method of Groszkowski *et al.* (6). The carboxylic acids (II and III) were converted to their corresponding acyl halides (IV and V) in nearly 95% yield using *N*,*N*-dimethylformamide as a catalyst (7). To avoid protecting the carboxylic function of L-proline, the direct *N*-acylation of L-proline with IV or V was carried out in 90% yield using the procedure of Hongo *et al.* (9). *N*-(*R*,*S*-3-Halogeno-2-methylpropanoyl)-L-proline (VIa or VIIa) was successfully separated into optically pure diastereoisomers using dicyclohexylamine. Treatment of halides of VIb or VIIb with methanolic ammonium hydrosulfide afforded captopril in 28% overall yield. This synthetic method is an improvement over that reported by Cushman *et al.* (12%) (2) and is more convenient than the method reported by Shimazaki *et al.* (3), which involves a fermentation step.

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Phosphorous-Containing Analogues of Aspartame

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Abstract □ Four analogues of aspartame (aspartylphenylalanine methyl ester) were prepared in which one of the carboxylate groups was replaced by a phosphonate group. None of the peptides so obtained was sweet, in contrast with the parent compound which is over 100 times sweeter than sucrose. These results contrast with several published reports of phosphonate analogues of amino acids and peptides which are potent inhibitors of enzymes containing acceptor sites for the parent compound.

Keyphrases □ Aspartame—phosphonate analogues, sweetness

Since the discovery in 1969 that the synthetic dipeptide aspartame (*S*-aspartyl-*S*-phenylalanine methyl ester, I) was over 100 times sweeter than sucrose (1), many analogues have been prepared to ascertain which structural features are responsible for the sweetness. Quite early it was shown that alteration of the aspartyl moiety often result in loss of sweetness, but the phenylalanine moiety can be modified significantly with retention of sweetness (1, 2). More recent work (3) has shown that there are many exceptions to this simple rule, and that the structure-taste relationships are subtle and complicated.

One modification not yet reported is the replacement of one of the two carboxylate groups with a phosphonic acid function. There are several examples in the literature of amino acid and peptide analogues in which carboxylate groups have been replaced by phosphonate groups with retention of biological activity. For example, 1-amino-2-phenylethanephosphonic acid, the phosphonic acid analogue of phenylalanine, is a competitive inhibitor of phenylalanyl-tRNA synthetase (4), 1-amino-3-phosphonobutyric acid inhibits glutamine synthetase (5), and a series of peptides incorporating 1-aminoethanephosphonic acid as the C-terminal residue are shown to have antibacterial properties (6).

These results prompted us to synthesize the following aspartame analogues as diastereomeric mixtures (Scheme I): *R*,*S*-2-amino-3-phosphonopropionyl-*S*-phenylalanine methyl ester (IV); the free acid thereof (V); *S*-aspartyl-*R*,*S*-aminobenzylmethanephosphonate diethyl ester (VIII) and its free acid (IX).

RESULTS AND DISCUSSION

The peptide analogues were made by a standard coupling method (7) from the blocked amino acids. Although other coupling methods have been used to prepare dipeptides incorporating amino phosphonic acids (8), in our hands the mixed anhydride method employing ethyl chloroformate as coupling agent (7, 9) proved most successful. Compound IV was synthesized from *S*-phenylalanine methyl ester and the *N*-benzoxycarbonyl derivative of racemic 2-amino-3-dimethylphosphonopropionic acid (II). After coupling, the fully blocked peptide was partially deblocked to the methyl carboxylate (IV) by treatment with hydrogen bromide in acetic acid. The remaining methyl group was shown to reside on the carboxylate rather than the phosphonate group by recovery of starting material after attempted reesterification with thionyl chloride and methanol, a method which esterifies carboxylic acids but not phosphonic acids. This ester was converted to the fully deblocked peptide (V) by treatment with methanolic sodium hydroxide. Compound VIII was synthesized by coupling *S*-aspartic acid in the form of its *N*-benzoxycarbonyl-γ-benzyl ester derivative (10) with the diethyl ester of racemic aminobenzylmethanephosphonic acid (VI) (11), followed by hydrogenolysis. Deblocking with hydrogen bromide in acetic acid yielded the fully deblocked peptide analogue IX. Spectral data for the blocked aminophosphonic acid (II), blocked peptides (IV and VII), and deblocked peptides (V and IX) are presented in the *Experimental Section*. No attempt was made to separate the diastereomers in each product mixture since a significantly sweet isomer could have been detected by taste in the presence of a large amount of tasteless material.

Following the method previously used for testing sweetness in aspartame analogues (1), cotton swabs were soaked in water solutions of compounds to be tested, and the solutions were sucked off the swab. Usually compounds are not considered significantly sweet unless solutions of ≤2% concentrations evoke a sweet taste. However, even with 5% solutions, each peptide was found to be tasteless or slightly bitter by three different people. The lack of sweetness in IV and V is perhaps not surprising when one considers which modifications of the aspartyl residue of aspartame have previously been found to destroy sweetness. Replacement with an α-aminomalonyl moiety (*i.e.*, removing a methylene group from the aspartyl side chain) yields a compound even sweeter than aspartame (12), while replacing the aspartate by a glutamate moiety (thus adding an extra methylene group to the side chain) destroys sweetness (1). All other modifications of the aspartyl residue have led to nonsweet compounds. This suggests (3) that the N-terminal end of the molecule must incorporate a five- or six-membered zwitterionic ring in order to exhibit

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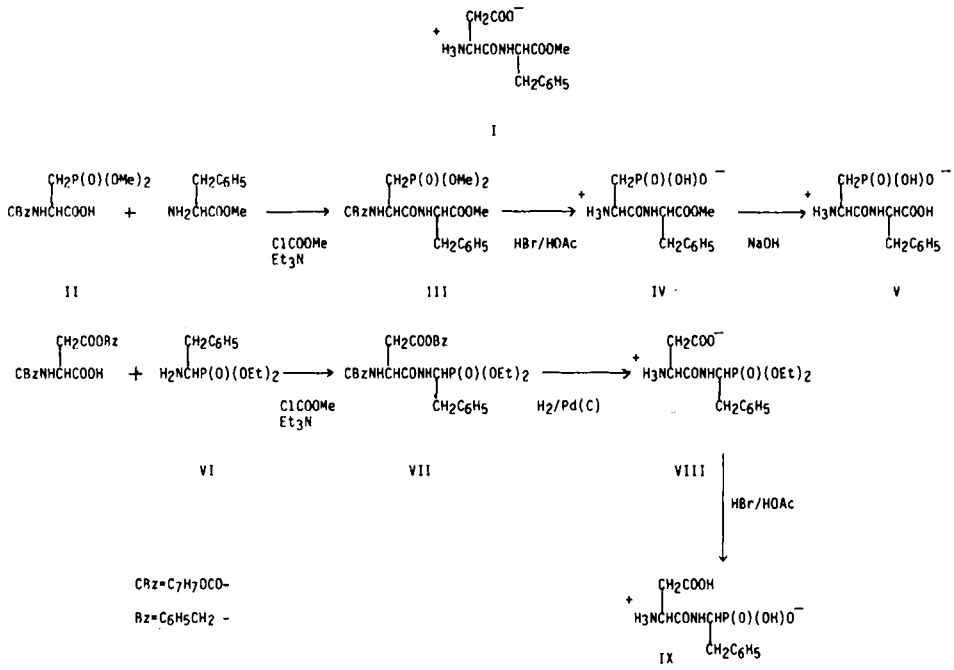

Scheme I

sweetness. According to this model, the negative carboxylate oxygen of the aspartyl moiety in aspartame is hydrogen bonded to the positive ammonium group, forming a six-membered ring. A seven-membered ring, which would be formed by a glutamate residue, is apparently too large. Since a phosphonate group is larger than a carboxylate group, replacement of the carboxylate group in the aspartyl side chain of aspartame would result in a six-membered ring larger than that in aspartame. Furthermore, the phosphonate group is pyramidal as opposed to the planar carboxylate group, so this ring could not be flat, as it is in aspartame.

Turning to the C-terminal end of aspartame, we find more leeway for modification. Many sweet analogues have been prepared incorporating a variety of amino acid esters and amides in place of phenylalanine methyl ester (13). However, in all these analogues two substituents of dissimilar size are attached to the carbon of the C-terminal residue. The orientation of the groups is always such that the large group corresponds to the benzyl side chain of aspartame and the smaller group corresponds to the methoxycarbonyl moiety. Apparently a difference in size between these two groups is important. For example, exchanging the methyl ester for the larger ethyl ester diminishes sweetness (1). Perhaps VIII and IX are not sweet because the phosphonate group, being larger than a methoxycarbonyl group, is too close in size to, or even larger than, the benzyl group. Also, it is noteworthy that replacement of the methoxycarbonyl group of aspartame with the free carboxylic acid or carboxamide destroys the sweetness. Evidently this part of the molecule must be relatively nonpolar. It is possible that both the diethylphosphonate group and the free phosphonic acid group are too polar to permit VIII and IX to be sweet.

In view of the exacting stereochemical requirements of the taste receptors it is not surprising that neither the charged, pyramidal phosphonic acid group nor the bulky diethylphosphonate group is a good replacement for the planar methylcarboxylate group of aspartame.

EXPERIMENTAL SECTION[1]

***N*-Benzyloxycarbonyl-2-*R*,*S*-amino-3-dimethylphosphonopropionic Acid (II)**—Racemic 2-amino-3-phosphonopropionic acid (14) (17 g, 0.1 mol) was stirred in water (100 mL) with KOH (33.5 g, 0.6 mol) at 0°C as a solution of carbobenzoxy chloride (35 g, excess) in ether was added in a dropwise manner. The solution was stirred overnight at room temperature, washed twice with ether, and acidified with hydrochloric acid to precipitate an oil. The aqueous layer was extracted with ethyl acetate and the combined oil and extract was dried with anhydrous sodium sulfate. Evaporation of the solvent yielded an oil, which was converted to the trimethyl ester by treatment for several hours with a methanolic solution of diazomethane according to a published method (15). After removal of volatile material, the oily triester (22.5 g, 0.065 mol) was refluxed in methanol (100 mL) with 0.55 M NaOH (118 mL, 0.065 mol) for 2 h. After evaporation of the solvent, the residue was triturated with hot ethyl acetate, dissolved in water, and neutralized with Dowex-50 (H^+). The water solution was extracted repeatedly with ethyl acetate, the combined extracts were dried, and the solvent was evaporated to yield the product as a glass (37%), which softens without melting at 150°C. TLC on silica gel (ethyl acetate–methanol, 9:1) shows one spot, R_f 0.3; ^{1}H-NMR ($CDCl_3$): δ 2.88 (m, CH_2), 4.03 (d, OCH_3), 5.48 (s, $ArCH_3$), 6.63 (m, NH), and 8.78 ppm (s, ArH); IR ($CHCl_3$): 3400–2400 (OH and NH), 1720 (C=O), 1500, 1210 (OH), and 1040 cm^{-1} (P=O).

Diethyl Aminobenzylmethanephosphonate (VI)—This material was prepared by diborane reduction (16) of the *O*-methyl oxime of diethyl phenylacetylphosphonate to give product identical with that of a published method (11).

Coupling Procedure—The blocked amino acids were coupled according to a published method (7), in which ethyl chloroformate is added to a solution of triethylamine and the blocked N-terminal amino acid in chloroform. The blocked C-terminal amino acid is added and the mixture is stirred for 12 h at room temperature.

Compound III was isolated as a thick oil in 78% yield. TLC on silica gel (ethyl acetate–methanol, 9:1) showed one spot, R_f 0.6; ^{1}H-NMR ($CDCl_3$): δ 2.73 (m, CH_2P), 3.48 (m, CHCO), 4.05 (m, OCH_3), 4.50 (m, CH), 5.05 (m, $ArCH_2$), 5.48 (s, $ArCH_2$), and 7.55 and 7.65 ppm (2 s, ArH); IR ($CHCl_3$): 2950–3420 (NH), 1680–1750 (C=O), 1500 (NH), 1190–1250 (OCH_3), and 1040 cm^{-1} (P=O).

Compound VII was isolated as a thick oil in 52% yield. TCL on silica gel (benzene–ethyl acetate, 7:3) showed one spot, R_f 0.2; ^{1}H-NMR ($CDCl_3$): δ 1.20 (m, CH_3), 3.00 (m, CH_2CO and CH), 4.03 (m, OCH_2), 4.60 (m, $ArCH_2$), 5.00 (m, $ArCH_2$ and CH), and 7.23 ppm (s, ArH); IR ($CHCl_3$): 3400 (NH), 3060 (ArH), 1740 (C=O), 1500 (NH), 1220 (OCH_2), and 1040 cm^{-1} (P=O).

Deblocking Procedures—Compound VII was deblocked to give VIII by hydrogenolysis over palladium on charcoal in methanol using 1 atm of hydrogen at room temperature. Filtration and evaporation of solvent yielded the product as a glass. Compounds III and VIII were deblocked to IV and IX, respectively, by a standard method (17) in which the blocked peptide was stirred at 60°C for 20 min in 48% hydrogen bromide–acetic acid (30 mL/g). The volatile material was evaporated, the residue was dissolved in ethanol, and the peptide was precipitated by addition of pyridine. Ester IV (0.0076 mol) was saponified to acid V by refluxing in 50 mL of methanolic sodium hydroxide (0.6 M) for 1 h. The solid so formed was isolated by filtration and

[1] NMR Spectra were obtained on a Tesla 80-MHz spectrometer, either in $CDCl_3$ with Me_4Si as standard, or in D_2O with sodium trimethylsilylpropanesulfonate as standard. IR spectra were obtained on a Specord spectrophotometer.

dissolved in aqueous acetic acid, and the product was precipitated by addition of ethanol.

Compound IV was an amorphous solid (59% yield) which did not melt up to 300°C. ^{1}H-NMR (D_2O): δ 2.50 (m, CH_2P), 3.48 (m, CHCO), 4.04 (s, OCH_3), 4.58 (m, ArCH_2), and 7.63 ppm (m, ArH). IR (KBr): 3700–2500 (NH and OH), 1680, 1740 (C=O), 1200 (OCH_3), and 1070 cm^{-1} (P=O).

Anal.—Calc. for $C_{13}H_{19}N_2O_6P \cdot 3.5\ H_2O$: N, 7.12; P, 7.87. Found: N, 6.64; P, 8.33.

Compound V was an amorphous solid (55% yield) which did not melt up to 300°C. ^{1}H-NMR (D_2O–D_2SO_4): δ 2.00–5.10 (m, aliphatic H) and 7.70 ppm (ArH); IR (KBr): 3700–2500 (NH and OH), 1650–1660 (C=O), and 1070 cm^{-1} (P=O).

Anal.—Calc. for $C_{12}H_{17}N_2O_6P$: N, 8.86; P, 9.80. Found: N, 8.18; P, 10.25.

Compound VIII was an amorphous gum which softened without melting at 90°C. TLC on silica gel (methanol) showed one spot, R_f 0.5.

Compound IX was an amorphous solid (50% yield) which did not melt up to 260°C. ^{1}H-NMR (D_2O–D_2SO_4): δ 2.75–3.88 (m, CH and CH_2), 4.38–5.25 (m, ArCH_2 and CH), and 7.62 cm^{-1} (s, ArH). IR (KBr): 2700–3500 (OH, ArH, and CH), 1700 (C=O), 1150 (C—O), and 1050 cm^{-1} (P=O).

Anal.—Calc. for $C_{12}H_{17}N_2O_6P \cdot H_2O$: N, 8.38; P, 9.26. Found: N, 8.40; P, 9.02.

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Induction of Hyperpyrexia by Dihydrocurvularin, a Metabolic Product of *Penicillium gilmanii*

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Abstract □ The previously reported rise in rectal temperature that follows the intravenous injection of the mixture of metabolic products (extractable with ether from the Czapek Dox medium on which *Penicillium gilmanii* has grown) is due to a single compound, dihydrocurvularin. Intravenous injection of 1–10 μg of dihydrocurvularin into rabbits causes a rise of at least one degree in rectal temperature of rabbits in 2–8 h. The degree of temperature rise depends more on the individual rabbit than on the quantity of dihydrocurvularin injected. Treatment with lipopolysaccharide abolishes the ability of dihydrocurvularin to cause a rise in rectal temperature. Treatment with dihydrocurvularin, however, does not abolish the ability of lipopolysaccharide to induce a temperature response or a leukocytosis. Rabbits respond to repeated treatment with dihydrocurvularin with a rise in rectal temperature that is indistinguishable from that observed on their first injection. Treatment with dihydrocurvularin does not affect differential counts or the concentration of leukocytes or red blood cells in the circulatory system.

Keyphrases □ Dihydrocurvularin—intravenous injection, hyperpyrexia, rabbits, effect of lipopolysaccharide

The intravenous injection of microgram quantities of the mixture of metabolic products elaborated by *Penicillium gilmanii* grown on a Czapek Dox medium induces a temporary rise in rectal temperature and leukocytosis in rabbits (1, 2). After extraction with ether the medium no longer induced a temperature rise but afforded a pure crystalline enolic heterocyclic compound, leucogenenol (2), of which 0.002 μg/kg, on intravenous injection, induces a temporary leukocytosis in animals (1, 3), but even milligram quantities do not induce hyperpyrexia. On the other hand, intravenous injection of microgram quantities of the mixture of compounds extractable from the culture medium with ether induces a hyperpyrexia in rabbits comparable to that observed following the injection of microgram quantities of lipopolysaccharides.

Investigations on leucogenenol established that it acted on cells in the peripheral circulation by increasing the rate at which committed cells of the bone marrow develop into functional cells such as neutrophils and lymphocytes (4–7). Undoubtedly as a consequence of its ability to increase the rate of development of cells involved in the immune response, treatment with leucogenenol enhances the immunocompetence of an animal and increases the rate at which immunosuppressed animals recover immunocompetence (8–13). Leucogenenol is also found as a thymo-thyroid hormone (14–16).

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