytocin, [6-homocysteine]oxytocin, [1-β-mercaptopropionic acid,6-homocysteine]oxytocin, [1,6-homocystine]oxytocin, and [1-γ-mercaptobutyric acid,6-homocysteine]oxytocin. The appropriate protected polypeptide intermediates were prepared by the solid-phase method of peptide synthesis. The protecting groups were removed by treatment with Na in NH_3 and the disulfide bond was formed by oxidation with ICH_2CH_2I in aqueous MeOH. Purification was effected by partition chromatography followed by gel filtration. The pharmacological activities of all five analogs are reported for the oxytocic, avian vasodepressor, and rat pressor assays. Compared to oxytocin, these analogs exhibited sharply reduced agonist potencies, and several exhibited antagonist activity.

The importance of the 20-membered disulfide ring to the biological activities of oxytocin and the vasopressins has been an object of continued investigation in this and other laboratories. Several studies have been done on analogs of oxytocin (Figure 1) in which the ring size has been increased in various ways.[3-12] None of these compounds showed more than a slight degree of biological activity.

One of the ways in which a change in the ring size has been accomplished without interrupting the α-amino acid sequence is by the formal insertion of a methylene group at position 1 to form the 21-membered ring analog [1-L-homocysteine]oxytocin.[6] This analog showed only a slight oxytocic activity and no avian vasodepressor (AVD) or rat pressor activity. In this analog the substitution of the homocysteine for the cysteine residue, while increasing the size of the ring, leads simultaneously to a change in the separation of the free amino group from the disulfide bond. The synthesis of the highly potent 1-deamino-oxytocin[13] ([1-β-mercaptopropionic acid]oxytocin), which possesses almost twice the oxytocic and AVD potency of oxytocin, showed that the free amino group is not requisite for these activities. The study of the effect of ring size without the involvement of the amino group was undertaken with the synthesis of several analogs of 1-deamino-oxytocin by the formal introduction of one or more methylene groups or the deletion of one methylene group at the β-mercaptopropionic acid residue at position 1.[7,8,11,12,14] In this manner analogs having 19-, 21-, 22-, and 28-membered rings were prepared and showed drastic reduction or total loss of activity.

Another possible way to study the effect of ring size without involvement of the free amino group at position 1 is to expand the ring size by the formal insertion of a methylene group at position 6. We report here the synthesis of five analogs of oxytocin in which the Cys[6] residue has been replaced by an L-homocysteine residue. Combining this modification with various residues in position 1, the following analogs of oxytocin have been prepared: [6-homocysteine]oxytocin (**3**)[15] ([Hcy[6]]oxytocin), [1,6-homocystine]oxytocin (**5**)[15] ([Hcy[1,6]]oxytocin), [1-α-mercaptoacetic acid,6-homocysteine]oxytocin (**7**)[16] ([α-Maa[1],Hcy[6]]oxytocin), [1-β-mercaptopropionic acid,6-homocysteine]oxytocin (**9**) ([β-Mpa[1],Hcy[6]]oxytocin), and [1-γ-mercaptobutyric acid,6-homocysteine]oxytocin (**11**) ([γ-Mba[1],Hcy[6]]oxytocin). The common intermediate, Boc-Tyr(Bzl)-Ile-Gln-Asn-Hcy(Bzl)-Pro-Leu-Gly-O-resin

Table I. Solid-Phase Procedure

Step no.	Reagent	Repetitions	Time, min
1	CH_2Cl_2	3	2
2	CF_3CO_2H–anisole–CH_2Cl_2 (48:2:50)	1	25
3	CH_2Cl_2	4	2
4	*i*-Pr_2NEt–CH_2Cl_2 (7:93)	2	2
5	CH_2Cl_2	4	2
6	Boc-amino acid in CH_2Cl_2 or DMF[a]	1	
7[b]	DCC in CH_2Cl_2	1	Variable[a]
8	CH_2Cl_2 or DMF[a]	3	2
9	EtOH	3	2

[a] See Experimental Section. DMF was the solvent whenever HBt was used. Different reaction times were employed depending on the use of HBt and on the amino acid residue. [b] This step is added to the reagent present from step 6. Only half of the normal solvent volume is used in steps 6 and 7, so that the total volume is the same as in all other steps.