Studies on Polypeptides. XXXVI. The Effect of Pyrazole–Imidazole Replacements on the S-Protein Activating Potency of an S-Peptide Fragment[1-3]

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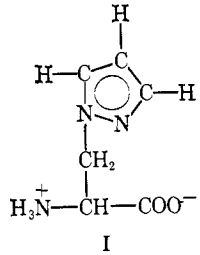
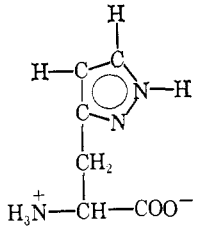
Abstract: Syntheses are described of two peptide amides [lysylglutamylthreonylalanylalanylalanyllysylphenylalanylglutamylarginylglutaminyl-β-(pyrazolyl-1)-alanine amide and lysylglutamylthreonylalanylalanylalanyllysylphenylalanylglutamylarginylglutaminyl-β-(pyrazolyl-3)-alanine amide] corresponding to positions 1–12 of S-peptide with the histidine moiety replaced by β-(pyrazolyl-1)-L-alanine or β-(pyrazolyl-3)-L-alanine. Both peptides fail to activate S-protein at high peptide–protein ratios. From these findings it is concluded that the ionization behavior of histidine 12 is responsible for the ability of S-peptide to activate S-protein.

The imidazole portion of histidine has assumed a key position in enzymology since there is considerable evidence to show that it constitutes an important structural element in the active site of a number of enzymes. Indeed the acid–base behavior of imidazole has provided the basis for proposed mechanisms of action of such enzymes as trypsin, chymotrypsin, and bovine pancreatic ribonuclease A.[4] In addition to its occurrence in enzymes histidine is also found in such biologically active peptides as α-MSH, β-MSH, the essential portion of the corticotropins, and hypertensin.[5] In order to explore the importance of the imidazole portion of histidine for biological function, we have initiated a systematic structure–function study involving replacement of histidine by other amino acid residues in biologically active peptides. As particularly intriguing histidine substitutes, we selected two pyrazolylalanines, *i.e.*, β-(pyrazolyl-1)-L-alanine [Pyr(1)Ala] (I) and β-(pyrazolyl-3)-L-alanine [Pyr(3)Ala] (II). Pyrazole is isosteric with imidazole but exhibits a markedly different acid–base behavior.



I



II

In previous communications[6] it was shown that the undecapeptide III, which corresponds to positions 1–11 of S-peptide, fails to bring about activation of S-protein[7] at molar ratios as high as 8000:1. The dodecapeptide amide IV which differs from III by the C-terminal histidine amide residue forms a fully active partially synthetic ribonuclease with S-protein at molar ratios of 200:1; the 50% activation ratio of this peptide amide is 88:1.[6] This finding and those of others[8] demonstrate that histidine in position 12 is essential for the catalytic activity of ribonuclease A. In order to explore further the functional significance of the imidazole portion of this histidine we have now synthesized two pyrazole analogs V and VI of IV and have tested their ability to reconstitute active ribonucleases with S-protein. Both compounds are inactive.

H·Lys·Glu·Thr·Ala_3·Lys·Phe·Glu·Arg·Gln·OH

III

H·Lys·Glu·Thr·Ala_3·Lys·Phe·Glu·Arg·Gln·His·NH_2

IV

H·Lys·Glu·Thr·Ala_3·Lys·Phe·Glu·Arg·Gln·Pyr(X)Ala·NH_2

V, $X = 1$
VI, $X = 3$

β-(Pyrazolyl-1)-L-alanine (I) is a naturally occurring amino acid which was isolated from watermelon (*Citrullus vulgaris*) seeds by Shinano and Kaya.[9] Noe and Fowden[10] confirmed the structure and described the first synthesis of the DL compound. Improved synthetic methods were developed later by Finar and Utting,[11] Sugimoto, Watanabe, and Ide,[12] and Reimlinger, *et al.*[13] β-(Pyrazolyl-3)-DL-alanine (II) was synthesized by Jones.[14] As far as we were able to

(1) The authors wish to express their appreciation to the U. S. Public Health Service and the American Cancer Society for generous support of this investigation.

(2) Except where noted otherwise amino acid residues in peptides are of the L variety. The following abbreviations are used: DMF = dimethylformamide, THF = tetrahydrofuran, TFA = trifluoroacetic acid, OPNP = *p*-nitrophenyl ester, O-*t*-But = *t*-butyl ester, Z = benzyloxycarbonyl, *t*-Boc = *t*-butoxycarbonyl, DCC = N,N′-dicyclohexylcarbodiimide, AP-M = aminopeptidase M, TEA = triethylamine.

(3) See K. Hofmann, M. J. Smithers, and F. M. Finn, *J. Am. Chem. Soc.*, **88**, 4107 (1966), for paper XXXV in this series.

(4) For reviews see F. H. Westheimer, *Advan. Enzymol.*, 24, 441 (1962); and M. L. Bender and F. J. Kézdy, *Ann. Rev. Biochem.*,**34**, 49 (1965).

(5) For a review see K. Hofmann and P. G. Katsoyannis in "The Proteins," Vol. I, H. Neurath, Ed., Academic Press Inc., New York, N. Y., 1963, p 54.

(6) (a) K. Hofmann, F. M. Finn, W. Haas, M. J. Smithers, Y. Wolman, and N. Yanaihara, *J. Am Chem. Soc.*, **85**, 833 (1963); (b) F. M. Finn and K. Hofmann, *ibid.*, **87**, 645 (1965).

(7) F. M. Richards, *Proc. Natl. Acad. Sci. U. S.*, **44**, 162 (1958); the following abbreviations will be used: RNAase-S, subtilisin-modified beef ribonuclease RNAase-A; S-peptide, the eicosapeptide obtained from RNAase-S; S-protein, the protein component obtained from RNAase-S; RNAase-S′, the reconstituted enzyme obtained by mixing equimolar proportions of S-peptide and S-protein; RNA, yeast ribonucleic acid.

(8) A. M. Crestfield, W. H. Stein, and S. Moore, *J. Biol. Chem.*, **238**, 2413 (1963).

(9) S. Shinano and T. Kaya, *J. Agr. Chem. Soc. Japan.* **31**, 759 (1957).

(10) F. F. Noe and L. Fowden, *Biochem. J.*, 77, 543 (1960).

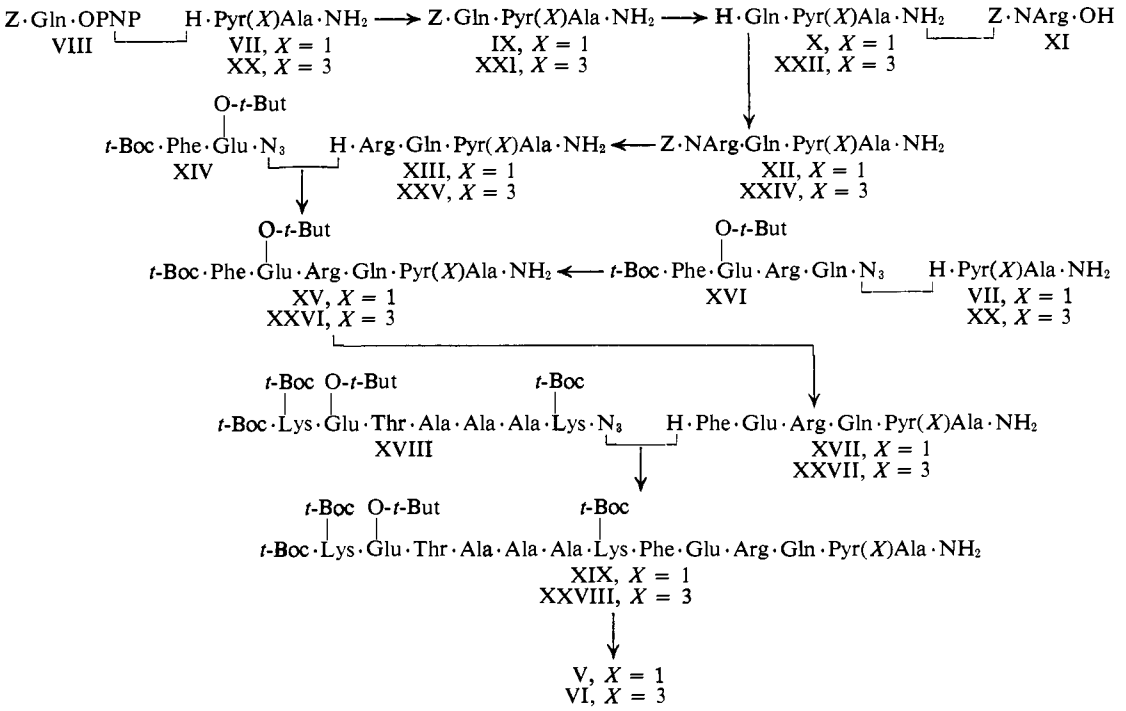
(11) I. L. Finar and K. Utting, *J. Chem. Soc.*, 5272 (1960).

(12) N. Sugimoto, H. Watanabe, and A. Ide, *Tetrahedron*, 11, 231 (1960).

(13) H. Reimlinger, J. F. M. Oth, and F. Billiau, *Ber.*, 97, 331 (1964).

(14) R. G. Jones, *J. Am. Chem. Soc.*, 71, 3994 (1949).

Chart I



ascertain, no synthetic peptides incorporating these two amino acids have as yet been described in the literature, but γ-L-glutamyl-β-(pyrazolyl-1)-L-alanine occurs naturally in cucumber seeds.[15] We prepared the DL form of the two amino acids along published lines and resolved them with acylase.[16] The L forms were then incorporated into peptides.

Synthetic Approach. The synthetic route to peptides V and VI is illustrated in Chart I. For preparation of V, L-Pyr(1)Ala was converted into the amide VII *via* the methyl ester dihydrochloride and the amide was treated with *p*-nitrophenyl benzyloxycarbonylglutaminate (VIII)[17] to give the protected dipeptide amide IX. Hydrogenolysis converted IX into X which was acylated with a mixed anhydride of N^{α}-benzyloxycarbonylnitroarginine (XI).[18] The ensuing protected tripeptide amide XII was exhaustively hydrogenated to give the tripeptide amide XIII which was coupled with the azide XIV[19] to form XV. Alternatively, compound XV was also obtained, in poor yield, from the reaction of the azide XVI[19] with β-(pyrazolyl-1)-alanine amide (VII). Compound XV was deblocked with TFA, trifluoroacetate ions were exchanged by acetate ions, and the pentapeptide dihydrate XVII was acylated with the azide XVIII[20] to give the protected dodecapeptide amide XIX. The latter compound was converted to the octahydrate of V by treatment with TFA followed by ion exchange. The dodecapeptide amide was purified by chromatography on carboxymethylcellulose (CMC) and was homogeneous as judged by paper chromatography. The compound was completely digestible with AP-M[21] and the digest contained the constituent amino acids in the ratios expected by theory. The dodecapeptide amide VI was prepared in an analogous manner from β-(pyrazolyl-3)-L-alanine amide (XX). Here again the final product was purified on CMC and its homogeneity assessed by paper chromatography and AP-M digestion.

Experimental Section

Materials. The ion exchangers, carboxymethylcellulose (CMC) (Cellex-CM), AG 1-X2, DEAE-cellulose (Cellex-D), and Bio-Rex 70 were purchased from Bio-Rad Laboratories, Richmond, Calif. The exchanger IRA-400 was supplied by Mallinckrodt Chemical Works, St. Louis, Mo. Aminopeptidase-M (AP-M) was obtained from Röhm and Haas GmbH, Darmstadt, West Germany. Hog kidney acylase was purchased from Sigma Chemical Co. Yeast RNA (Sigma Chemical Co.) was purified by precipitation from 0.1 *M* sodium acetate as described by Klee and Richards.[22] Ribonuclease A was prepared from bovine pancreatic ribonuclease A (Sigma Chemical Co., five times crystallized) by the procedure of Crestfield, *et al.*[23] Ribonuclease S,S-protein, and S-peptide were prepared as described previously.[6]

General Procedures. Optical rotations were determined with a Zeiss precision polarimeter 0.005°. Measurements were carried out with a mercury lamp at 546 and 578 mμ and extrapolated mathematically to the 589-mμ sodium line. Procedures for thin layer and paper chromatography were those given in ref 20. Enzyme assays were performed at 20° as described in ref 21b. Liberation of the pyrazolylalanines and their amides was monitored on the amino acid analyzer. The two pyrazolylalanine isomers are widely separated on the long column; Pyr(1)Ala emerges with threonine, Pyr(3)Ala appears as a sharp peak after the buffer change and is well separated from methionine. Pyr(1)Ala amide emerges from the short column between ammonia and arginine; Pyr(3)Ala amide is eluted with arginine. For analytical convenience, digestion times of peptides were chosen so as to liberate Pyr(1)Ala amide

(15) P. M. Dunnill and L. Fowden, *Biochem. J.*, **86**, 388 (1963).
(16) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1961, p 715.
(17) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
(18) K. Hofmann, W. D. Peckham, and A. Rheiner, *ibid.*, **78**, 238 (1956).
(19) K. Hofmann, W. Haas, M. J. Smithers, R. D. Wells, Y. Wolman, N. Yanaihara, and G. Zanetti, *ibid.*, **87**, 620 (1965).
(20) K. Hofmann, R. Schmiechen, R. D. Wells, Y. Wolman, and N. Yanaihara, *ibid.*, **87**, 611 (1965).
(21) (a) G. Pfleiderer and P. G. Celliers, *Biochem. Z.*, **339**, 186 (1963); (b) K. Hofmann, F. M. Finn, M. Limetti, J. Montibeller, and G. Zanetti, *J. Am. Chem. Soc.*, **88**, 3633 (1966).
(22) W. A. Klee and F. M. Richards, *J. Biol. Chem.*, **229**, 489 (1957).
(23) A. M. Crestfield, W. H. Stein, and S. Moore, *ibid.*, **238**, 618 (1963).

and free Pyr(3)Ala because of their unique positions on the analyzer, *i.e.*, 5 hr for peptide amides V and XVII, 20 hr for peptide amides VI and XXVII.

Syntheses. **β-(Pyrazolyl-1)-L-alanine (I).** β-(Pyrazolyl-1)-DL-alanine synthesized according to Finar and Utting[11] was resolved with hog kidney acylase[16] (for a representative experiment see resolution of N^{α}-acetyl-β-(pyrazolyl-3)-DL-alanine). The L-amino acid was recrystallized from water–ethanol, 1:1; mp 243° with decomposition; $[\alpha]^{29}D$ −72.0° (*c* 1.0, water); single ninhydrin-positive spot with R_f^1 0.42 and R_f^3 1.9 × His (lit.[24] mp 241–243° dec; $[\alpha]^{17}D$ −72.0°).

Racemization of Acetyl-β-(pyrazolyl-1)-D-alanine. The mother liquors from the acylase reaction were concentrated to a small, volume *in vacuo;* the residue was acidified with hydrochloric acid, and extracted with several portions of ethyl acetate. The extracts were dried over sodium sulfate and evaporated to dryness. This material was dried and dissolved in a tenfold amount of glacial acetic acid, and acetic anhydride (1 ml per gram of solid) was added. The mixture was kept at room temperature for 24 hr. The solvents were removed *in vacuo;* the residue was evaporated several times with water and dried over P_2O_5 and potassium hydroxide pellets; mp 152–154°; ninhydrin negative; no measurable optical rotation.

β-(Pyrazolyl-3)-DL-alanine. The hydrochloride was prepared essentially as described by Jones.[14] For conversion to the free amino acid, the crude hydrochloride (from 15.3 g of 3-chloromethylpyrazole) was dissolved in water (300 ml), IRA-400 (acetate cycle, 300 ml settled in water) was added, and the suspension was shaken for 2 hr at room temperature. The resin was removed by filtration and the filtrate passed through a column (3 × 25 cm) of the same resin which was washed with 2% acetic acid until the effluent was ninhydrin negative. Ninhydrin-positive fractions were pooled and concentrated to a syrup *in vacuo.* β-(Pyrazolyl-3)-DL-alanine precipitated upon the addition of ethanol; yield 9.5 g; mp 228–229°; R_f^1 0.30; R_f^3 1.5 × His (lit.[14] mp 226–228°).

N^{α}-Acetyl-β-(pyrazolyl-3)-DL-alanine. β-(Pyrazolyl-3)-DL-alanine (2.1 g) was dissolved in hot glacial acetic acid (24 ml), and acetic anhydride (1.6 ml) was added. The solution was refluxed for 2 min, the solvents were removed *in vacuo*, and the resulting oil was evaporated with several portions of water to give a crystalline residue. Recrystallization from water gave clusters of prisms; yield 2.3 g (86%); mp 179–181°; single ninhydrin-negative, chlorine-positive spot with R_f^1 0.75; R_f^{VI} 0.60.

Anal. Calcd for $C_8H_{11}O_3N_3$: C, 48.8.; H, 5.6; N, 21.3. Found: C, 48.5; H, 5.9; N, 21.2.

N^{α},N^{pyr}-Diacetyl-β-(pyrazolyl-3)-DL-alanine Dicyclohexylammonium Salt. β-(Pyrazolyl-3)-DL-alanine was acetylated in the manner described above. The oil which remained after evaporation of acetic acid and acetic anhydride was dissolved in ethyl acetate, and dicyclohexylamine was added. The crystalline precipiate was collected, washed with ethyl acetate and ether, and dried; mp 188–189° dec.

Anal. Calcd for $C_{22}H_{36}O_4N_4$: C, 62.8; H, 8.6; N, 13.3; O, 15.2. Found: C, 63.2; H, 8.4; N, 13.9; O, 15.2.

β-(Pyrazolyl-3)-L-alanine (II). N^{α}-Acetyl-β-(pyrazolyl-3)-DL-alanine (16.3 g) was dissolved in water (1000 ml), and the pH of the solution was adjusted to 7.2 by addition of 3% ammonium hydroxide. The solution was kept at room temperature for 4 hr, then the pH, which had dropped, was readjusted to 7.2. Acylase (160 mg) and toluene (a few drops) were added, and the solution was incubated at 37° for 12 hr. Following readjustment of the pH an additional quantity of acylase (160 mg) was added and incubation continued for 12 hr. The reaction mixture was then acidified to pH 5 with glacial acetic acid, boiled for 5 min, and concentrated *in vacuo* to a volume of approximately 400 ml. This solution was applied to a column (4 × 40 cm) of AG 1-X2 (acetate cycle) which was eluted with water. Fractions containing ninhydrin-positive material were combined and evaporated to a volume of approximately 30 ml, and ethanol (500 ml) was added. The crystalline material was collected and dried; yield 6.1 g (95%). Recrystallization from water–ethanol gave colorless needles; mp 247–248°; $[\alpha]^{25}D$ −42.0° (*c* 1.0, water); single ninhydrin-positive spot with R_f^1 0.33 and R_f^3 1.5 × His.

Anal. Calcd for $C_6H_9O_2N_3$: C, 46.5; H, 5.8; N, 27.1. Found: C, 46.5; H, 6.0; N, 26.8.

For isolation of N^{α}-acetyl-β-(pyrazolyl-3)-D-alanine, the column was eluted with 15% acetic acid. Chlorine-positive fractions were pooled and evaporated to dryness. The resulting material was racemized as described above, and the racemate resolved with acylase.

Methyl β-(Pyrazolyl-1)-L-alaninate Dihydrochloride. This derivative was prepared in the usual manner from 2.7 g of the amino acid; yield 3.9 g (93%); mp 173–174°; $[\alpha]^{28}D$ −8.5° (*c* 1.0, water); R_f^1 0.61; R_f^3 3.6 × His.

Anal. Calcd for $C_7H_{11}O_2N_3 \cdot 2HCl$: C, 34.7; H, 5.4; N, 17.3. Found: C, 35.3; H, 5.2; N, 17.3.

Repeated precipitation of the ester dihydrochloride from methanol with ether results in losses of hydrochloric acid.

Methyl β-(Pyrazolyl-3)-L-alaninate Dihydrochloride. This derivative was prepared in the usual manner from 2.5 g of the amino acid; recrystallized from methanol–ether; yield 3.8 g (97%); mp 112–114°, sealed capillary; $[\alpha]^{23}D$ +6.1° (*c* 3.0, water); R_f^3 2.5 × His; R_f^V 0.70; R_f^{VI} 0.40; dried at 60° for analysis.

Anal. Calcd for $C_7H_{11}O_2N_3 \cdot 2HCl$: C, 34.7; H, 5.4; N, 17.3. Found: C, 34.5; H, 5.6; N, 17.2.

β-(Pyrazolyl-1)-L-alanine Amide (VII). A solution of β-(pyrazolyl-1)-L-alanine methyl ester dihydrochloride (3.34 g) in methanol (34 ml) was saturated with ammonia at −5° and kept in a pressure bottle at room temperature for 7 days. The solvent was evaporated, the residue dissolved in approximately 5 ml of water, and the solution layered with ethyl acetate. After cooling in an ice bath, sodium hydroxide (2 g) in a small volume of water was added, and solid potassium carbonate to give a heavy slurry. The mixture was shaken vigorously, the ethyl acetate layer was removed, and the residue was extracted with several additional portions of ice-cold ethyl acetate. The ethyl acetate extracts were combined, and dried over potassium carbonate and sodium sulfate, and the solvent was removed. The colorless solid residue was washed with ether and recrystallized from ethyl acetate; yield 1.95 g (92%); mp 91–92°; $[\alpha]^{28}D$ +4.8° (*c* 1.0, water); R_f^1 0.48; R_f^3 2.5 × His.

Anal. Calcd for $C_6H_{10}ON_4$: C, 46.7; H, 6.5; N, 36.3. Found: C, 46.8; H, 6.8; N, 36.2.

β-(Pyrazolyl-3)-L-alanine Amide Monoacetate (XX). β-(Pyrazolyl-3)-L-alanine methyl ester dihydrochloride (3.5 g) was dissolved in methanol saturated at −5° with ammonia (30 ml), and the mixture was kept in a pressure bottle at room temperature for 7 days. The solvent was removed, the residue was dissolved in water (250 ml), and the solution was added to an AG 1-X2 column (3 × 25 cm) (acetate cycle) which was eluted with water. Ninhydrin-positive fractions were combined and evaporated to dryness, and the residue was recrystallized from methanol; yield 2.05 g mp 158–160°; $[\alpha]^{27}D$ +7.5° (*c* 1.0, water); R_f^1 0.43; R_f^3 (66%); 1.9 × His.

Anal. Calcd for $C_6H_{10}ON_4 \cdot CH_3COOH$: C, 44.9; H, 6.6; N, 26.1. Found: C, 45.1; H, 6.8; N, 26.2.

Benzyloxycarbonylglutaminyl-β-(pyrazolyl-1)-alanine Amide (IX). To an ice-cold solution of β-(pyrazolyl-1)-L-alanine amide (VII) (1 g) in DMF (20 ml) *p*-nitrophenyl benzyloxycarbonylglutaminate[17] (2.5 g) was added, and the mixture was kept at room temperature for 48 hr. The resulting precipitate was collected, washed with several portions of hot ethyl acetate and ethanol, and dried; yield 1.91 g (71%). A sample for analysis was recrystallized from 50% acetic acid; needles; mp 241–242°; $[\alpha]^{27}D$ −25.3° (*c* 0.5, 90% ethanol).

Anal. Calcd for $C_{19}H_{24}O_5N_6$: C, 54.8; H, 5.8; N, 20.2; O, 19.2. Found: C, 55.0; H, 6.0; N, 19.9; O, 19.7.

Benzyloxycarbonylglutaminyl-β-(pyrazolyl-3)-alanine Amide (XXI). To an ice-cold solution of β-(pyrazolyl-3-)-L-alanine amide acetate (XX) (1.5 g) in DMF (30 ml), water (3 ml), and triethylamine (0.98 ml) was added *p*-nitrophenyl benzyloxycarbonylglutaminate[17] (2.8 g), and the solution was stirred for 48 hr at room temperature. The resulting crystalline precipitate was collected, washed with ethyl acetate, and dried; yield 2.25 g (77%). A sample for analysis was recrystallized from aqueous acetic acid; mp 207–208°; $[\alpha]^{26}D$ −14.6° (*c* 1.0, 90% acetic acid); R_f^1 0.82; R_f^{VI} 0.70.

Anal. Calcd for $C_{19}H_{24}O_5N_6$: C, 54.8; H, 5.8; N, 20.2; O, 19.2. Found: C, 54.9; H, 6.4; N, 20.2; O, 19.5.

Glutaminyl-β-(pyrazolyl-1)-alanine Amide Monoacetate (X). The protected dipeptide amide IX (2.03 g) was suspended in water–methanol–acetic acid (1:1:1) (60 ml) and hydrogenated over palladium in the usual manner. The catalyst was removed by filtration and the residue evaporated *in vacuo* to give an amorphous solid; yield quantitative; $[\alpha]^{29}D$ +13.8° (*c* 0.5, 10% acetic acid); R_f^1 0.31; R_f^{VI} 0.24; slightly contaminated with a ninhydrin-negative material with R_f^1 0.58 and R_f^{VI} 0.45 ("diketopiperazine"?).

(24) M. Takeshita, Y. Nishizuka, and O. Hayaishi, *J. Biol. Chem.* 238, 660 (1963).

Anal. Calcd for $C_{11}H_{18}O_3N_6 \cdot CH_3COOH$: C, 45.6; H, 6.5; N, 24.5; O, 23.4. Found: C, 46.0; H, 7.0; N, 24.3; O, 22.9.

Glutaminyl-β-(pyrazolyl-3)-alanine Amide Monohydrate (XXII). The protected dipeptide amide XXI (2.16 g) was hydrogenated over palladium in acetic acid–methanol–water (2:2:1) (50 ml) in the usual manner. The catalyst was removed by filtration and the filtrate evaporated to dryness *in vacuo*. The residue was dissolved in water (200 ml) and the solution applied to a Bio-Rex 70 column (1.8 × 15 cm, hydrogen form) which was eluted first with water, then with 1% ammonium hydroxide. The ammonium hydroxide eluates were evaporated to dryness to give a hygroscopic solid; yield 0.80 g (51%); $[\alpha]^{26}D$ +36.8° (*c* 1.0, 10% acetic acid); R_f^{I} 0.32; R_f^{VI} 0.24.

Anal. Calcd for $C_{11}H_{18}O_3N_6 \cdot H_2O$: C, 44.0; H, 6.7; N, 28.0; O, 21.3. Found: C, 43.1; H, 7.0; N, 28.5; O, 21.6.

Evaporation of the water eluates gave 0.53 g of the "diketopiperazine" which was recrystallized from ethanol; mp 211–212°; $[\alpha]^{26}D$ −19.0° (*c* 1.0, 90% acetic acid); R_f^{I} 0.55; R_f^{VI} 0.43; ninhydrin negative, chlorine positive.

Anal. Calcd for $C_{11}H_{15}O_3N_5$: C, 49.8; H, 5.7; N, 26.4; O, 18.1. Found: C, 50.3; H, 6.0; N, 26.0; O, 18.5.

N^{α}-Benzyloxycarbonylnitroarginylglutaminyl-β-(pyrazolyl-1)-alanine Amide (XII). a. **By the Mixed Anhydride Procedure.** A mixed anhydride was prepared at −5° from N^{α}-benzyloxycarbonylnitroarginine (XI)[18] (1.99 g) in THF (20 ml) containing tri-*n*-butylamine (1.36 ml) and ethyl chloroformate (0.542 ml). The solution was cooled at −10°, and a precooled solution of glutaminyl-β-(pyrazolyl-1)-alanine amide acetate (X) (1.93 g) in DMF (50 ml) containing TEA (0.788 ml) was added. After stirring for 1 hr at room temperature the solvents were removed, and the residue was dissolved in 1-butanol (200 ml). The butanol solution was extracted with six 60-ml portions of 1 *N* ammonium hydroxide and evaporated to dryness. The solid residue was dissolved in hot ethanol, and the precipitate which formed on cooling was collected, washed with ice-cold ethanol, and dried; yield 1.96 g (56%); $[\alpha]^{28}D$ −22.8° (*c* 1.0, DMF); ninhydrin-negative, chlorine-positive spot with R_f^{I} 0.70 and R_f^{VI} 0.54.

Anal. Calcd for $C_{25}H_{35}O_8N_{11}$: C, 48.6; H, 5.7; N, 24.9; O, 20.7. Found: C, 48.9; H, 6.0; N, 25.2; O, 20.3.

b. By the DCC Method. A DMF solution (10 ml) containing glutaminyl-β-(pyrazolyl-1)-alanine amide acetate (X) (0.578 g) and N^{α}-benzyloxycarbonylnitroarginine (XI)[18] (0.760 g) was cooled at 0°, and DCC (0.508 g) was added slowly with stirring. The mixture was stirred at room temperature for 16 hr, the N,N′-dicyclohexylurea was removed by filtration, and the filtrate was evaporated to dryness. The residue was then dissolved in 1-butanol and the product isolated in the manner described above; yield 0.55 g (53%); $[\alpha]^{26}D$ −22.8° (*c* 1.0, DMF); R_f^{I} 0.70; R_f^{VI} 0.58.

N^{α}-Benzyloxycarbonylnitroarginylglutaminyl-β-(pyrazolyl-3)-alanine Amide Ethanol Solvate (XXIV). This material was prepared by the mixed anhydride procedure essentially in the manner described above for preparation of the β-(pyrazolyl-1)-alanine analog. From 1.3 g of glutaminyl-β-(pyrazolyl-3)-alanine amide (XXII) 2.29 g (80%) of the desired product was obtained; recrystallized from ethanol; mp 179–181°; $[\alpha]^{26}D$ −15.0 (*c* 1.0, 90% acetic acid); R_f^{I} 0.80; R_f^{VI} 0.77.

Anal. Calcd for $C_{25}H_{35}O_8N_{11} \cdot C_2H_5OH$: C, 48.9; H, 6.2; N, 23.2; O, 21.7. Found: C, 48.8; H, 6.2; N, 23.8; O, 21.6.

Arginylglutaminyl-β-(pyrazolyl-1)-alanine Amide Diacetate Monohydrate (XIII). The protected tripeptide XII (1.76 g) was hydrogenated in the usual manner in acetic acid–methanol–water (1:2:1) (60 ml); yield 1.60 g (97%); $[\alpha]^{28}D$ −8.7° (*c* 1.0, 10% acetic acid); single chlorine- and ninhydrin-positive spot with R_f^{2} 0.23 and R_f^{3} 1.44 × His.

Anal. Calcd for $C_{17}H_{30}O_4N_{10} \cdot 2CH_3COOH \cdot H_2O$: C, 43.7; H, 7.0; N, 24.3; O, 25.0. Found: C, 43.6; H, 7.2; N, 24.3; O, 24.3.

Arginylglutaminyl-β-(pyrazolyl-3)-alanine Amide Triacetate Tetrahydrate (XXV). The protected tripeptide amide XXIV (2.06 g) was suspended in 50 ml of a mixture of acetic acid–water–methanol (2:2:1) and hydrogenated in the usual manner; yield 1.9 g (89%); $[\alpha]^{28}D$ +3.4° (*c* 1.0, 10% acetic acid); single ninhydrin-, Sakaguchi-, and chlorine-positive spot with R_f^{2} 0.30 and R_f^{3} 1.2 × His.

Anal. Calcd for $C_{17}H_{30}O_4N_{10} \cdot 3CH_3COOH \cdot 4H_2O$: C, 40.0; H, 7.3; N, 20.3; O, 32.4. Found: C, 39.9; H, 7.7; N, 20.8; O, 31.8.

***t*-Butoxycarbonylphenylalanyl-γ-*t*-butylglutamylarginylglutaminyl-β-(pyrazolyl-1)-alanine Amide Diacetate (XV).** This compound was prepared from *t*-butoxyphenylalanyl-γ-*t*-butylglutamic acid hydrazide (XIV)[19] (1.5 g) and the tripeptide diacetate monohydrate XIII (1.59 g) by the Rudinger procedure.[25] (See ref 21b for pertinent examples.) The crude reaction product was distributed between 1-butanol and 2% acetic acid, and the material from the butanol extracts was lyophilized; yield 2.89 g of a material with R_f^{VI} 0.45 contaminated with impurities exhibiting R_f^{VI} values of 0.70 and 0.80, respectively. For purification, a 200-mg sample of the material was dissolved in 100 ml of the solvent mixture 2-propanol–methanol–water (2:2:1), and the solution was added to a Bio-Rex 70 column (1.2 × 30 cm; hydrogen form). The column was eluted first with the same solvent mixture (60 ml), then with 2-propanol–methanol–2% acetic acid (2:2:1); the latter solvent eluted the desired compound. Sakaguchi-positive fractions were pooled, evaporated to a small volume *in vacuo*, and lyophilized; yield 112 mg (59%); $[\alpha]^{27}D$ −21.0° (*c* 0.5, DMF); R_f^{IV} 0.45.

Anal. Calcd for $C_{40}H_{62}O_{10}N_{12} \cdot 2CH_3COOH$: C, 53.3; H, 7.1; N, 17.0; O, 22.6. Found: C, 53.0; H, 7.3; N, 17.0; O, 22.4.

***t*-Butoxycarbonylphenylalanyl-γ-*t*-butylglutamylarginylglutaminyl-β-(pyrazolyl-3)-alanine Amide Diacetate Dihydrate (XXVI).** This compound was prepared from *t*-butoxyphenylalanyl-γ-*t*-butylglutamic acid hydrazide (XIV)[19] (1.88 g) and the tripeptide triacetate tetrahydrate XXV (1.83 g) by the Rudinger procedure.[25] Following distribution between 1-butanol and 2% acetic acid, the butanol phases were evaporated to give 3.5 g of crude product. This material (600 mg) was dissolved in 2-propanol–methanol–water (1:1:1) (300 ml), and the solution was applied to a Bio-Rex 70 column (1.8 × 16 cm; hydrogen form). The column was eluted with the same solvent mixture (150 ml), then the desired peptide was eluted with the solvent system 2-propanol–methanol–5% acetic acid (1:1:1). Sakaguchi-positive fractions were pooled, evaporated to a small volume, and lyophilized; yield 380 mg (68%); $[\alpha]^{26}D$ −8.0° (*c* 1.0, DMF); single chlorine- and Sakaguchi-positive spot with R_f^{I} 0.89 and R_f^{VI} 0.60.

Anal. Calcd for $C_{40}H_{62}O_{10}N_{12} \cdot 2CH_3COOH \cdot 2H_2O$: C, 51.5; H, 7.3; N, 16.4; O, 24.9. Found: C, 51.3; H, 7.5; N, 16.6; O, 24.7.

Phenylalanylglutamylarginylglutaminyl-β-(pyrazolyl-1)-alanine Amide Dihydrate (XVII). Procedure a. The protected pentapeptide XV (968 mg) was dissolved in TFA (15 ml), and the solution was kept at room temperature for 30 min. The TFA was then evaporated and the residue lyophilized several times from small volumes of water. The residue was dissolved in water (100 ml), IRA-400 (50 ml settled in water, acetate cycle) was added, and the suspension was stirred for 30 min. The resin was removed by filtration and washed with 2% acetic acid, and filtrate and washings were evaporated to a small volume and lyophilized; yield 749 mg. For purification this material was dissolved in water (500 ml), and the solution was applied to a DEAE column (3 × 33 cm) which was eluted with water. Ninhydrin-positive fractions were pooled, evaporated to a small volume *in vacuo*, and lyophilized; yield 512 mg (70%); $[\alpha]^{27}D$ −28.0° (*c* 1.0, 10% acetic acid); R_f^{2} 0.45; R_f^{3} 1.8 × His; amino acid ratios in acid hydrolysate: $Phe_{1.00}Glu_{2.13}Arg_{0.95}Pyr(1)Ala_{0.92}NH_{3(1.9)}$; amino acid ratios in 5-hr AP-M digest: $Phe_{1.00}Glu_{1.00}Arg_{1.10}Gln_{0.97}Pyr(1)Ala$ amide$_{0.92}$.

Anal. Calcd for $C_{31}H_{46}O_8N_{12} \cdot 2H_2O$: C, 49.6; H, 6.7; N, 22.4; O, 21.3. Found: C, 49.6; H, 6.6; N, 22.0; O, 20.2.

Procedure b. A DMF solution, cooled at −50°, containing the azide XVI (derived from 1.5 g of *t*-butoxycarbonylphenylalanyl-γ-*t*-butylglutamylnitroarginylglutamine "α-carbobenzoxyhydrazide"[19]) and 1.04 ml of 5.6 *N* hydrogen chloride in dioxane plus *t*-butyl nitrite (0.183 ml) was neutralized at −60° with 0.8 ml of triethylamine and a solution of VII (400 mg) in 3 ml of DMF was added. The mixture was stirred for 1 hr at −20°, for 68 hr at 4°, and finally for 3 hr at room temperature. The solvent was removed *in vacuo*, and the residue was distributed in the usual manner between 2% acetic acid and 1-butanol. The butanol phases were pooled and evaporated to dryness *in vacuo*, and the residue, dissolved in water (1000 ml), was added to a CMC column (3 × 30 cm). The column was eluted with water and 0.005 *M* ammonium acetate, and Sakaguchi-positive eluates were pooled, evaporated to a small volume, and lyophilized to give crude XV; yield 980 mg. This material was deblocked in the usual manner with TFA (20 ml) and trifluoroacetate ions were exchanged for acetate ions with IRA-400. Of the resulting 738 mg of material, a portion (258 mg) was dissolved in a mixture of 2-butanol–3% ammonia (3:1) (25 ml) and added to a DEAE column (3 × 34 cm) which was

(25) J. Honzl and J. Rudinger, *Collection Czech. Chem. Commun.*, **26**, 2333 (1961).

eluted with the same solvent. Eluents containing the desired component (R_f^2 0.45) were pooled and evaporated to dryness, and the residue was lyophilized from water; yield 131 mg (18%); $[\alpha]^{27}D$ −28.0° (*c* 0.5, 10% acetic acid); R_f^2 0.45; R_f^3 1.9 × His; amino acid ratios in acid hydrolysate: $Phe_{1.04}Glu_{2.03}Arg_{0.92}Pyr(1)\text{-}Ala_{1.00}$.

Anal. Calcd for $C_{31}H_{46}O_8N_{12}\cdot 3.5H_2O$: C, 47.9; H, 6.9; N, 21.6; O, 23.7. Found: C, 47.5; H, 6.9; N, 21.1; O, 23.2.

Phenylalanylglutamylarginylglutaminyl-β-(pyrazolyl-3)-alanine Amide Trihydrate (XXVII). The protected pentapeptide XXVI (2.0 g) was dissolved in TFA (20 ml), and the solution was kept at room temperature for 30 min. The TFA was then evaporated, and the residue was lyophilized from small volumes of water. The material was dissolved in water (150 ml) and IRA-400 (100 ml settled in water, acetate cycle) was added, and the suspension was stirred for 30 min. The resin was removed by filtration and washed with 2% acetic acid until the washings were Sakaguchi negative, and filtrate and washings were pooled, evaporated to a small volume, and lyophilized; yield 1.66 g. This material was dissolved in water (500 ml), and the solution was added to a DEAE-cellulose column (3 × 33 cm) which was eluted with water. Sakaguchi-positive fractions were combined, evaporated to a small volume, and lyophilized; yield 1.1 g (71%); $[\alpha]^{24}D$ −19.5° (*c* 1.5, 10% acetic acid); R_f^1 0.35; R_f^3 1.75 × His; amino acid ratios in acid hydrolysate: $Phe_{0.99}Glu_{2.06}Arg_{0.94}Pyr(3)Ala_{1.01}NH_{3(1.87)}$; amino acid ratios in 20-hr AP-M digest: $Phe_{1.03}Glu_{1.05}Arg_{0.97}Gln_{0.91}Pyr(3)Ala_{1.05}\text{-}NH_{3(0.73)}$.

Anal. Calcd for $C_{31}H_{46}O_8N_{12}\cdot 3H_2O$: C, 48.4; H, 6.8; N, 21.9; O, 22.9. Found: C, 47.9; H, 7.1; N, 21.4; O, 22.8.

N^{α},N^{ϵ}-Di-*t*-butoxycarbonyllysyl-γ-*t*-butylglutamylthreonylalanylalanylalanyl-N^{ϵ}-*t*-butoxycarbonyllysylphenylalanylglutamylarginylglutaminyl-β-(pyrazolyl-1)-alanine Amide (XIX). N^{α},N^{ϵ}-Di-*t*-butoxycarbonyllysyl-γ-*t*-butylglutamylthreonylalanylalanylalanyl-N^{ϵ}-*t*-butoxycarbonyllysine azide (XVIII),[20] prepared from 495 mg of the hydrazide dissolved in ice-cold DMF (10 ml), was added to an ice-cold solution of the pentapeptide XVII (154 mg) in DMF (10 ml) containing TEA (0.03 ml). The mixture was stirred for 3 hr at 0° and for 20 hr at 4° when a second portion of azide (from 495 mg of hydrazide) was added. Stirring was continued for 20 hr at 4° and for 3 hr at room temperature when the solvents were removed *in vacuo*. The residue was dissolved in 1-butanol (100 ml), and the solution was extracted with 2% acetic acid. The butanol phases were evaporated and the residue lyophilized from water–dioxane; yield 1.10 g. This material dissolved in a mixture of 2-propanol–methanol–water (1:1:1) (950 ml) was added to a Bio-Rex 70 column (3 × 35 cm; hydrogen form) which was washed with the same solvent system (300 ml), then with a mixture of 2-propanol–methanol–2% acetic acid (1:1:1). Sakaguchi-positive fractions were pooled and evaporated *in vacuo*, and the residue was lyophilized from water–dioxane; yield 427 mg; $[\alpha]^{28}D$ −23.5° (*c* 0.5, DMF); R_f^{VI} 0.58 with minor chlorine-positive impurities with R_f^{VI} 0.70 and 0.48, respectively; amino acid ratios in acid hydrolysate: $Lys_{2.06}Glu_{2.09}[Thr + Pyr(1)Ala]_{1.95}Ala_{3.05}Phe_{0.86}Arg_{0.88}NH_{3(2.32)}$.

Lysylglutamylthreonylalanylalanylalanyllysylphenylalanylglutamylarginylglutaminyl-β-(pyrazolyl-1)-alanine Amide Octahydrate (V). The protected dodecapeptide amide XIX (426 mg) was deblocked with TFA at room temperature in the usual manner and TFA ions were exchanged for acetate ions with Amberlite IRA-400 (acetate cycle). The resulting product (352 mg) was dissolved in water (100 ml), and the solution was added to a CMC column (2.2 × 55 cm) which was eluted with the following ammonium acetate (*M*) solutions: 0.03 (150 ml), 0.04 (150 ml), 0.05 (250 ml), and 0.06 (250 ml). The 0.06 *M* eluates were analyzed by paper chromatography in the pyridine system and fractions containing the desired compound (R_f^3 0.87 × His) were pooled, desalted on a column of Bio-Rex 70 (1.8 × 6 cm; hydrogen form), and lyophilized; yield 119 mg (33%); $[\alpha]^{25}D$ −66.0° (*c* 0.05, 10% acetic acid); R_f^2 0.25; R_f^3 0.87 × His; single ninhydrin-, chlorine-, and Sakaguchi-positive spot; amino acid ratios in acid hydrolysate: $Lys_{1.94}Glu_{3.22}Thr_{0.94}Ala_{2.97}Phe_{1.00}Arg_{1.09}Pyr(1)Ala_{0.94}$; amino acid ratios in 5-hr AP-M digest: $Lys_{2.07}Glu_{2.03}Thr_{1.00}Ala_{3.07}Phe_{1.00}\text{-}Arg_{1.00}Gln_{0.93}Pyr(1)Ala\ amide_{0.86}$.

Anal. Calcd for $C_{61}H_{99}O_{18}N_{21}\cdot 8H_2O$: C, 47.0; H, 7.4; N, 18.9; O, 26.7. Found: C, 46.7; H, 7.6; N, 18.8; O, 26.0.

Lysylglutamylthreonylalanylalanylalanyllysylphenylalanylglutamylarginylglutaminyl-β-(pyrazolyl-3)-alanine Amide (VI). The protected dodecapeptide amide XXVIII was prepared from N^{α},N^{ϵ}-di-*t*-butoxycarbonyllysyl-γ-*t*-butylglutamylthreonylalanylalanylalanyl-N^{ϵ}-*t*-butoxycarbonyllysine azide[20] (from 600 mg of the hydrazide) and the pentapeptide XXVII (160 mg) in DMF in the manner described above for preparation of the Pyr(1)Ala analog; yield 278 mg (73%); R_f^1 0.93; R_f^{VI} 0.70 with minor impurities with R_f^{VI} 0.57 and 0.83, respectively. This material (278 mg) was deblocked with TFA at room temperature for 25 min. Trifluoroacetate ions were exchanged for acetate ions in the usual manner. The resulting product (232 mg) was dissolved in water (100 ml), and the solution was added to a CMC column (2.2 × 55 cm) which was eluted with the following ammonium acetate (*M*) solutions: 0.03 (100 ml), 0.04 (100 ml), 0.05 (250 ml), 0.06 (250 ml), and 0.07 (250 ml). Fractions from the 0.07 *M* eluates which contained the desired material (R_f^3 0.7 × His) were pooled, desalted on a column of Bio-Rex 70 (1.8 × 5 cm; hydrogen form), and lyophilized; yield 74 mg; $[\alpha]^{25}D$ −63.3° (*c* 1.0, water); R_f^2 0.30; R_f^3 0.7 × His; ninhydrin-, chlorine-, and Sakaguchi-positive spot; amino acid ratios in acid hydrolysate: $Lys_{2.02}Glu_{3.02}Thr_{0.90}Ala_{3.20}Phe_{0.96}\text{-}Arg_{0.92}Pyr(3)Ala_{0.97}NH_{3(2.10)}$; amino acid ratios in 20-hr AP-M digest: $Lys_{1.95}Thr_{1.01}Glu_{2.12}Ala_{3.28}Phe_{0.95}Arg_{1.01}Gln_{0.83}Pyr(3)Ala_{0.86}\text{-}NH_{3(1.07)}$.

Discussion

In this and previous studies we have resorted to chromatography on CMC for purification of peptides using ammonium acetate solutions of increasing ionic strength for elution. Removal of the ammonium acetate from the final products was accomplished by the very laborious process of repeated lyophilization. In the course of this work we have employed the ion exchanger Bio-Rex 70 for rapid desalting of basic peptides. This resin retains basic amino acids and peptides considerably firmer than CMC, *i.e.*, ammonium acetate of higher ionic strength is required for elution. Effluents from CMC columns containing the desired peptides plus ammonium acetate were passed through Bio-Rex 70, and the salt was removed by exhaustive washing with water. Elution of the salt-free peptides was readily accomplished with freshly prepared 1% aqueous ammonium hydroxide.

During evaluation of the stereochemical homogeneity of compounds V, VI, XVII, and XXVII it was observed that 5-hr digests of the materials containing Pyr(1)Ala amide contained, in addition to the other expected amino acids, Pyr(1)Ala amide rather than the free amino acid. This was not the case in the Pyr(3)Ala amide series of compounds where Pyr(3)Ala was recovered quantitatively.

To resolve this discrepancy, the rates of hydrolysis of Pyr(3)Ala amide and Pyr(1)Ala amide with AP-M were investigated with the results shown in Figure 1. The widely different rates of hydrolysis of these simple amides account for the results obtained with the more complex compounds. There is at present no obvious explanation for the markedly different behavior toward AP-M of these closely related amino acid amides.

Among conceivable histidine substitutes the β-pyrazolylalanines command considerable interest. Pyrazole, like imidazole, is a five-membered planar aromatic ring system which contains two nitrogens. Its molecular dimensions are very similar, if not identical, with those of imidazole, and the geometry of histidine and the pyrazolylalanines is the same. However, the different spacing of the nitrogens endows the two ring systems with remarkably different acid–base properties. The p*K* of the imidazole portion of histidine is 5.97[26] in contrast to the p*K*'s of the pyrazole por-

(26) J. P. Greenstein and M. Winitz, "The Chemistry of the Amino Acids," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1961, p 486.

tion of Pyr(1)Ala andPyr (3)Ala which are 2.2 and ~2.1, respectively.[27,28]

While causing a minimal alteration in the stereochemistry pyrazole–imidazole replacements appear to provide a means to assess the role of ionization in function of biologically active peptides.

We have evaluated in a systematic manner the relation between chain length and ability to regenerate active enzymes with S-protein of a series of S-peptide fragments.[6,21b] Removal of amino acid residues 15–20 had no effect on the protein-activating potential. Further shortening of the peptide chain from either the C- or N-terminus resulted in a decrease of activity. Only in one instance however was complete deactivation observed, namely when the histidine residue in position 12 was missing. On the basis of this and other evidence[8] it was concluded that histidine 12 is vital for enzymatic function. Two possible explanations come to mind for the role of this histidine. By virtue of its aromatic character it may make a profound contribution to the binding of the peptides to S-protein or because of its unique acid–base behavior it may be essential for function.

The dodecapeptide amides V and VI were synthesized in order to distinguish between these possibilities. We have added increasing amounts of peptide VI to S-protein up to peptide–protein ratios of 1500:1 without observing any activation. Similarly peptide V at ratios as high as 5500:1 was without effect. Thus, these two pyrazolylalanines cannot function in lieu of histidine 12 in the S-peptide–S-protein system. It remains to be determined whether this lack of activity is attributable to inability to function catalytically, inability to bind, or both.

(27) F. Schneider and W. Schaeg, *Z. Physiol. Chem.*, 327, 74 (1962).
(28) In our laboratory we obtained p*K* values of 2.2 and 2.5 for the two pyrazolylalanines.

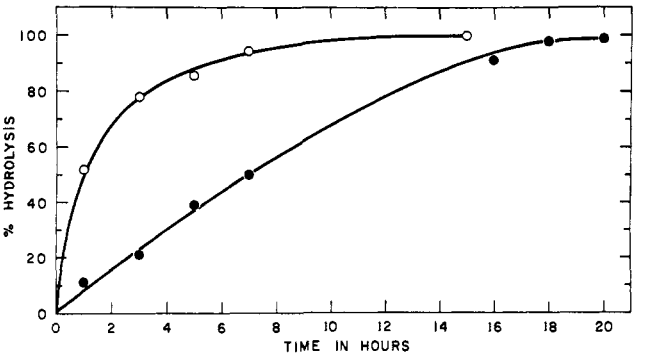


Figure 1. Rates of hydrolysis of β-(pyrazolyl-1)-L-alanine amide, ●, and β-(pyrazolyl-3)-L-alanine amide, ○, by aminopeptidase M. For experimental conditions see ref 21b.

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2-Multiprenylphenols and 2-Decaprenyl-6-methoxyphenol, Biosynthetic Precursors of Ubiquinones[1]

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Abstract: In the biosynthetic sequence of the conversion of *p*-hydroxybenzoic acid to ubiquinone, two intermediates have been isolated in pure form and structurally elucidated. These two compounds, 2-decaprenylphenol and 2-decaprenyl-6-methoxyphenol, are in the pathway to ubiquinone-10 of *Rhodospirillum rubrum*. Two other 2-multiprenylphenols, 2-tetraprenylphenol and 2-nonaprenylphenol, were also isolated and characterized. 2-Nonaprenylphenol is a precursor of ubiquinone-9 which is also known to be biosynthesized by *R. rubrum*. However, 2-tetraprenylphenol is somewhat surprising as a naturally occurring lower homolog; the corresponding ubiquinone-4 has not yet been reported from any source. Ubiquinone-4 may be a trace constituent of *R. rubrum*, but is as yet undetected. Radioactivity incorporation experiments have shown that all four of these 2-multiprenylphenols as isolated from *R. rubrum* are derived from *p*-hydroxybenzoic acid. Although none of the tocopherols or plastoquinones has been isolated from *R. rubrum*, the isolation of 2-tetraprenylphenol, showing its existence in nature, may forecast its precursor relationship to the tocopherols and plastoquinone-4 in other living systems.

Four new biosynthetic precursors of ubiquinone have been isolated from the photosynthetic bacterium *Rhodospirillum rubrum* and structurally elucidated as 2-decaprenylphenol (III, n = 10),[5,6] 2-nonaprenylphenol (III, n = 9), 2-tetraprenylphenol (III,

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(4) Research Career Awardee, GM-K6-993, U. S. Public Health Service.

(5) The nomenclature in this paper is based on a recommendation of an IUPAC–IUB Commission of Biochemical Nomenclature, *Biochim. Biophys. Acta*, 107, 5 (1965). 2-Decaprenylphenol is also named 2-(3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl)phenol (*Chemical Abstracts*) or 2-[3′-methyl-2-butenylenakis(3′-methyl-2′-butenylene)]phenol (IUPAC).
(6) R. K. Olsen, J. L. Smith, G. D. Daves, Jr., H. W. Moore, K. Folkers, W. W. Parson, and H. Rudney, *J. Am. Chem. Soc.*, 87, 2298 (1965).