Table I. *In Vitro* Antiplaque Activity

Compd[b]	% inhibition[a] at 10^{-2} *M*		% inhibition at 10^{-3} *M*.	
	24 hr	48 hr	24 hr	48 hr
2	80	60	0	0
4	60	60	0	0
5	80	0	0	0
6	60	60	40	20

[a]Percentage of teeth which did not show plaque formation after stated incubation period. Subjective estimates were made of plaque formation using a scale from 0 (no growth) to 4 (maximum growth). Because in an overwhelming number of tests growth ratings were either 0 or 4, the findings were expressed as either plaque formation or inhibition. [b]All compounds were evaluated as their HCl salts in 95% EtOH solution on five teeth. The solvent served as a control. The teeth were immersed in solutions of the test compounds, air-dried, and washed with distilled H_2O before anaerobic incubation with *S. mutans* No. 6715. Procedural details are given in ref 4.

this mixture incubated under anaerobic conditions (BBL-Gaspak§) for 48 hr. All four compounds inhibited bacterial growth at a concentration of 10^{-5} *M* but not at 10^{-6} *M*.

The *in vitro* antiplaque activity of these compounds was evaluated using the method of Turesky and coworkers[4] which utilizes sterilized extracted human teeth. As shown in Table I, only compound **6**, containing the *m*-trifluoromethylphenyl substituent, had any activity at a concentration of 10^{-3} *M*. With a tenfold increase in concentration, compounds **4** and **6** had activities comparable with chlorhexidine, while **5** had activity of shorter duration.

These results show that it is possible to enhance the *in vitro* antiplaque activity of chlorhexidine-type molecules through proper modification of the N^5 substituent. Since all four compounds were comparable in antibacterial activity, the increase in antiplaque activity exhibited by compound **6** may reflect better binding to the tooth surface by this compound.

Further analogs must be synthesized and tested to determine the degree to which the activity can be enhanced. In addition, clinical studies will be necessary to determine if chlorhexidine analogs with increased *in vitro* activity are also more active *in vivo* and/or possess fewer side effects.

Experimental Section#

1,6-Bis(N^5-cyclohexyl-N^1-biguanido)hexane (**4**) was prepared in 35% yield as previously described.[9]

1,6-Bis(N^5-1-adamantyl-N^1-biguanido)hexane (5). A mixture of 2.50 g (0.100 mol) of 1,6-bis(N^5-cyano-N^1-guanidino)hexane (**1**) and 3.78 g (0.0200 mol) of 1-aminoadamantane·HCl in 16 ml of 2-ethoxyethanol was stirred under reflux for 16 hr, cooled, and filtered, and the solid product was washed on the filter with EtOH to give 0.70 g (13%) of **5** as the dihydrochloride. Recrystallization (MeOH–EtOAc) gave pure **5**·2HCl as a white solid, mp 264–268°. *Anal.* ($C_{30}H_{52}N_{10}$·2HCl·0.5H_2O) C, H, N.

1,6-Bis(N^5-*m*-trifluoromethylphenyl-N^1-biguanido)hexane (6). This biguanide was prepared analogously to **5** with **6**·2HCl being obtained in a yield of 14%, mp 247–250°. *Anal.* ($C_{24}H_{30}N_{10}F_6$·2HCl) C, H, N.

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#Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Scandinavian Microanalytical Laboratory, Herlev, Denmark. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

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Synthesis and Pharmacology of Position 6 Analogs of Angiotensin II†

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Since elucidation of the structure of angiotension II (Figure 1) by Skeggs, *et al.*,[2] over a hundred analogs and homologs of this polypeptide have been synthesized and tested

1 2 3 4 5 6 7 8
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

Figure 1. Structure of angiotensin II.

for biological activity.[3-6] It has still not been clear, however, exactly what role the His^6 residue plays in the biological activity. The finding that Ang may be inactivated by photooxidation suggests, however, that the imidazole side chain is important.[7] Substitution of Ala or β-2-thienyl-L-alanine (Thi) at this position has been reported to give relatively inactive compounds.[4] [Phe^6]-Ang^8 and [Lys^6]-Ang^9 have also been found to possess low pressor activity, and [Arg^6]-Ang was reported to be inactive.[10] In contrast, Andreatta and Hofmann[11] reported that substitution of the isosteric β-(3-pyrazolyl)-L-alanine (Pza) in the 6 position gave an analog with good pressor activity (57–79%) in the rat despite its lack of basicity. Khosla, *et al.*,[10] feel that "neither positive charge alone nor merely aromatic character in position 6 is responsible for the pressor activity of Ang." The studies reported here were undertaken with the hope of further clarifying the role of the His residue in the biological activity of Ang.

Results

All of the peptides used in this study, with the exception of [Val^5,Pza^6]-Ang (a generous gift of Dr. Klaus Hofmann), were synthesized by the Merrifield solid-phase method essentially as described by Stewart and Young[12] and are listed in Table I. All analogs were the Asp^1,Ile^5 species, except [Val^5,-Pza^6]-Ang. The relative potencies of the analogs with respect to [Asp^1,Ile^5]-Ang are listed in Table II.

All of the compounds were also tested as possible competitive inhibitors of Ang on both rat uterus and guinea pig ileum. In each assay a standard Ang dose–response curve was run both

†All amino acid residues are of the L configuration. Abbreviations used: Boc = *tert*-butyloxycarbonyl; Thi = β-(2-thienyl)-L-alanine; Pza = β-(3-pyrazolyl)-L-alanine; Ang - angiotensin II. Standard abbreviations are used for the other amino acids; see ref 1.

Table I. Synthetic [Asp[1],Ile[5]]angiotensin II Analogs

Residue at position 6	CCD, k^a	Tlc		Electrophoresis		Amino acid composition[f] (mol/mol of peptide)							
		R_fF^b	R_fI^c	E_{lys} 5[d]	E_{lys} 2.8[e]	Phe	Pro	Ile	Tyr	Val	Arg	Asp	Other
Thi	1.64	0.81	0.81		0.37	1.00	1.06	1.03	0.94	1.05	1.00	0.94	0.63
Phe	1.56	0.82	0.66		0.47	2.00	1.01	1.02	0.83	1.00	1.04	0.94	
Met	1.44	0.77	0.59		0.44	1.00	1.02	0.98	0.94	0.96	1.0-	0.94	0.88
Leu	1.44	0.74	0.54		0.41	1.00	1.00	0.83	0.94	0.97	1.02	0.98	1.01
Orn	0.19	0.57	0.35	0.38	0.68	1.00	1.07	1.03	0.95	1.04	0.98	0.96	1.02
Arg	0.22	0.63	0.45	0.38	0.59	1.00	1.05	1.07	0.98	1.06	2.02	0.93	
Glu	0.59	0.68	0.50		0.47	1.00	0.97	0.98	0.97	0.98	0.98	0.85	0.94

[a] k is the partition coefficient found after countercurrent distribution for 100 transfers in n-BuOH–AcOH–H_2O (4:1:5, by volume). [b] On cellulose in system F: 1-BuOH (15), AcOH (3), H_2O (12), pyridine (10). [c] On cellulose in system I: 1-BuOH (4), AcOH (1), H_2O (1). [d] Mobility relative to Lys at pH 5.0. [e] Mobility relative to Lys at pH 2.8. [f] Peptides were hydrolyzed and analyzed for amino acid content as described in the text.

Table II. Relative Potency of Angiotensin II Analogs[a]

Compound	Rat blood pressure		Rat uterus	Guinea pig ileum
	Normal	Ganglion-blocked		
[Asp[1],Ile[5]]-Ang	100	100	100	100
[Val[5],Pza[6]]-Ang	34.4 (20–43)	49.6 (31–84)	20.7 (10–29)	5.5 (4.1–6.2)
[Thi[6]]-Ang	10.1 (6.3–14)	19.3 (11–29)	4.2 (3.3–6.5)	1.8 (1.1–2.5)
[Phe[6]]-Ang	3.2 (1.6–4.5)	5.1 (1.8–7.8)	1.85 (0.72–2.8)	0.39 (0.28–0.60)
[Met[6]]-Ang	0.22 (0.1–0.3)	0.23 (0.1–0.35)	0.36 (0.08–0.60)	0.14 (0.1–0.2)
[Leu[6]]-Ang	0.02 (0.01–0.03)	0.02 (0.015–0.025)	0.025 (0.02–0.034)	No effect at 10 μg/ml
[Orn[6]]-Ang	0.03 (0.02–0.05)	0.04 (0.03–0.05)	0.015 (0.013–0.02)	0.02 (0.01–0.024)
[Arg[6]]-Ang	0.01 (0.003–0.02)	0.012 (0.008–0.014)	No effect at 10 μg/ml	No effect at 10 μg/ml
[Glu[6]]-Ang	No effect at 100 μg iv	No effect at 100 μg iv	No effect at 10 μg/ml	No effect at 10 μg/ml

[a] Assays were carried out as described in the text. Relative potency was calculated by comparison of the dose necessary to give a 50% maximal contraction, and the results are expressed as per cent of the activity of [Asp[1],Ile[5]]-Ang. Blood pressure potency in both normal and ganglion-blocked rats was determined by comparison of the doses of Ile[5]-Ang and the analog required to produce a rise in mean arterial pressure of 25 mm. These results are also expressed as percentages. Each value is the mean of at least three assays, and the range is included in parentheses.

in the presence and absence of each of the analogs. In the inhibition tests the analogs were present in the bath in a concentration just below that which would elicit a response or at 10 μg/ml in the case of the inactive analogs. In no case was any significant inhibition observed.

Discussion

The results of these studies would tend to support the suggestion of Andreatta and Hofmann[11] that the stereochemistry of the histidine side chain is more important in determining the biological activity of Ang than is the basic nature of this residue. Indeed, it was found that the presence in this position of a basic residue which would be protonated at the pH of the assay destroyed the biological activity. This is consistent with the prior report that the Lys[6] analog had only 0.1% pressor activity in the rat[9] and that [Arg[6]]-Ang was inactive.[10] Similarly, an acidic (Glu) residue in this position also gave an inactive analog. Insertion of neutral aliphatic residues in this position (*e.g.*, Leu) also yields inactive analogs. This is consistent with the finding of Bumpus, *et al.*,[4] that the Ala[6] analog had only 1% of the pressor activity of angiotensin II. It is of interest, however, that the Leu[6] was approximately two orders of magnitude less active than the Ala[6] analog. Substitution of aromatic residues (Phe and Thi) in this position gave analogs with higher potency. The findings on the Phe[6] analog are in reasonable agreement with those reported earlier.[8] The Thi[6] analog was quite active on the isolated rat uterus, although it was not the equal of the Pza[6] analog. Its pressor activity was good in both the normal (10.1%) and the ganglion-blocked (19.3%) animal. Our findings show this analog to be considerably more active than reported by Bumpus, *et al.*[4] It is of interest that the Thi[6] analog is approximately 2–3 times more active on all bioassay systems than is the Phe[6] analog despite the fact that the thiophene and benzene ring systems are isosteric.[13] This is reminiscent of the finding that replacing the Phe residues in bradykinin with Thi resulted in analogs possessing greater activity in some assay systems than bradykinin itself.[14] This may mean that the nucleophilic character of the thiophene S atom is beneficial with respect to the inherent biological activity of both angiotensin and bradykinin. Further support for this suggestion comes from the finding that [Met[6]]-Ang is more active than the Leu[6] analog.

There has lately been much work on the conformation of angiotensin II in solution.[15,16] Unfortunately, as recent discussions point out,[17] there is as yet no clear picture of what the actual conformation of Ang may be in physiological media and at biologically realistic concentrations. Although Weinkam and Jorgensen[15] have postulated an H-bonding role for the imidazole of Ang in solution in DMSO, the model proposed by the French group (see particularly ref 17, p 545) depicts the imidazole as free of interactions with other parts of the peptide molecule in organic solvents. This latter

situation has also been proposed by Glickson, *et al.* (ref 17, p 563), for Ang in aqueous solution. As yet there is no evidence to indicate that the histidine side chain plays a role in determining the conformation of Ang under physiological conditions or at the receptor. For these reasons we have attempted to interpret our results with the 6-position analogs in terms of receptor interaction rather than conformation. Combined with other studies of pH effects on smooth muscle response to Ang (ref 17, p 521) and of alkylating derivatives of Ang,[18] this interpretation has led to a better understanding of hormone-receptor interaction and a new interpretation of tachyphylaxis.[19]

In summary, these results indicate that a structure resembling the imidazole side chain of His^6 is required for good biological activity of Ang. Both aromatic and nucleophilic character are useful properties of residues at this position. In contrast, substitution of strongly basic or acidic residues results in essentially total inactivation of the peptide.

Experimental Section

All *tert*-butyloxycarbonylamino acids were purchased from Schwarz BioResearch except Boc-Pro, Boc-Orn(Z), and Boc-Glu(Bzl) which were purchased from Fox Chemical Co. *tert*-Butyloxycarbonylthienylalanine was a gift of Dr. Floyd W. Dunn. Other special amino acid derivatives used were Boc-Tyr(Bzl), Boc-Arg(NO_2), Boc-His(Tos), and Boc-Asp(Bzl). Reagent grade $CHCl_3$ was used as obtained.

Solid-Phase Synthesis of Peptides. The essentials of the procedures used have been published previously.[12] Boc-Phe was esterified to 1% crosslinked chloromethyl resin (Bio-Rad Laboratories, 1.7 mequiv of Cl/g) by the standard procedure. Hydrolysis and amino acid analysis of an aliquot of the resin showed the degree of substitution to be 0.52 mmol/g. All syntheses were carried out on an improved model of the automatic instrument previously described.[20] For each synthesis an amount of Boc-Phe resin (0.77 g) was used which contained a total of 0.4 mmol of Phe. The Boc-protecting group was removed by a 30-min treatment with 25% CF_3CO_2H in $CHCl_3$ (v:v) (also containing 1 mg/ml of 2-mercaptoethanol or indole) following a prewash with the same reagent. After six $CHCl_3$ washes the trifluoroacetate form of the α-NH_2 group was neutralized with 10% Et_3N in $CHCl_3$ (v:v) for 10 min following a prewash with the same reagent. The resin was again washed six times with $CHCl_3$ and the coupling of the next *tert*-butyloxycarbonylamino acid was carried out with DCI in $CHCl_3$. The DCI and *tert*-butyloxycarbonylamino acids were present in 2.5-fold excess (1.0 mmol total) and the coupling time was 2 hr. Because of its limited solubility in $CHCl_3$, Boc-Arg(NO_2) was coupled in 1:1 DMF–$CHCl_3$. After the last residue was coupled, the peptide-resin was subjected to a final deprotection step. The resin was then washed with EtOH and CH_2Cl_2 and dried *in vacuo*. Cleavage of the remaining blocking groups and removal of the peptide from the resin were accomplished in anhydrous HF (10 ml per g of resin) in the presence of 50 equiv of anisole for 30 min at 0°. After evaporation of the HF, the resin and cleaved peptide were dried *in vacuo* and then washed with EtOAc to extract the remaining anisole and its reaction products. The peptides were extracted from the resin with glacial AcOH and dried by lyophilization.

The crude peptides were purified by countercurrent distribution in *n*-BuOH–AcOH–H_2O (4:1:5). Paper electrophoresis was carried out at either pH 5 (pyridine–AcOH; 0.1 *M* in pyridine) or pH 2.8 (1 *M* AcOH). Tlc was done on commercially prepared (Brinkmann or Eastman) cellulose plates developed in solvents F and I of ref 12, p 59. Electropherograms and tlc plates were developed with ninhydrin, Sakaguchi, and Pauly reagents.[12] Quantitative amino acid analyses were carried out on samples hydrolyzed in sealed N_2-flushed tubes for 72 hr at 110° in 6 *N* HCl containing 1 mg/ml each of PhOH and 2-mercaptoethanol. Partial destruction of thienylalanine occurs even in the presence of these protective reagents. The analyses were carried out on a Beckman 120C amino acid analyzer.

Bioassays. Rat uterus assays were carried out in modified de Jalon's solution containing 20 mg/l. of $CaCl_2$. Guinea pig ileum assays were carried out in Tyrode's solution containing 0.1 μg/ml of atropine sulfate. The details of the procedure were as described earlier[21] except that both solutions were bubbled with 98% O_2–2% CO_2 to give a final pH of 7.4. All assays were done at a bath temperature of 28°.

Rat blood pressure assays were carried out as previously described.[14] The peptides were administered by intrajugular injection. In those experiments where ganglion blockade was used, pentolinium tartrate was administered at a dose of 5 mg/kg ip.

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L-5-Oxo-2-pyrrolidinecarboxamides from Phenethylamines

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The L-pyroglutamide of *d-trans*-2-phenylcyclopropylamine recently was found inactive as a monoamine oxidase (MAO) inhibitor *in vitro*, though readily cleaved by a rat brain enzyme to the free amine.[1] The latter is a clinically useful antidepressant acting *via* MAO inhibition.

It thus seemed possible that the L-pyroglutamide of dopamine and/or similar compounds might cross the blood-brain barrier more readily than its usual amino acid precursor and that subsequent enzymatic cleavage of the amide might give