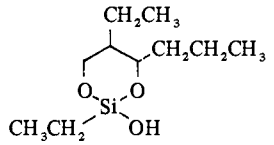
afford 2-ethyl-1,3-hexanediol. If compounds 1–4 were hydrolyzed rapidly enough to release the minimum effective dose of the repellent, then compounds 5–7 might also be expected to hydrolyze at such a rate that *some* repellent activity would be seen unless, of course, the cleavage of these agents did not release diol 8. In the preparation of 18, a compound was obtained which, from spectral data (nmr, ir), was shown to be silanol 22. The isolation of 22 and its



22

unexpected stability (as well as that of the corresponding methyl and butyl silanols) suggests that in the instance of 5–7, diol 8 is not a product of hydrolysis—only the corresponding silanols. However, in the instance of compounds 1–4, if hydrolysis to their corresponding silanols occurs, 1 mol of repellent diol 8 would still be released for every mole of compound—hence their activity. However, in the case of compounds 5–7, hydrolysis would not afford a repellent moiety (22 was found devoid of repellent activity). The lack of insectifugal activity of the silyl ether precursor molecules 5–7 is believed to be due to their hydrolysis to the inactive silanols (*e.g.*, 22) (Table I).

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1,6-Bis(N^5-*m*-trifluoromethylphenyl-N^1-biguanido)-hexane and Related Analogs of Chlorhexidine as Inhibitors of Dental Plaque†

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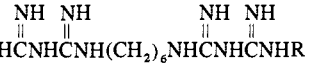
Dental plaque is a soft, tenacious bacterial deposit which forms on the surface of teeth. A close correlation exists between the formation of plaque and the development of caries, gingivitis, and subsequent periodontal disease.[1] Mechan ical cleansing is the principal means of removing plaque and its clinical effectiveness is limited. Since plaque is composed mainly of bacteria, numerous antibacterial agents have been investigated for their ability to inhibit plaque formation and several compounds have been reported to be active.[1-4] Clinical reports[2,3,5] have established that chlorhexidine (2), an antibacterial bisbiguanide, is one of the more effective inhibitors of plaque formation. The toxicity of chlorhexidine is low;[6] however, it does produce minor side effects that preclude general clinical use.[7]

The observation[8] that the phenyl analog 3 of chlorhexidine did not inhibit plaque formation, coupled with the earlier report[6] that variations at this position radically changed antibacterial activity, prompted us to synthesize chlorhexidine analogs 4–6 in an attempt to optimize plaque inhibition.

The synthesis of the analogs was based on the method of Rose and Swain.[9] The general procedure involved treating 1,6-diaminohexane with sodium dicyanamide to give 1,6-bis(N^3-cyano-N^1-guanidino)hexane (1) which on treatment with the appropriate amine gave the desired bisbiguanides.

1



2, R = *p*-ClC_6H_4
3, R = C_6H_5
4, R = cyclohexyl
5, R = 1-adamantyl
6, R = *m*-$CF_3C_6H_4$

Biological Results. Antiplaque activity as displayed by chlorhexidine requires that a compound be an antibacterial agent and therefore the antibacterial activity of the compounds prepared in this work was evaluated *in vitro* against *Streptococcus mutans* No. 6715, a pure strain of plaque forming bacteria.‡ Chlorhexidine (Ayerst Laboratories, Inc.) was tested concurrently.

A solution of the test compound (1 ml) was added to 7.85 ml of trypticase broth, 1 ml of 50% sterile sucrose solution, and 0.15 ml of a 24-hr culture of *S. mutans* No. 6715, and

†A preliminary account of this work was presented at the 163rd National Meeting of the American Chemical Society, Boston, Mass., April 1972, Abstract No. MEDI 16.

‡Isolated at and made available to us by the National Institute of Dental Research.

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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§BBL, Division of BioQuest, Cockeysville, Md.

#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.