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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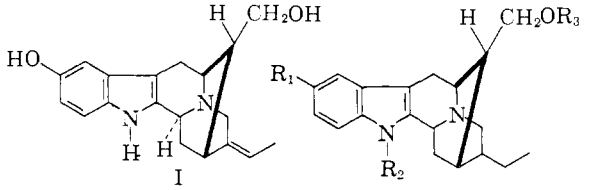
## RAUWOLFIA ALKALOIDS. XXXIII. THE STRUCTURE AND STEREOCHEMISTRY OF SARPAGINE

*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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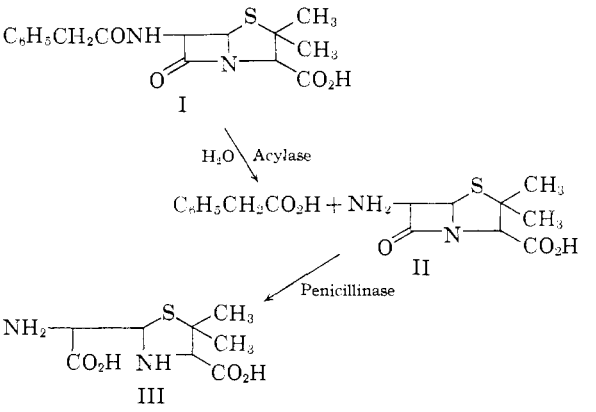
## ENZYMATIC HYDROLYSIS OF THE SIDE CHAIN OF PENICILLINS

*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.

thiazolidineacetic acid, formed by quantitative paper chromatography using ninhydrin as the color reagent. From a suitable batch of reaction mixture 6-aminopenicillanic acid (II) has been isolated by absorption on IR-120 ($H^+$), elution with $NH_4OH$ at *p*H 7.0, concentration *in vacuo*, and adjusting the *p*H to 4.4. The recrystallized product had m.p. 207–208° (dec.) and $[\alpha]_D^{25}$ +277 (*C* 1.0 in 0.1 *N* hydrochloric acid).[5] It assayed approximately 2750 u./mg. based on sodium benzylpenicillin by the hydroxylamine colorimetric procedure[6] and by microbiological determination after phenylacetylation[1] (theor., 2752 u./mg.). Acylation of (II) with the appropriate acid chlorides in aqueous acetone buffered at *p*H 7.0 to 7.5 has given good yields of crystalline potassium salts of benzylpenicillin and phenoxymethylpenicillin, which are identical in all respects to the product prepared by fermentation.

Both phenoxymethylpenicillin (V) and allylmercaptomethylpenicillin (O) are hydrolyzed by this microbial acylase system. Details on the distribution of this acylase in microörganisms, and its activity on a series of penicillins, including a large number of new semi-synthetic penicillins will be reported elsewhere.

(5) J. C. Sheehan and K. R. Henery-Logan, THIS JOURNAL, **81**, 5835 (1959), report $[\alpha]^{21}D$ +273 (*C* 1.2 in 0.1 *N* hydrochloric acid).
(6) G. Boxer and P. M. Everett, *Anal. Chem.*, **21**, 670 (1949).

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## SEPARATION OF ALKALOIDS BY GAS CHROMATOGRAPHY

*Sir:*

In the past, the separation of alkaloids from crude alkaloid mixtures has depended upon fractional crystallization, precipitation, countercurrent extraction and either adsorption or liquid phase partition chromatography. Several recent communications have reported the use of gas phase chromatographic techniques for the separation and identification of steroids[1,2] and high molecular weight fatty primary amines.[3] This communication demonstrates the feasibility of this method for the isolation, separation and identification of alkaloids. Our attention has been focused on alkaloids with molecular weights above 250, since suitable modifications of the conditions should permit separations of lower molecular weight substances without difficulty.[4]

Alkaloids listed in the table gave single component sharp peaks, consistent with the absence of decomposition. A typical sample was 1–3 $\mu$l. of a 0.5–1.0% solution of the alkaloid in methanol, acetone or chloroform. In several cases (N-methylcytisine, crinine, ibogaine and solanidine) macro samples were chromatographed and the product was identified as unchanged starting material by standard methods. The power of this analytical tool is illustrated in a separation of *Papaveraceae* alkaloids (Fig. 1).

TABLE I
ALKALOID RETENTION TIMES

| Compound | Time, min.[a,b] |
|---|---|
| 1. Lupin alkaloids | |
| Cytisine | 5.1 |
| Methylcytisine | 4.3 |
| Methylcytisine N-oxide | 5.8 |
| Lupanine | 5.5 |
| 13-Hydroxylupanine | 11.6 |
| Matrine | 8.5 |
| Lupinine | 1.5[d] |
| Sparteine | 5.9[d] |
| α-Isosparteine | 5.2[d] |
| 13-Hydroxysparteine | 14.3[d] |
| 2. Amaryllidaceae | |
| Galanthine | 19.0 |
| Acetylcaranine | 10.5 |
| Lycorenine | 10.6 |
| Galanthamine | 7.8 |
| Crinine | 9.5 |
| Powelline | 15.8 |
| Tazettine | 15.2 |
| Belladine | 8.7 |
| 3. *Papaveraceae* | |
| Codeine | 8.2 |
| Gnoscopine | 90.6 |
| Laudanosine | 21.0 |
| Morphine | 11.0 |
| Neopine | 9.1 |
| Papaverine | 35.3 |
| Thebaine | 13.2 |
| 4. Indole Alkaloids | |
| Brucine | 80.0[c] |
| Coronaridine | 8.2[c] |
| Ibogamine | 15.4 |
| Ibogaine | 35.1 |
| Serpentine | 16.8[c] |
| Strychnine | 25.9[c] |
| Voacangine | 40.3 |
| 5. Steroidal alkaloids | |
| Solanidine | 40.6[c,e] |
| Solasodine | 74.3[c,e] |
| Tomatidine | 77.3[c,e] |
| 6. Miscellaneous | |
| Atopine | 5.0 |
| Caffeine | 1.6 |
| Cinchonine | 6.7[c] |
| Cocaine | 4.8[c] |
| Corydaline | 16.2[c] |
| Cryptopine | 50.8 |
| Himbacine | 12.7[c] |
| Piperine | 33.0 |
| Protopine | 44.7 |
| Quinine | 11.8[c] |

[a] Argon ionization detector, 6 ft. × 4 mm. i.d. columns. [b] Pressure 15 psi., 2–3/100 SE-30 on Chromosorb W, 80–100 mesh, temperature 204° unless otherwise noted. [c] Temperature 222°. [d] Temperature 160°. [e] Pressure 10 psi.

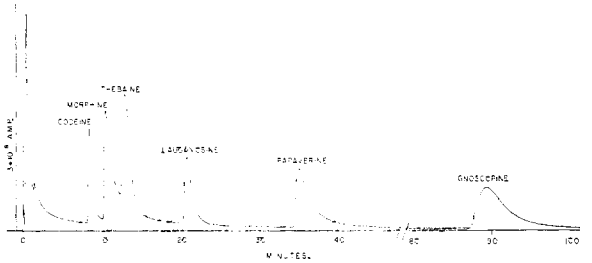


Fig. 1.

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## THE ENTROPY OF ACTIVATION OF ADDITION OF METHYL RADICALS TO UNSATURATED COMPOUNDS POSSESSING THE SAME REACTION CENTER[1]

*Sir:*

Addition of methyl radicals to ethylene,[2] propylene,[2] isobutene,[2] styrene,[3] α-methylstyrene,[3] butadiene[4] and isoprene[4] was studied in this

(1) W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning, THIS JOURNAL, **82**, 3481 (1960).
(2) R. K. Beerthuis and J. H. Recourt, *Nature*, **186**, 372 (1960).
(3) J. Nelson and A. Milun, *Chemistry & Industry*, 663 (1960).
(4) *Cf.* L. D. Quin, *J. Org. Chem.*, **24**, 911 (1959).

(1) This work was supported by a grant from the National Science Foundation.
(2) R. P. Buckley and M. Szwarc, *Proc. Roy. Soc.*, **A240**, 396 (1957).
(3) F. Leavitt, M. Levy and M. Szwarc, THIS JOURNAL, **77**, 5493 (1955).
(4) A. Rajbenbach and M. Szwarc, *Proc. Roy. Soc.*, **A251**, 1266 (1959).