parameters given in Tables I and II of *Reference 1*. The results are shown here in Tables I and II. The probabilities for the NF XIII and USP XVII plans as previously reported are included in parentheses. (The new values are taken from tabled probabilities for all combinations of the four parameters where δ_p, δ_y go from -0.10 to 0.10 in steps of 0.01 and γ_p, γ_y go from 0 to 0.06 in steps of 0.005.)

As may be seen in Tables I and II, the probability of passing a lot, when the coefficients of variation increase, is greater for the new plan than for the old.

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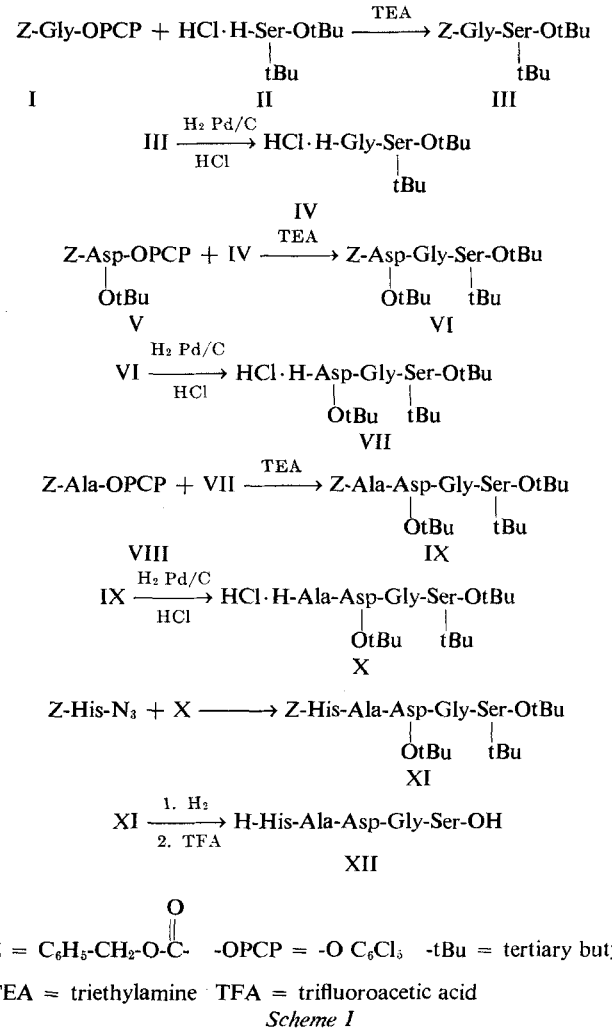
Enzyme-Active Sites: Importance of Aspartic Acid in Peptide Esterase Models

Keyphrases □ Peptide esterase models, catalytic activity—aspartic acid residue influence □ Proteolytic enzyme active sites—peptide catalytic activity determination



Scheme I

Sir:

Recent investigations point out that the serine proteinases, notably chymotrypsin and trypsin, require serine and histidine residues in some type of close steric relationship for their catalytic activity. While there are 246 amino acids in the total sequence of chymotrypsinogen, the inactive precursor of chymotrypsin, the suitable folding of this enzyme brings two histidines from positions 40 and 57 and one serine from position 195 close enough to act as the active center responsible for catalyzing the hydrolysis. Trypsin affords the same possibilities, where two histidines from positions 29 and 46 and a serine from position 183 act as the active site of the enzyme (1, 2).

One approach to studying the active sites of enzymes is the synthesis and evaluation of the catalytic activity of relatively simple peptides that embody as many known features of proteolytic enzymes as possible. A number of polymers and copolymers of histidine and serine (3) and small peptides incorporating histidine and serine were reported (4, 5). Photaki and Moschopedis (6) carried out the same type of studies with cysteine proteinases with peptides incorporating histidine and cysteine. A relatively more potent esterase model, L-histidyl-glycyl-L-aspartyl-L-seryl-L-phenylalanine, was reported recently (7). The catalytic coefficient (3) for this pentapeptide was 179 l. mole^{-1} min.$^{-1}$ compared with 147 l. mole^{-1} min.$^{-1}$ for L-seryl-γ-aminobutyryl-L-histidyl-γ-aminobutyryl-L-aspartic acid (5) and 10^4 l. mole^{-1} min.$^{-1}$ for α-chymotrypsin (3). Although the catalytic coefficient of these peptides, when compared with chymotrypsin, is considerably low, further evaluation is definitely warranted of a series of peptides where the molecule can be made more flexible. Moreover, the role played by individual amino acids in the peptide esterase models should be studied.

Among a number of peptides subjected to the catalytic activity determination, the peptides incorporating histidine and serine without aspartic acid showed very little or no catalytic activity. Examples are: L-histidyl-L-alanyl-glycyl-L-serine (catalytic coefficient 15 l. mole^{-1} min.$^{-1}$), L-histidyl-glycyl-L-tyrosyl-L-serine (catalytic coefficient 21 l. mole^{-1} min.$^{-1}$), and glycyl-L-histidyl-glycyl-L-serine (catalytic coefficient 17 l. mole^{-1} min.$^{-1}$).

In this communication, we report the importance of aspartic acid in the peptide esterase models. A comparative study of catalytic activity of peptides incorporating histidine and serine, differing only in the aspartic acid residue, was conducted. To provide more or less the same physicochemical environments, the comparison was made with the peptide incorporating glutamic acid instead of aspartic acid. The

pentapeptide, L-histidyl-glycyl-L-glutamyl-L-seryl-L-phenylalanine, had a much lower catalytic activity (93 l. mole^{-1} min.$^{-1}$) when compared with the corresponding pentapeptide containing aspartic acid in the sequence. The same observation was true in the case of two tetrapeptides, L-histidyl-L-aspartyl-glycyl-L-serine (catalytic coefficient 150 l. mole^{-1} min.$^{-1}$) and L-histidyl-L-glutamyl-glycyl-L-serine (catalytic coefficient 63 l. mole^{-1} min.$^{-1}$). The importance of aspartic acid in the peptide esterase models was further confirmed by comparing two pentapeptides, L-histidyl-L-alanyl-L-aspartyl-glycyl-L-serine (catalytic coefficient 210 l. mole^{-1} min.$^{-1}$) and L-histidyl-L-alanyl-L-glutamyl-glycyl-L-serine (catalytic coefficient 87 l. mole^{-1} min.$^{-1}$). The increased catalytic activity of peptide esterase models containing aspartic acid may be justified on the basis that, in the case of chymotrypsin, aspartic acid occupies position 194, which is adjacent to the postulated active serine at position 195. The same is true of trypsin, where aspartic acid is at position 184 adjacent to active serine at position 183.

The catalytic activity of the peptide esterase models was determined by the liberation of *p*-nitrophenol from *p*-nitrophenylacetate following the procedure used by Sheehan *et al.* (4). The synthesis of the pentapeptide, L-histidyl-L-alanyl-L-aspartyl-glycyl-L-serine, which has the highest catalytic coefficient of reported peptide esterase models, is outlined in Scheme I. To limit the degree of racemization, the peptide chains were extended from the C-terminal residues of amino acids, and all peptide bonds, except histidine, were formed using the pentachlorophenyl active ester method (8, 9). Histidine residues were incorporated in the peptide chain using the azide method (10). All the protecting groups used for unreacting functionalities during the reaction sequences were acid labile or removable by hydrogenolysis. This approach excluded the use of alkali treatment during the synthesis, thereby further limiting the degree of racemization and problems of transpeptidation (11). The elemental analyses of the intermediates and final peptides reported in this work were within experimental tolerance, and they were homogeneous to paper chromatography and paper electrophoresis under a variety of conditions.

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BOOKS

REVIEWS

Antitussive Agents, Volumes I, II, and III. *Section 27, International Encyclopedia of Pharmacology and Therapeutics.* Edited by H. SALEM and D. M. AVIADO, Pergamon Press Inc., Maxwell House, Fairview Park, Elmford, NY 10523, 1970. li + 833 pp. 16 × 23.5 cm.

All but three of the 15 chapters in this three-volume series are authored by the editors. In this ambitious undertaking, the author-editors have extensively reviewed the pharmacological and clinical literature pertaining to antitussives up through 1966 and systematically organized their findings into a readily usable format. The first portion of Volume I, an exhaustive tabular listing of patents issued for antitussive compounds, should be of great assistance to medicinal chemists as well as patent lawyers interested in searching for new chemical entities with this activity. The 453 entries include names of consignees, countries issuing, and references. A comprehensive review of antitussive testing methods in humans and animal species is also presented in tabular form. Pharmacologists should find the 100 referenced entries most useful because each includes information about the cough stimulus used, the method of assessing the cough, as well as other pertinent specifications. The last part of Volume I consists of two chapters. The first discusses physiology of the cough reflex as a basis for understanding factors causing cough and pharmacologic approaches to alleviating cough. The last chapter is an interesting historical review of the changing concepts of causes and approaches to treating pathological cough.

Volumes II and III of this series present a review of the experimental and clinical literature about specific antitussive compounds. Volume II deals with derivatives of opium (three chapters on codeine, *d*-methorphan, and miscellaneous opiates) and four chapters on classes of nonopiates with central action. Volume III considers nonopiates with peripheral actions (chapters on local anesthetics, bronchodilators, expectorants, and mucolytic agents). The format of each chapter includes chemistry and dosage, pharmacodynamics, and clinical usefulness of the compound in question, as well as a tabular listing of all domestic and foreign commercially available preparations containing those particular compounds.

The carefully designed format of these three volumes provides a comprehensive look at the presently available experimental and clinical information about antitussive compounds. Areas needing further study are clearly identified so that these volumes provide a