with 10 ml. of 0.2 M $NaHCO_3$ and the insoluble material was removed by centrifuging at 2,000 × g for 30 min. Co A was removed from the supernatant by treatment with Dowex-1.[8] The crude extracts respond to the alumina procedure[3] for the removal of significant quantities of bond thioctic acid from the enzymes. Although assay[9] shows that only about 50% of the thioctic acid is removed by the alumina procedure, treated enzymes possess significantly less acetate activating activity, as measured by the hydroxamic acid method,[10] than do untreated enzymes (Table I). This indicates that although the cofactor has not been completely removed, the concentration has been decreased to below enzyme saturation level. Activity of the acetate-activating system may be restored to alumina treated preparations by the addition of 7.5 Mμg. of synthetic thioctic acid.[11]

Two fractions of the pigeon liver extracts have been separated by ammonium sulfate precipitation; the fractions were collected by centrifugation at 18,000 × g for 15 min. Fraction I (0–35% saturated) contains only very slight acetate activating activity. Fraction II (35–70% saturation) contains almost the entire activity contained in the crude extract; but it does not respond to alumina treatment. Removal of thioctic acid by the alumina procedure is achieved upon combination of the two fractions. Heating Fraction I for 10 min. at 65° completely destroys the activity. It thus appears that Fraction I functions by splitting protein-bound thioctic acid from the enzyme; liberated thioctic acid is then adsorbed and removed by the alumina.

TABLE I

Incubation mixture contained, per 1.4 ml.: 100 μM. Na acetate; 20 units Co A; 200 μM. TRIS buffer, pH 8.2; 10 μM. glutathione; 200 μM. hydroxylamine; 10 μM. Na-ATP; 10 μM. $MgCl_2$; 60 μM. NaF; enzyme solution. Incubated 90 min. at 32°. Glutathione, Na-ATP and hydroxylamine were adjusted to pH 8 with 1 M TRIS before use. Thioctic acid content of crude untreated material was 20 Mμg. per mg. protein; after alumina treatment the content was 11 Mμg. per mg. Fractions I and II contained 17 Mμg. thioctic acid per mg. protein before alumina treatment and 9 Mμg. per mg. after treatment. Alumina treatment of Fraction II alone resulted in no change in thioctic acid content.

Additions and treatment	μM. hydroxamic acid formed: Crude (0.3 ml.)	Fraction I (0.3 ml.)	Fraction II (0.2 ml.)	Fractions I + II (0.1 ml.) (0.2 ml.)
None	0.96	0.02	0.52	0.54
7.5 Mμg. thioctic acid	.95	.03	.50	.53
Alumina treated	.42	..	.53	.34
Alumina treated + 7.5 Mμg. thioctic acid	.80	..	..	.47
2 μM. arsenite	.88	..	.48	.49
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite	.51	..	..	.39
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite + 20 μM. cysteine	.49	..	..	.40
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite + 20 μM. addnl. glutathione	.50	..	..	.39
Alumina treated + 7.5 Mμg. thioctic acid + 2 μM. arsenite + 10 μM. BAL	.84	..	..	.50

(8) E. R. Stadtman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, **191**, 365 (1951).

(9) I. C. Gunsalus, M. I. Dolin and L. Struglia, *ibid.*, **194**, 849 (1952).

(10) T. C. Chou and F. Lipmann, *ibid.*, **196**, 89 (1952).

(11) Kindly supplied by Dr. E. L. R. Stokstad.

As anticipated[12] with a reaction requiring thioctic acid, the acetate-activating system is inhibited by arsenite, but not by arsenate. The arsenite sensitivity of systems containing alumina-treated enzyme plus added thioctic acid is much greater than is the sensitivity in mixtures with untreated enzymes containing an excess of the cofactor. The inhibition is reversed by BAL, but not by such monothiols as cysteine or glutathione.

(12) I. C. Gunsalus, *J. Cell. Comp. Physiol.*, **41** (Suppl. I), 113 (1953).

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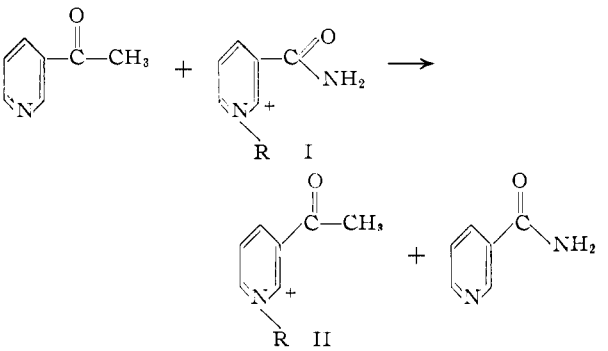
THE 3-ACETYLPYRIDINE ANALOG OF DPN[1]

Sir:

3-Acetylpyridine has been reported to produce symptoms of nicotinamide deficiency in animals but is without effect on the growth of microörganisms.[2] It has been found that the DPNase from pig brain will catalyze an exchange between isonicotinic acid hydrazide and the nicotinamide moiety of DPN (I) to form the isonicotinic acid hydrazide analog of DPN.[3] We have in a similar manner been able to demonstrate the synthesis of the 3-acetylpyridine analog of DPN (II) by the pig brain DPNase according to the equation



R represents the adenosinediphosphate ribose moiety of the DPN molecule (I). II has been isolated from a reaction mixture containing pig brain enzyme, I, and 0.1 M 3-acetylpyridine. Under these conditions, I is almost quantitatively converted to II. II was isolated by precipitation with acid acetone.

II is cleaved by the pig brain DPNase at the same rate as is I. This cleavage results in the forma-

(1) Contribution No. 76 of the McCollum-Pratt Institute aided in part by grants from the American Cancer Society as recommended by the Committee on Growth of the National Research Council, the Williams Waterman Fund, the American Trudeau Society Medical Section of the Natl. Tuberculosis Assoc., and the Rockefeller Foundation.

(2) D. W. Woolley, *Ann. N. Y. Acad. Sci.*, **52**, 1235 (1950).

(3) L. J. Zatman, N. O. Kaplan, S. P. Colowick and M. M. Ciotti, THIS JOURNAL, **75**, 3293 (1953).

tion of 3-acetylpyridine and adenosinediphosphate ribose and the two components can be separated on a Dowex formate column. 3-Acetylpyridine can be washed off the column with water and can be identified spectrophotometrically as illustrated in Fig. 1. The absorption spectrum presented is identical with free 3-acetylpyridine as shown by the peak at 230 mμ.[4] The spectrum of nicotinamide released from DPN by the same treatment is compared with the product of cleavage of the acetylpyridine analog in the figure. From the curves in Fig. 1, it is evident that II contains 3-acetylpyridine in place of the nicotinamide moiety of I.

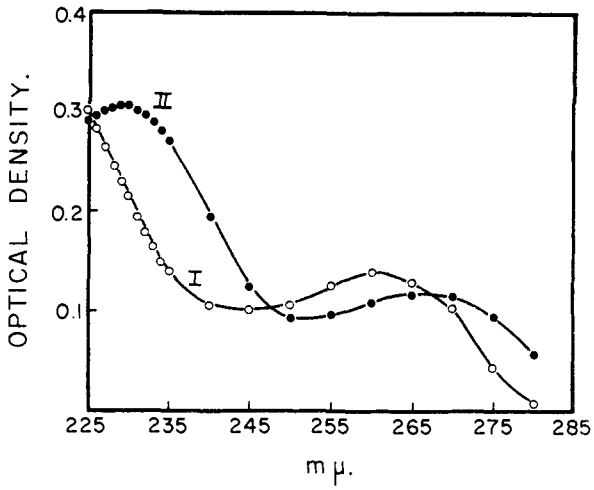


Fig. 1.—Absorption spectra of pyridine compounds released by pig brain DPNase from (I) DPN and (II) the 3-acetylpyridine analog of DPN; concentration of compound equal to 7×10^{-5} *M* in each case. Optical density values represent readings in 3 ml. at *p*H 7.0.

(4) W. T. Beher, S. P Marfey, W. L. Antony and O. H. Gaebler, *J. Biol. Chem.*, **205**, 521 (1953).

Neurospora DPNase[5] does not attack II. However, II reacts with crystalline yeast alcohol dehydrogenase at a rate of about 1/20 that of I. The reduced acetylpyridine analog has a maximum absorption at 365 mμ in contrast to the 340 mμ peak of reduced DPN. This is the only case thus far noted in which an analog of DPN is enzymatically reducible. It is of interest to note that the equilibrium constant with yeast alcohol dehydrogenase is different with I and II; II appears to have an oxidation–reduction potential considerably closer to the alcohol system than does I. In the presence of nicotinamide the pig brain DPNase can catalyze the conversion of II to I.

After injection of 3-acetylpyridine into mice, evidence has been obtained for the presence of II in brain.[6] It appears that formation of II may account for the deficiency symptoms produced by the acetylpyridine. In this connection, it is of interest to note that bacteria in general do not appear to contain DPNases of the animal tissue type, and this may explain why 3-acetylpyridine is not inhibitory to bacteria.

Details of the chemistry, enzymatic activity, nutritional significance and pharmacology of the 3-acetylpyridine analog of DPN will be published elsewhere.

(5) N. O. Kaplan, S. P. Colowick and A. Nason, *ibid.*, **191**, 473 (1951).

(6) N. O. Kaplan, A. Goldin, S. R. Humphreys and M. M. Ciotti, in preparation.

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BOOK REVIEWS

Relationship Between Chemical Structure and Toxic Action on Rats. By JAMES B. DEWITT, ERVIN BELLACK, CLARENCE W. KLINGENSMITH, JUSTUS C. WARD and RAY TREICHLER. **Relationship Between Chemical Structure and Rat Repellency.** By ERVIN BELLACK, JAMES B. DEWITT AND RAY TREICHLER; United States Department of Interior, Fish and Wildlife Service, Patuxent Research Refuge, Laurel, Maryland. Publications Office, National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D. C., 1953. iii + 156 pp. 17 × 25 cm. Price, $1.75.

This interesting little book is a highly condensed summary of the vast amount of investigation carried out by the Fish and Wildlife Service on rodenticides and rat repellents.

As indicated by the title, the first part of this volume gives data on the toxicity of about 1000 compounds to rats while the second part gives the results of examination of more than 2700 materials as rodent repellents. Interpretations are, in general, cautious and limited to an empirical approach. The chemical configurations, which appear to be most effective biologically, are identified and briefly discussed.

Some idea of the condensation of this pesticide review may be gained from the fact that only 14 of its 156 pages are text while the remainder contains tables of experimental results.

The type size is smaller than desirable for comfortable reading, although clearly reproduced. There are remarkably few typographical errors. A minor inconsistency is the representation of the carboxylic acid group variously as –COO–, –C(O)O–, and –C(:O)O–.

This summary is a valuable reference source for those working in this field and should furnish guidance for future studies on rodenticides and repellents.

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Organic Syntheses. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals. Volume 33. By CHARLES C. PRICE (Editor-in-Chief). John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1953. vi + 115 pp. 15.5 × 23.5 cm. Price, $3.50.

"Organic Syntheses" has become a priceless publication for the organic chemist. The syntheses are concisely, but adequately, described such that anyone skilled in the science of chemistry can do the preparations with relative ease.

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Every chemist concerned with organic reactions should have ready access to this volume.

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