suggestion is supported by preliminary data obtained with copolypeptides of helix-forming and β-forming amino acids.[14]

(14) R. K. Kulkarni, G. D. Fasman and E. R. Blout, to be published.

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RECEIVED JUNE 27, 1960

THE NATURE OF THE SPECTRAL CHANGES ACCOMPANYING THE DEACYLATION OF MONOACETYL-α-CHYMOTRYPSIN[1]

Sir:

Evidence was presented[2] which indicated that two stable monoacetyl-α-chymotrypsin intermediates,[3] AC-I and AC-A, existed in the α-chymotrypsin catalyzed hydrolysis of *p*-nitrophenyl acetate and that AC-I was the direct intermediate in the catalytic reaction. Spectroscopic studies did not reveal acetyl-imidazolyl as intermediate in the deacylation of AC-I.[2] However, acetyl-imidazolyl has been implicated in the deacylation of monoacetyl-δ-chymotrypsin.[4] The difference spectrum of this acetyl enzyme at *p*H 8.9, where it deacylates, *versus* *p*H 3.5, showed a rapid increase in absorption at 245 mμ and a spectral peak with a maximum near 245 mμ.[4] It was noted[4] that the position of the peak and the rate of decrease in absorbancy are characteristic of acetyl-imidazole and its rate of hydrolysis. The nature of these spectral changes is an important consideration in attempts to understand chymotrypsin catalyzed reactions[5] and is the subject of this communication.

The difference spectra of AC-A, AC-I, monoacetyl-δ-chymotrypsin and, *significantly*, *α-chymotrypsin* at *p*H 9.0 *versus* *p*H 3.5 all show a maximum at approximately 245 mμ (Fig. 1). The *p*H dependence of ΔD at 245 mμ indicates that the principal component of these difference spectra is due to tyrosyl ionization. The difference spectrum of N-acetyl-L-tyrosine ethyl ester at *p*H 9.0 *versus* *p*H 3.5 is shown in Fig. 1. This spectrum, modified through incorporation of tyrosine into a protein and by *p*H dependent electrostatic effects on other chromophores, could easily explain the *p*H difference spectra observed with monoacetyl-chymotrypsin and chymotrypsin.

An absorption peak near 245 mμ is not observed in a difference spectrum of AC-A *versus* deacylated AC-A at a *p*H where AC-A is known to deacylate, providing that the *p*H and ionic strength of the solutions are the same (Fig. 1 inset). In these experiments an increase followed by a slow decrease in absorbancy near 245 mμ is observed with AC-A and monoacetyl-δ-chymotrypsin[4] (but not with AC-I).[2] However, the absorbancy changes are not only observed at 245 mμ but also in the visible region where chymotrypsin is known not to absorb[6] (Fig. 1 inset). It was observed[7,8] that absorbancy in difference spectra at wave length regions where the protein does not absorb can result from Rayleigh light scattering due to differences in molecular aggregation. In the visible region a log–log plot ΔD *versus* λ is linear, and extrapolation of this linear portion into the region of absorption allows correction of the scattering contribution.[8] The linearity of the plot in Fig. 1 (inset) indicates that the absorbancy at 245 mμ accompanying the deacylation of AC-A is due to light scattering. Accordingly, the rate of change in absorbancy is the same at all wave lengths between 240 mμ and 500 mμ in the *p*H range studied (*p*H 5.5–9.0) (Fig. 1 inset). It should be noted that both the association-dissociation of chymotrypsin[9]

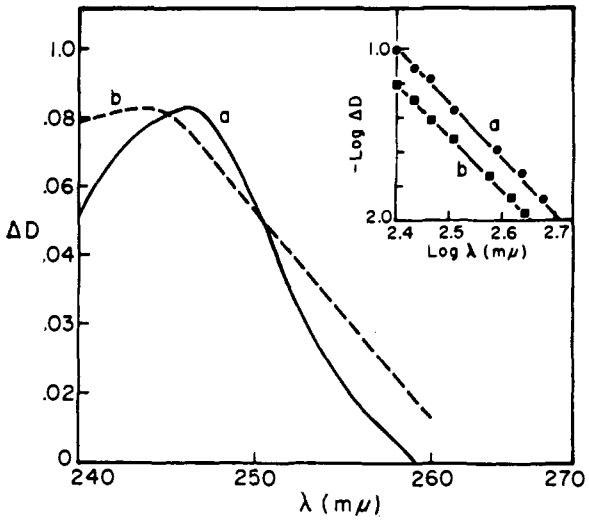

Fig. 1.—Ultraviolet *p*H difference spectra, *p*H 9.0 (0.033 *M* tris-(hydroxymethyl)-aminomethane-HCl buffer) *versus* *p*H 3.5 (HCl): curve a, α-chymotrypsin (6×10^{-5} *M*); curve b, N-acetyl-L-tyrosine ethyl ester (2×10^{-5} *M*). INSET: Ultraviolet and visible difference spectra of AC-A (monoacetyl-α-chymotrypsin) *versus* deacylated enzyme, *p*H 6.3 (0.05 *M* tris-(hydroxymethyl)-aminomethane-HCl, 0.05 *M* $CaCl_2$), 25 ± 0.5°; curve a, 1 minute after adjustment of *p*H of AC-A solution from *p*H 3.5 (HCl) to *p*H 6.3; curve b, 15 minutes after adjustment of AC-A solution to *p*H 6.3. All experiments were performed in presence of 0.05 *M* $CaCl_2$ to prevent the spectral changes at 245 mμ observed by Spencer and Sturtevant[5] with α-chymotrypsin. A Cary Model 14 self-recording spectrophotometer was used for these studies. Three times crystallized α-chymotrypsin, purchased from Worthington Biochemicals, was used. Enzyme concentrations were determined spectrophotometrically at 280 mμ by using $E_{1\%}^{280} = 20.0$ to relate extinction to enzyme concentration.[10] A molecular weight of 25,000 was assumed for α-chymotrypsin.

(1) This work was aided by grants from the National Science Foundation, and the Office of Naval Research.

(2) G. P. Hess and M. A. Marini, *4th Intern. Congr. Biochem.*, p. 42, Vienna, (1958); M. A. Marini and G. P. Hess, THIS JOURNAL, **81**, 2594 (1959); *ibid.*, in press (1960); *Nature*, **184**, 113 (1959).

(3) A. K. Balls and H. N. Wood, *J. Biol. Chem.*, **219**, 245 (1956).

(4) G. H. Dixon and H. Neurath, THIS JOURNAL, **79**, 4558 (1957).

(5) G. H. Dixon, H. Neurath and J. F. Pechére, *Ann. Rev. Biochem.*, **27**, 489 (1958); A. Tinoco, Jr., *Archiv. Biochem. Biophys.*, **76**, 148 (1958); T. Spencer and J. M. Sturtevant, THIS JOURNAL, **81**, 1874 (1959); H. Gutfreund and B. R. Hammond, *Biochem. J.*, **73**, 526 (1959).

(6) These observations have also been made by G. R. Schonbaum and M. L. Bender, unpublished observations, 1960.

(7) M. Laskowski, Jr., S. J. Leach and H. A. Scheraga, THIS JOURNAL, **82**, 571 (1960).

(8) S. J. Leach and H. A. Scheraga, *ibid.*, in press (1960).

(9) R. F. Steiner, *Archiv. Biochem. Biophys.*, **53**, 457 (1954).

(10) G. H. Dixon and H. Neurath, *J. Biol. Chem.*, **225**, 1049 (1957).

and the change in absorbancy at 245 mμ[5] are independent of temperature between 10° and 25°.

These data indicate that the reported[4] spectral peak and the absorbancy changes at 245 mμ are independent phenomena. The former is a component of the *p*H difference spectrum of chymotrypsin. The latter is due to light scattering caused by changes in molecular aggregation of the acetyl enzyme. This implies that the monoacetyl-enzyme (AC-A) and chymotrypsin are in a different state of aggregation at *p*H 5.5 to 9.0, and that the deacylation and deaggregation of AC-A are intimately related events.

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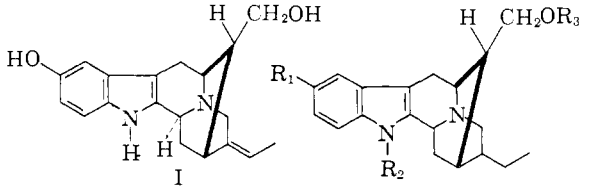
RECEIVED APRIL 9, 1960

RAUWOLFIA ALKALOIDS. XXXIII. THE STRUCTURE AND STEREOCHEMISTRY OF SARPAGINE

Sir:

Sarpagine, the only phenolic alkaloid so far isolated from the genus Rauwolfia, is believed to have the structure (I, stereochemistry unspecified), biogenetic considerations playing a principal role in arriving at this conclusion.[1] The experimental evidence for structure (I) was by no means compelling, based as it was upon the recognition of the functional groups and the fact that the chromophoric systems present in the dehydrogenation products could have arisen from such a system. However we have confirmed these ideas as well as establishing the detailed stereochemistry shown in (I) by a conversion of sarpagine into a degradation product of ajmaline. Sarpagine[2] was reduced in the presence of palladium to its dihydro derivative, m.p. 350°, $[\alpha]_D$ +31° (MeOH), which was converted into its monoacetyl compound[3] (II), m.p. 278°, $[\alpha]_D$ +29° (MeOH). Cleavage of its phenolic O-tosyl derivative (III), m.p. 198–203°, through the use of Raney nickel in boiling ethanol[4] furnished the deoxy acetate (IV), m.p. 253–254°, $[\alpha]_D$ +1° (MeOH). Treatment of the sodio



I

II, R_1 = OH; R_2 = H; R_3 = Ac
III, R_1 = O-tosyl; R_2 = H; R_3 = Ac
IV, R_1 = R_2 = H; R_3 = Ac
V, R_1 = R_3 = H; R_2 = Me

derivative of (IV) with methyl iodide in liquid ammonia yielded an amorphous N_a methyl compound which could not be induced to crystallize. Upon hydrolysis, however, it gave a compound (V) with physical properties indistinguishable from deoxyajmalol-B.[5] Since the absolute stereochemistry of this compound is known,[5] the stereoformula (I) for sarpagine is established with the exception of configuration of the ethylidene group.

(1) The Chemistry of Sarpagine and its congeners is summarized in two recent reviews: (a) K. Bernauer, *Fortschr. Chem. Organ. Naturstoff*, **17**, 183 (1959); (b) A. R. Battersby and H. F. Hodson, *Quart. Reviews*, **14**, 77 (1960).

(2) We are grateful to Dr. Kiang ai Kim for a generous sample of this alkaloid.

(3) By the same method as monoacetylsarpagine was prepared from sarpagine: D. Stauffacher, A. Hofmann and E. Seebeck, *Helv. Chim. Acta*, **40**, 508 (1957).

(4) G. W. Kenner and M. A. Murray, *J. Chem. Soc.*, S, 178 (1949).

(5) M. F. Bartlett, E. Schlittler, R. Sklar, W. I. Taylor, R. L. S. Amai and E. Wenkert, THIS JOURNAL, **82**, 3792 (1960).

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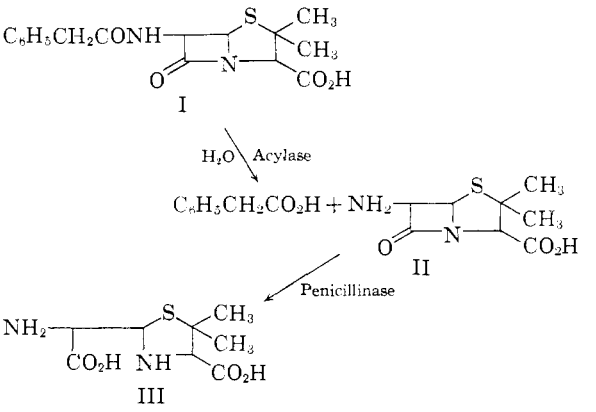
RECEIVED JUNE 1, 1960

ENZYMATIC HYDROLYSIS OF THE SIDE CHAIN OF PENICILLINS

Sir:

The isolation of 6-aminopenicillanic acid (II) from submerged cultures of *Penicillium chrysogenum* has been described by Batchelor, *et al.*[1] We wish to report that 6-aminopenicillanic acid (II) can also be prepared conveniently by the microbial hydrolysis of benzylpenicillin (I). The occurrence



I

II

III

in *P. chrysogenum* of a hydrolytic enzyme which cleaves the acyl side chain of (I) has been claimed by Sakaguchi and Murao[2]; but no confirmation of this work has, thus far, appeared. We have found high levels of penicillin "acylase" activity are produced by widely distributed members of the *Schizomycetes*, including species from such genera as *Escherichia*, *Bordetella*, *Alcaligenes*, *Micrococcus*, *Pseudomonas*, and *Nocardia*.

When sodium benzylpenicillin at 5 g./l. was shaken with 2 g./l. of freeze-dried cells of *Nocardia* F. D. 46973 in 0.05 *M* potassium phosphate buffer[3] at *p*H 7.5 and 28° for 16 hours in the presence of 0.2% toluene, the reaction mixture was found to contain 2.4 g./l. (*i.e.*, 80% of theoretical) of 6-aminopenicillanic acid (II). The latter was determined by treating a filtered sample with penicillinase[4] and assaying the amount of penicic acid (III),[2] *i.e.*, *d*-4-carboxy-5,5-dimethyl-α-amino-2-

(1) F. R. Batchelor, F. P. Doyle, J. H. C. Nayler and G. N. Rolinson, *Nature*, **183**, 257 (1959).

(2) K. Sakaguchi and S. Murao, *J. Agric. Chem. Soc. Japan*, **23**, 411 (1950); S. Murao, *Nippon Nogei-Kagaku Kaishi*, **29**, 400, 404 (1955).

(3) The medium contained yeast extract 4 g., malt extract 10 g., and glucose 4 g., made up to 1 l. with tap water; 500 ml. of medium in a 3 l. fernbach flask was inoculated and incubated at 28° on a rotary shaker for 48 hours.

(4) Baltimore Biological Laboratory.