chlorobenzene (1.33 g) at 130° for 24 hr gave 48.3 mg (80%) of triptycene, and about 5 mg of triptoic acid.

Decarbonylation of Triptycene-1-carboxaldehyde.—Typically 63.0 mg of aldehyde, 59.5 mg of *t*-butyl peroxide, and 4.1815 g of carbon tetrachloride were degassed, sealed, and held at 130° for 24 hr, the volatile products removed by bulb-to-bulb distillation, 1 ml of methanol added, and the mixture let stand 48 hr before vpc analysis. Standard mixtures of chlorotriptycene and methyl triptoate were used to obtain the ratio of these two products; k_1/k_2 was calculated by the method of Applequist and Kaplan.

Attempted Decarbonylation of Benzaldehyde.—Using freshly distilled benzaldehyde, the product after the methanol treatment contained no chlorobenzene to the limits of vpc analysis. Methyl benzoate was formed in high yield.

Registry No.—1, 21343-33-9.

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The Use of Acetylenic Substrates in Enzyme Chemistry. Reaction of the Active Site of α-Chymotrypsin with N-Phenylpropioloylimidazole

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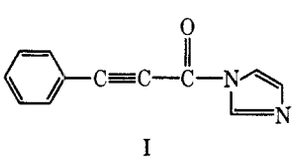
A new series of acetylenic substrates has been synthesized in this laboratory for the purpose of probing the reactions of a variety of enzymes. As an example of the wide applicability of the acetylenic substrates, the chromophoric compound N-phenylpropioloylimidazole (I) is introduced in the present note as a substrate for α-chymotrypsin, which can be employed for the direct spectrophotometric assay of this proteolytic enzyme.



I

We have found that I reacts very rapidly and stoichiometrically with chymotrypsin in an acylation step to give N-phenylpropioloyl-α-chymotrypsin. This reaction is followed by a deacylation step sufficiently slow to render I serviceable as an active site titrant.

Since there is no dearth of active site titrants for α-chymotrypsin,[2] the demonstration that N-propioloylimidazole rapidly acylates chymotrypsin is important, not only because it has allowed us to use I as an active site titrant, but also since it will permit us to delve further into the specificity of the active site. Considerable literature[3] exists on the reactions of olefinic substrates such as cinnamoylimidazole and cinnamate esters with chymotrypsin, and we can now embark on a thorough comparison of these reactions with those of acetylenic substrates, which obviously have a very different geometry. From such a comparison, a better picture of the stereochemistry of chymotrypsin's active site should emerge.[4]

Experimental Section

N-Phenylpropioloylimidazole (I) was synthesized by the reaction of N-phenylpropioloyl chloride with imidazole in benzene. After recrystallization from cyclohexane, I had mp 118–119° dec.

Anal. Calcd for $C_{12}H_8N_2O$: C, 53.63; H, 3.00. Found: C, 53.42; H, 3.14.

Titration of the Active Site of Chymotrypsin with I.—A wavelength of 310 mμ was determined to be most convenient for the spectrophotometric titration measurements. The stock solutions of N-propioloylimidazole were 1×10^{-2} *M* in this substrate and were prepared in acetonitrile which had been distilled over P_2O_5. Stock solutions of chymotrypsin in 0.05 *M* acetate buffer of pH 5.06 which were approximately 1×10^{-3} *M* in the enzyme were generally used.

In a typical experiment, 3 ml of 0.05 *M* acetate buffer was first placed in a 1-cm path-length spectrophotometer cell. After adjusting the base line of the spectrophotometer, 25 μl of the substrate stock solution was added and the absorbance of the resultant solution at 310 mμ was determined. Then 100 μl of the enzyme stock solution was added, and a sharp drop in the absorbance at 310 mμ was observed, which is ascribed to the consumption of the substrate by its reaction with the enzyme, leading to the formation of the acyl-enzyme, N-phenylpropioloyl-α-chymotrypsin. Finally, a slow further decrease in the absorption was seen due to the slow deacylation of the acyl-enzyme with attendant additional consumption of the substrate.

Using the above experimental procedure, the normality of enzyme-active sites in the reaction solution can be calculated from eq 1. In this equation, A_1 is the absorbance of the substrate solution at the time of addition of the enzyme. A_2 is the absorbance of the solution resulting after the addition of the enzyme, extrapolated back to the time of addition of the enzyme. A_3 is the absorbance of a solution prepared by adding 100 μl of the enzyme stock solution to 3.0 ml of 0.05 *M* acetate buffer at pH 5.06.

$$\text{normality} = \frac{0.968A_1 + 0.992A_3 - A_2}{250.2} \qquad (1)$$

Duplicate titrations with N-propioloylimidazole (I) agree to ±2%, a precision comparable to that obtained with the excellent chymotrypsin active site titrants, cinnamoylimidazole[5] and 5-nitro-1,2-benzoxathiole 2,2 dioxide.[6] Furthermore, excellent agreement was found between the normalities of chymotrypsin solutions determined by titration with I or with cinnamoylimidazole.

Registry No.—I, 21473-06-3.

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(2) M. L. Bender, M. L. Begue-Canton, R. L. Blakely, L. J. Brubacher, J. Feder, C. R. Gunter, F. J. Kezdy, J. V. Kilheffer, Jr., T. H. Marshall, C. G. Miller, R. W. Roeske, and J. K. Stoops, *J. Amer. Chem. Soc.*, **88**, 5880 (1966).

(3) M. L. Bender and F. J. Kezdy, *Ann. Rev. Biochem.*, **34**, 49 (1965).

(4) We have found that acetylenic substrates are useful reagents for other enzymes as well. For example, *O*-phenylpropioloyl-DL-β-phenylacetic acid has been synthesized and has been shown to be a reactive substrate for the action of carboxypeptidase A. (Unpublished studies of B. L. Kaiser and N. Latif).

(5) G. R. Schonbaum, B. Zerner, and M. L. Bender, *J. Biol. Chem.*, **236**, 2930 (1961).

(6) J. H. Heidema and E. T. Kaiser, *Chem. Commun.*, 300 (1968).