E(His) 0.83 (pH 1.95), *E*(His) 1.10 (pH 8.6). *Anal.* ($C_{47}H_{73}N_{13}O_{11}\cdot 1AcOH\cdot 1H_2O$) C, H, N. Amino acid ratio in the acid hydrolysate was Asp 1.00, Arg 1.04, Val 1.00, Phe 1.00, Ile 2.01, His 1.00, Pro 1.01, and after incubation with L-amino acid oxidase His 0.07, Pro 1.00.

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Synthesis of Diethyl *N*-Dodecylphosphoramidate Analogs as Potential Inhibitors of Dental Plaque†

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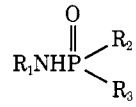
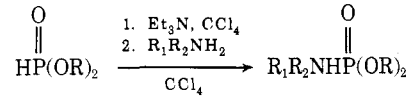
Dental plaque is a soft, tenacious bacterial deposit which forms on the surface of teeth. A close correlation exists between dental plaque and the principal diseases of the mouth: caries, gingivitis, and periodontal disease.[1] The high incidence of these diseases among the general population[2] is ample evidence that the current approaches to plaque control based on the use of mechanical aids are not achieving effective results. 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,[1-6] including several phosphoramidates (1-3) investigated in our laboratories.[5] Monoethyl *N*-dodecylphosphoramidate (1), either as the free acid or the dodecylammonium salt, has both *in vitro*[5] and *in vivo*[7,‡] antiplaque activity. Used clinically, 1 significantly reduced plaque formation during short-term trials.[7,‡] The toxicity of 1 is apparently low;[7,‡] however, its use at the concentration necessary for inhibition of plaque formation (1%, 0.034 *M*) was associated with a bad taste and stinging sensation.‡ This finding, along with the knowledge that 0.1% (2.2 m*M*) solutions of chlorhexidine, an antibacterial bisbiguanide, have *in vivo* antiplaque activity comparable to 1,[3,4] led us to investigate other phosphoramidates in the search for a more active compound.



1, $R_1 = n\text{-}C_{12}H_{25}$; $R_2 = OC_2H_5$; $R_3 = OH$ or $^-O^+NH_3\text{-}n\text{-}C_{12}H_{25}$
2, $R_1 = n\text{-}C_{14}H_{29}$; $R_2 = OC_2H_5$; $R_3 = {}^-O^+NH_3\text{-}n\text{-}C_{14}H_{29}$
3, $R_1 = n\text{-}C_{12}H_{25}$; $R_2 = R_3 = OC_2H_5$
4, R_1 = cyclohexyl; $R_2 = R_3 = OCH_3$

Initial studies[5] found that 2 and 3 inhibited *in vitro* plaque formation while 4 did not, showing that (1) a free OH (or O^-) was not necessary in the phosphoramidate molecule for antiplaque activity, and (2) changes in the N substituent can affect antiplaque activity. We thus synthesized a number of dialkyl- and diaryl-N-substituted phosphoramidates (**5-19**) in an attempt to optimize plaque inhibition.

Synthesis. The phosphoramidates were synthesized using the general procedure developed by Atherton, Openshaw, and Todd.[8] Treatment of diethyl, diphenyl, or dibenzyl phosphite with carbon tetrachloride and triethylamine followed by the appropriate amine gave the desired phosphoramidates.



$R = C_2H_5$, C_6H_5, $CH_2C_6H_5$
R_1R_2 = R_1R_2, Table I

Biological Results. The *in vitro* antiplaque activity of the test compounds was evaluated on extracted human teeth using the method of Turesky and coworkers.[5] *Streptococcus mutans* No. 6715, a pure strain of plaque-forming bacteria, was employed as the challenge organism (see Experimental Section). Chlorhexidine (Ayerst Laboratories, Inc.) and diethyl *N*-dodecylphosphoramidate (3) were tested concurrently. All the new compounds **5-19** were inactive at the highest concentration tested (10^{-1} *M*). At this concentration, chlorhexidine completely inhibited visible plaque formation during the entire 48-hr incubation period, while at a concentration of 10^{-2} *M*, chlorhexidine produced 60% inhibition for 24 hr, while diethyl *N*-dodecylphosphoramidate (3) produced 40% inhibition for 48 hr and 20% inhibition for 24 hr at a concentration of 10^{-1} and 10^{-2} *M*, respectively.

This primary study has found that the following relationships existed between the structure of the phosphoramidate diesters and antiplaque activity. (1) *N*-Aryl and *N*-adamantyl substituents were detrimental to activity. (2) In the diethyl ester series, chain lengthening of the N substituent gave an inactive compound. (3) All diphenyl esters prepared were inactive. On the basis of these results it would appear that it will probably take more than a simple modification of the ester moiety or the N substituent to achieve a clinically acceptable phosphoramidate antiplaque agent.

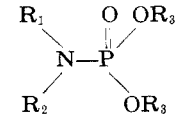
Experimental Section§

N-Substituted Phosphoramidate Esters 5-19. Phosphoram-

†A preliminary account of this work was presented at the 165th National Meeting of the American Chemical Society, Dallas, Texas, April 1973, Abstract No. MEDI 49.
‡S. S. Turesky, unpublished results.

§Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Midwest Microlab, Ltd., Indianapolis, Ind.

Table I. Phosphoramidates Evaluated for *in Vitro* Antiplaque Activity



Compd	R_1	R_2	R_3	Reaction time,[a] hr: Reflux	Reaction time,[a] hr: Room temp	Yield, %[c]	Mp (solvent)[b] or bp (mm), °C	Formula	Analyses
5	H	C_6H_5	C_2H_5	0	16	85	94–96[d] (A/B)	$C_{10}H_{16}NO_3P$	C, H, N
6	H	4-ClC_6H_4	C_2H_5	1	0.5	50	76–78[e] (C)	$C_{10}H_{15}ClNO_3P$	C, H, N
7	H	3-$CF_3C_6H_4$	C_2H_5	0	144	42	137 (0.5)	$C_{11}H_{18}F_3NO_3P$	C, H, N
8	H	1-Naphthyl	C_2H_5	6	66	51	110–113 (D/E)	$C_{14}H_{15}NO_3P$	C, H, N
9[f]	H	1-Adamantyl	C_2H_5	1	1	58	97.5–102 (C)	$C_{14}H_{26}NO_3P$	C, H, N
10		$-(CH_2)_5-$	C_2H_5	2	0	53	110 (4)	$C_9H_{20}NO_3P \cdot 0.5H_2O$	C, H, N
11	H	*n*-$C_{14}H_{29}$	C_2H_5	2	0	24	47.5–50 (C)	$C_{18}H_{40}NO_3P$	C, H, N, P
12	H	C_6H_5	C_6H_5	2	17	54	128–130[g] (D/E)	$C_{18}H_{16}NO_3P$	C, H, N
13	H	4-ClC_6H_4	C_6H_5	1.5	16	32	115–118.5[h] (D/F)	$C_{18}H_{15}ClNO_3P$	C, H, N, P
14	H	3-$CF_3C_6H_4$	C_6H_5	1	16	15	89–93 (D/G)	$C_{19}H_{15}F_3NO_3P$	C, H, N
15[f]	H	1-Adamantyl	C_6H_5	1	16	46	125–127 (D/G)	$C_{22}H_{26}NO_3P$	C, H, N, P
16	H	*n*-$C_{12}H_{25}$	C_6H_5	2.5	0	46	62–63.5 (C/D)	$C_{24}H_{36}NO_3P$	C, H, N
17	H	*n*-$C_{14}H_{29}$	C_6H_5	2.5	0	55	65.5–67.5 (C/D)	$C_{26}H_{40}NO_3P$	C, H, N, P
18	H	Cyclohexyl	$CH_2C_6H_5$	0	22	42	76.5–80[i] (C/D)	$C_{20}H_{25}NO_3P$	C, H, N
19	$(C_2H_5O)_2PN$ (O, CH_2CH_2, CH_2CH_2)		C_2H_5	2	0	58	Oil[j]	$C_{12}H_{28}N_2O_6P_2 \cdot 0.75H_2O$	C, H, N

[a] Length of reaction at reflux and/or room temperature. [b] Recrystallization solvents: A = ethanol; B = water; C = *n*-pentane; D = hexane; E = benzene; F = cyclohexane; G = 1,2-dichloroethane. [c] No attempt was made to optimize the yields. [d] Lit. mp 95.5–96.5°: H. McCombie, B. C. Saunders, and G. J. Stacey, *J. Chem. Soc.*, 380 (1945). [e] Lit. mp 76°: P. Otto, Ber., **28,** 616 (1895). [f] L. A. Cates, University of Houston, informed us subsequent to the completion of our synthetic work that he had independently prepared the adamantyl compounds for other purposes. [g] Lit. mp 128–129°: A. B. Foster, W. G. Overhead, and M. Stacey, *J. Chem. Soc.*, 980 (1951). [h] Lit. mp 116°: J. I. G. Cadogan and W. R. Foster, *ibid.*, 1079 (1957). [i] Lit.[8] mp 79–80°. [j] Purified by chromatography on silica gel (Woelm dry column grade) by eluting with C_6H_6–$CHCl_3$ (50:50) and 1% EtOH–$CHCl_3$. This compound decomposed on attempted vacuum distillation.

idates **5**–**19** were prepared using the general method developed by Atherton, Openshaw, and Todd.[8] To an ice-cooled and well-stirred solution of Et_3N (0.1 mol) in 50 ml of CCl_4 under N_2 was added the appropriate phosphite diester (0.1 mol) in 75 ml of CCl_4. The solution was heated to reflux with a precipitate forming. A solution of the appropriate amine (0.1 mol) in 50 ml of CCl_4 (0.05 mol in 25 ml of $CHCl_3$ for **19**) was added dropwise and additional precipitation occurred. The reaction mixture was stirred at reflux and/or at room temperature for a total of 1.5–144 hr. The mixture was cooled and filtered, and the filtrate was extracted with 5% HCl (2 × 25 ml), 5% $NaHCO_3$ (2 × 25 ml), and H_2O (25 ml) and dried (K_2CO_3). Concentration *in vacuo* gave the crude phosphoramidates which were purified by recrystallization or distillation (**5**–**18**) or by chromatography (**19**). Details are given in Table I.

Assay for *in Vitro* Antiplaque Activity.[5] Sterilized extracted human teeth were immersed in 1,2-dimethoxyethane solutions of the test compounds for two 1-min periods, each of which was followed by a 1-min exposure to air. These treated teeth were washed with 250 ml of distilled water for 5 min and then incubated under anaerobic conditions (BBL-Gaspak, BBL, Division of Bioquest, Cockeyville, Md.) in a sucrose-containing trypticase broth with *Streptococcus mutans* No. 6715, a pure strain of plaque-forming bacteria (isolated at and made available to us by the National Institute of Dental Research). The teeth were suspended in test tubes on orthodonic wire (Rocky Mount, 0.71 mm in diameter) threaded through a hole in the root so that the entire tooth was completely immersed. After 24 and 48 hr subjective estimates were made of adherent microbial growth on the test tube walls, wires, and the teeth and of nonadherent growth in the broth using a scale from 0 (no growth) to 4 (maximum growth). The total microbial accumulation was considered as *in vitro* plaque. For our results, growth ratings of 0 or 1 have been scored as plaque inhibition. Each compound was evaluated on five teeth with per cent inhibition values being reported. This indicated the percentage of teeth which did not show plaque formation after the stated incubation period. The solvent served as the control.

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Fibrin-Stabilizing Factor Inhibitors. 10. Thiol Esters as Potent Enzyme Inhibitors

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A series of thiol esters has been prepared and tested as inhibitors of the fibrin-stabilizing factor. Two of the compounds were found to be the most active inhibitors so far described in the literature.

Synthetic anticoagulants which selectively inhibit the fibrin-stabilizing factor (FSF, factor XIII) have not been clinically tried so far. However, because such inhibitors