Notes

Solid-Phase Synthesis of [3-Proline,8-isoleucine]-, [1-Sarcosine,3-proline,8-isoleucine]-, and [4-Phenylalanine,8-isoleucine]angiotensin II as the Antagonists of the Parent Hormone

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Continuation of our studies to investigate the factors which influence antagonistic properties of angiotensin II antagonists[1-4] has led us to determine the effect of variations in positions 3 and 4 of [8-isoleucine]angiotensin II. We reported earlier[5] that replacement of valine (position 3) with proline in angiotensin II reduced the pressor activity to 53% of that of the parent hormone. Recently, we observed that [Pro^3]angiotensin II when present in high concentration (100 ng/ml) in the bath inhibited the myotropic response of rabbit aortic strips to angiotensin II. This was the first 3-substituted analog of angiotensin II in which antagonistic properties were observed without a simultaneous modification in the 8 position. Since an aliphatic residue in position 8 of angiotensin II produced good antagonistic activity and sarcosine in the one position enhanced this effect, we synthesized [Pro^3,Ile^8]- and [Sar^1,Pro^3,Ile^8]angiotensin II to determine whether these substitutions enhanced the antagonistic properties of these analogs. However, compared to [Ile^8]angiotensin II (Table I), both these compounds were found to have low antagonistic activity against angiotensin II.

Replacement of tyrosine (position 4) with alanine reduced the antagonistic activity of [Ile^8]angiotensin II.[4] In order to determine if the phenolic hydroxyl group is important in this position, we synthesized [Phe^4,Ile^8]angiotensin II. The antagonistic activity of this compound (Table I) was found to be between that of [Ile^8]angiotensin II and [Ala^4,Ile^8]angiotensin II. This indicates that both the aromatic ring and the phenolic hydroxyl group in position 4 are required.

The phenolic group of tyrosine in angiotensin II is not involved in intramolecular hydrogen bonding and is therefore available for hydrogen bonding to a suitable group on the receptor.[6,7,†] With the limited evidence available for the antagonistic peptide, [Ile^8]angiotensin II,[8] it is reasonable to assume that the same situation prevails with regard to the phenolic group of this peptide.

The results available suggest that an angiotensin II analog is agonistic or antagonistic to myotropic response depending on the nature of the C-terminal amino acid[1-4] while the functional groups in the remainder of the chain play an important role in determining the potency of the peptide. This may be because of their role in determining the peptide's conformation, in altering the binding onto the receptor, or in increasing resistance to metabolism or it may be due to all of these phenomena. We synthesized the C-terminal tetrapeptide of [Ile^8]angiotensin II (Ile-His-Pro-Ile) and found it to have very weak inhibitory activity.

† S. Fermandjian, J. L. Morgat, P. Fromageot, C. Lutz, and J. P. Leicknam, unpublished results.

Table I. Comparative Pressor and Antagonistic Properties of Analogs of Angiotensin II[a,b]

Analog of angiotensin II	Log K_2	Pressor activity[e]
[Ile^8]-[g]	9.21 ± 1.02[c]	
[Pro^3,Ile^8]-	6.92 ± 0.18 (6)[d]	0.80
[Sar^1,Pro^3,Ile^8]-	7.17 ± 0.05 (6)	1.50
[Phe^4,Ile^8]-	7.17 ± 0.15 (6)	0.01
[Pro^3]-[h]	6.80 ± 0.11 (6)	53.00[f]

[a] Ile-His-Pro-Ile caused inhibition of angiotensin II at a dose level of 1 μg/ml. This tetrapeptide did not inhibit response of epinephrine. [b] The antagonistic activity was determined on rabbit aortic strips[4] and, for comparison, has been expressed as log K_2 value. Log K_2 values were calculated by the equation [H. O. Schild, *Pharmacol. Rev.*, **9**, 242 (1957)] $\log (x - 1) = n \log B + \log K_2$. When $x = 2$, then $-\log B = pA_2$ and if $n = 1$, $pA_2 = \log K_2$. [c] This was reported as pA_2 value by M. Yamamoto, R. K. Türker, P. A. Khairallah, and F. M. Bumpus, *Eur. J. Pharmacol.*, **18**, 316 (1972). [d] The number in parentheses represents the number of experiments. [e] Relative to [Asp^1,Ile^5]angiotensin II = 100. [f] Pressor activity reported earlier was 40%.[5] [g] See ref 2, 4, and 12. [h] See ref 5.

The compounds were synthesized by the solid-phase procedure[9] as reported earlier.[3] Purification of the desired peptides and the determination of their biological activities were carried out as reported in a preceding paper.[4] [Pro^3]angiotensin II was synthesized, as reported earlier.[5] Purification of this compound by the new procedure[4] gave enhanced pressor activity (Table I). In general, the overall yield of the purified octapeptides, based on initial amino acid attached to the polymer, was 40%. Comparative data for the pressure response and the antagonism to the myotropic response to angiotensin II are given in Table I. Synthesis and conformational studies with the C-terminal tetrapeptide would be reported elsewhere.

Experimental Section

Solvents used for tlc were (a) *n*-BuOH-AcOH-H_2O (BAW) (4:1:5); (b) *n*-BuOH-AcOH-H_2O-Pyr (BAWP) (30:6:24:20); (c) *n*-BuOH-AcOEt-AcOH-H_2O (BEAW) (1:1:1:1); (d) *n*-BuOH-Pyr-H_2O (BPW) (10:2:5); (3) *n*-PrOH-H_2O (PW) (1:1). Buffers used for ionophoresis were HCO_2H-AcOH (pH 1.95)[4] and Beckman barbiturate buffer B-2 (pH 8.6). The optical homogeneity was determined by the procedure reported earlier.[4] Angiotensin analogs reported in this paper contain L-isoleucine in position 5. Other experimental details for analyses are described in a preceding paper.[4] Where analyses are indicated only by symbols of the elements or function, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

[Pro^3,Ile^8]angiotensin II: tlc (cellulose) R_f 0.19 (BAW), R_f 0.62 (BEAW), R_f 0.54 (BAWP), R_f 0.10 (BPW), R_f 0.66 (PW); *E*(His) 0.87 (pH 1.95), *E*(His) 1.21 (pH 8.6). *Anal.* ($C_{47}H_{71}N_{13}O_{12}$·1.5AcOH) C, H, N. Amino acid ratio in the acid hydrolysate was Asp 1.00, Arg 0.97, Pro 1.82, Tyr 0.99, Ile 1.99, His 0.96, and after incubation with L-amino acid oxidase His 0.04, Pro 1.00.

[Sar^1,Pro^3,Ile^8]angiotensin II: tlc (cellulose) R_f 0.40 (BAW), R_f 0.61 (BEAW), R_f 0.60 (BAWP), R_f 0.16 (BPW), R_f 0.69 (PW); *E*(His) 0.82 (pH 1.95), *E*(His) 0.99 (pH 8.6). *Anal.* ($C_{46}H_{71}N_{13}O_{10}$·2.5AcOH) C, H, N. Amino acid ratio in the acid hydrolysate was Sar 1.11, Arg 1.01, Pro 2.00, Tyr 1.06, Ile 2.00, His 0.99, and after incubation with L-amino acid oxidase His 0.04, Pro 1.00, Sar 1.00.

[Phe^4,Ile^8]angiotensin II: tlc (cellulose) R_f 0.43 (BAW), R_f 0.69 (BEAW), R_f 0.54 (BAWP), R_f 0.00 (BPW), R_f 0.83 (PW);

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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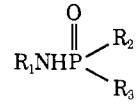
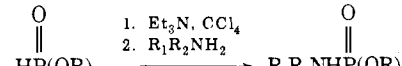
Dental plaque is a soft, tenacious bacterial deposit which forms on the surface of teeth. A close correlation exists between dental plaque and the principal diseases of the mouth: caries, gingivitis, and periodontal disease.[1] The high incidence of these diseases among the general population[2] is ample 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.