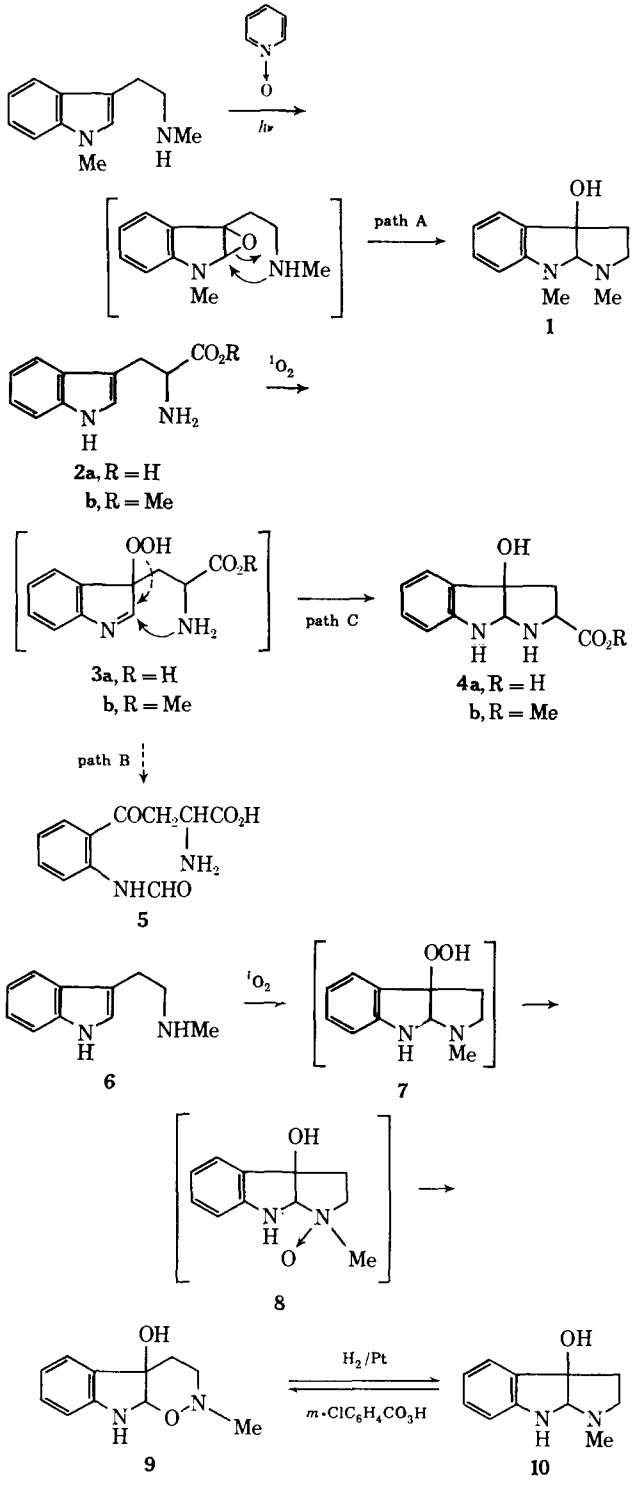

1

2a, R = H
b, R = Me

3a, R = H
b, R = Me

4a, R = H
b, R = Me

5

6

7

8

9

10

of easy rearrangement of **8** to **9** at room temperature.[14] The oxidation of physostigmine by hydrogen peroxide and rearrangement to geneserine has been reported.[15] The isolation of **4b** and **9** provides new evidence for 3a-hydroperoxyindolenines as intermediates in the reaction of tryptophan derivatives with singlet oxygen.

The fact that both dioxygenase and monooxygenase model reactions produce the 3a-hydroxyhexahydropyrrolo[2,3-*b*]indole ring system[16] suggests that the hydroxyl group at the **3a** position in the sporidesmins and brevianamides most likely arises biogenetically *via* path A or path C. Kynurenine derivatives were not formed under our reaction conditions.

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Sulfite Esterase Activity of Pepsin Modified at Active Site Carboxyl Groups

Sir:

Since the discovery was made that pepsin catalyzes the hydrolysis of sulfite esters,[1] the following lines of evidence have been adduced in support of the hypothesis that the active site requirements for the sulfite esterase action of the enzyme are the same as those for its peptidase action. (a) Peptides which bind to the active site of pepsin have been shown to act as competitive inhibitors toward the sulfite esterase activity of the enzyme, and the inhibition constants obtained with these peptides correspond closely with the Michaelis constants calculated from their pepsin-catalyzed hydrolysis.[1,2] (b) The diazocarbonyl reagent *N*-diazoacetyl-D,L-norleucine methyl ester which is known to inactivate pepsin as a peptidase[3] has also been reported to cause the inactivation of the enzyme as a catalyst for the hydrolysis of diphenyl sulfite and methyl phenyl sulfite at pH 2.[1] (c) The pH dependency of the rate parameter k_{cat}/K_m for the pepsin-catalyzed hydrolysis of the reactive sulfite ester substrate bis-*p*-nitrophenyl sulfite[4] corresponds fairly closely to the pH dependency of this parameter for the hydrolysis of the neutral dipeptide *N*-acetyl-L-phenylalanyl-L-phenylalanylamide.[5]

We now wish to report our discovery that pepsin modified by treatment with either the diazoketone α-diazo-*p*-bromoacetophenone (I) in the presence of cupric ion[6] or the epoxide 1,2-epoxy-3-(*p*-nitrophenoxy)-propane (II)[7,8] at pH 5 and 25°, retaining less than 1% activity toward the peptide substrate hemoglobin,[9] remains very active over a range of pH values as a catalyst for the hydrolysis of a variety of symmetrical and unsymmetrical sulfite ester substrates, including bis-*p*-nitrophenyl sulfite (III), phenyl *p*-nitrophenyl sulfite (IV), and methyl *p*-nitrophenyl sulfite (V).

Pseudo-first-order kinetics were obtained, and no evidence for enantiomeric specificity was seen when the rates of hydrolysis of the latter sulfite ester catalyzed by

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pepsin modified by I (pepsin M-I) or by II (pepsin M-II) were examined at 25.0° from pH 3.05 to 5.8, under conditions of enzyme in excess, following *p*-nitrophenol release at 320 nm with a Cary 15 spectrophotometer. Measurements at 430 nm on the reduction of *o*-chloranil by inorganic sulfite[4b] generated by the solvolysis of V catalyzed by pepsin M-I at pH 3.6 and 3.75 and catalyzed by pepsin M-II at pH 3.5 indicated that the rates of inorganic sulfite formation corresponded to those of *p*-nitrophenol release. Proceeding from more acidic to less acidic reaction conditions and monitoring *p*-nitrophenol release, the k_{cat}/K_m values for the action of the modified pepsin species on V showed ascending sigmoidal pH dependencies. The pK_a values of the ionizing groups on the enzyme which are reactive in their basic forms and which appear to be responsible for this behavior are 3.9 in the case of pepsin M-I and 4.3 for pepsin M-II, and the $(k_{cat}/K_m)_{lim}$ values are $(9.1 \pm 2.4) \times 10^2\ M^{-1}\ sec^{-1}$ and $(1.7 \pm 0.2) \times 10^3\ M^{-1}\ sec^{-1}$, respectively.

In contrast to the observations with the modified pepsin species, native pepsin shows marked enantiomeric specificity when it reacts with racemic IV and V. Over a considerable acidic pH range, with the native enzyme in excess, observation of the release of *p*-nitrophenol reveals that the rates of hydrolysis of these compounds show biphasic character. The rate data can be analyzed in terms of a fast and a slow reaction, both following pseudo-first-order rate laws and accounting, respectively, for 50% of the decomposition of the ester. Measurements on the hydrolysis of racemic V at pH 4.0 (acetate buffer, $\mu = 0.05$), for instance, using enzyme concentrations ranging from 2×10^{-4} to $1.1 \times 10^{-3}\ M$ gave values of $k_{cat}/K_m = 2.5 \times 10^3\ M^{-1}\ sec^{-1}$ for the fast reaction and $4.5 \times 10^2\ M^{-1}\ sec^{-1}$ for the slow reaction. In marked contrast to our findings on the reaction of V with modified pepsin, measurements on the variation of k_{cat}/K_m for the pepsin-catalyzed hydrolysis of the fast-reacting isomer of V indicated that, as in the enzymatic hydrolysis of III,[4a] this parameter decreases at the higher pH values.[10]

While sequencing information concerning the site in pepsin which reacts with I is incomplete, the stoichiometric modification of the enzyme by several other diazocarbonyl compounds has been determined to occur at the β-carboxyl group of an active site aspartate residue[11-13] in the sequence Ile-Val-Asp-Thr-Gly-Thr-Ser-Leu, and it seems reasonable to assume that this is the residue with which I also reacts.[14] A different active site aspartate group which is in the sequence Ile-Phe-Asp-Thr-Gly-Ser-Ser-Asn is modified by II (revised sequence, privately communicated to us by Professor J. Tang).

(10) In the case of the native pepsin-catalyzed hydrolysis of III, it was found that the values of k_{cat}/K_m decreased when an enzyme-bound group with pK_a = 5.2 lost a proton.[4a]

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(14) Professor B. F. Erlanger has informed us (private communication) that two peptide fragments containing the aspartate residue which reacts with I have been isolated. In addition to aspartate, one includes isoleucine and valine, and the other contains threonine and valine. These observations are consistent with the assignment of the site modified as the aspartate group which reacts with other diazocarbonyl compounds.[11-13]

Our results with pepsin M-I and pepsin M-II demonstrate that neither of the two aspartate β-carboxyl groups in pepsin which are believed on the basis of the chemical modification data to be important active site residues in the enzyme's peptidase action is essential to the catalytic action of the enzyme on sulfite ester substrates. The k_{cat}/K_m–pH profiles obtained with pepsin M-I and pepsin M-II suggest strongly that only a single active site carboxylate group is necessary for the enzymatic hydrolysis of sulfite esters.[15] Furthermore, although the k_{cat}/K_m–pH dependencies for the action of the modified pepsins and of pepsin itself on V are quite different, the $(k_{cat}/K_m)_{lim}$ values found in our work with the former species lie between the values obtained for the hydrolysis of the fast- and slow-reacting enantiomers of V catalyzed by the native enzyme.

A reasonable interpretation of our observations on the action of modified pepsins on sulfite esters is that the carboxylate group required for pepsin's sulfite esterase activity differs from those necessary for peptidase activity in spite of the evidence that the active sites for the two types of activities overlap. It must be pointed out, however, that the elimination of peptidase activity by the esterification of either of the aspartate β-carboxyl groups modified in pepsin by I or by II does not constitute positive proof that these groups in their unmodified forms are catalytic participants in the peptidase action of the enzyme. The possibility remains that although esterification of these carboxylate functions blocks the peptidase and not the sulfite esterase action, the carboxylate group crucial to the hydrolysis of sulfite esters is one of those required for the action of the enzyme on peptides.[16] Further studies which should clarify the interpretation and the implications of our discovery that pepsin M-I and pepsin M-II can function as catalytically active species are in progress in our laboratory.

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(15) Despite the apparent dependence of the rate parameter k_{cat}/K_m for the pepsin-catalyzed hydrolysis of bis-*p*-nitrophenyl sulfite (III) on the state of ionization of two carboxyls in the native enzyme,[4] only one of these may be directly involved in the catalytic process while the ionization of the other could be reflected in the kinetics of reaction primarily because of its inhibitory effect on catalysis.

(16) If this postulate were correct, then the differences between the k_{cat}/K_m–pH profiles for the action of pepsin M-I and pepsin M-II on V and the action of the native enzyme on a variety of substrates (J. S. Fruton in "The Enzymes," Vol. III, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N. Y., 1971, pp 120–164) would indicate that esterification of a carboxyl group in the vicinity of the active site causes a substantial alteration in the pK_a for the ionization of the catalytically active carboxylate function.

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Reactions of Tungsten and Molybdenum Atoms with 1,3-Butadiene. Tris(butadiene)tungsten and -molybdenum

Sir:

In studying the reactions of atomic tungsten and atomic molybdenum in the condensed phase, we have found that they react readily with a variety of olefinic