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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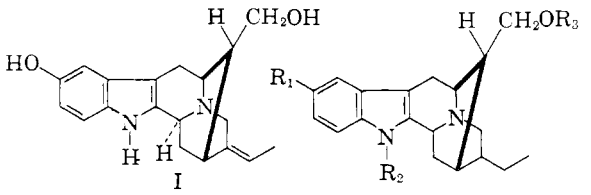
RECEIVED APRIL 9, 1960

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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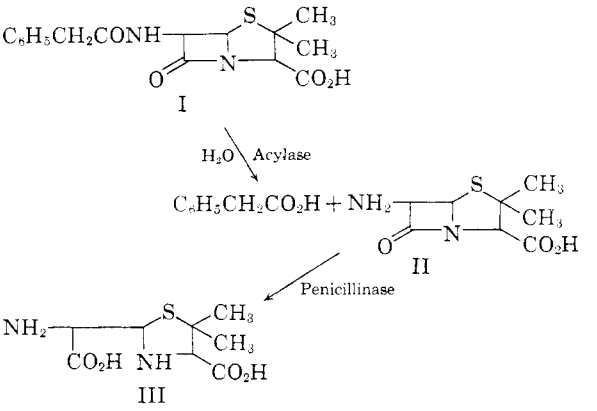
RECEIVED JUNE 1, 1960

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.