sibility of rearrangement during breakdown is not being overlooked.

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, N. Y.
LYMAN C. CRAIG
WERNER HAUSMANN
JAMES R. WEISIGER

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A NEW SYNTHESIS OF CYCLOHEPTATRIENE

Sir:

Methods presently available[1] for the preparation of cycloheptatriene (I) involve ring expansion of a six-carbon cycle as the key reaction step. We wish to report a novel and convenient synthesis of I which involves opening of the cyclobutane ring in a derivative of bicyclo[3.2.0]heptane. Reduction of the readily prepared (from cyclopentadiene and ketene)[2] bicyclo[3.2.0]hept-2-ene-6-one with lithium aluminum hydride afforded (95%) bicyclo[3.2.0]-hept-2-ene-6-ol (II), b.p. 96–98° (38 mm.), $n^{25}D$ 1.4987 (Calcd. for $C_7H_{10}O$: C, 76.32; H, 9.15. Found: C, 76.20; H, 8.87). Treatment of II with methanesulfonyl chloride in pyridine afforded the methanesulfonate (III) as a crude oil which was not purified further owing to its thermal instability. Solvolysis of III in hot acetic acid containing two mole equivalents of sodium acetate, or preferably sodium dihydrogen phosphate monohydrate, afforded I in approximately 50% yield based upon II. Identification of I was made through comparison of its physical constants (b.p. 60.5° (122 mm.), $n^{25}D$ 1.5208) and absorption spectra (λ_{max} 260 mμ; major infrared absorption bands at 3.31, 3.37, 3.48, 3.53, 6.20, 6.97, 7.17, 7.68, 10.98, 12.57, 13.44 and 14.02 μ) with those of authentic cycloheptatriene[3] and by comparison (mixture m.p. 103.5–104.5°) of its maleic anhydride adduct (obtained in 64% yield; m.p. 104.2–105.0°) with that obtained (also 64% yield; m.p. 103.8–104.8°)[4] from authentic cycloheptatriene.

We are currently investigating the possibility of preparing substituted cycloheptatrienes as well as other types of unsaturated seven-carbon ring compounds from cyclopentadiene–ketene adducts.

(1) (a) E. P. Kohler, M. Tishler, H. Potter and H. T. Thompson, THIS JOURNAL, **61**, 1057 (1939); (b) W. von E. Doering and L. H. Knox, *ibid.*, **75**, 297 (1953).

(2) A. T. Blomquist and J. Kwiatek, *ibid.*, **73**, 2098 (1951).

(3) This was prepared by a modification of the procedure of ref. 1a. (H. L. Dryden, Jr., and B. E. Burgert, unpublished work).

(4) Reported (ref. 1a) m.p. 102–104°.

DEPARTMENT OF CHEMISTRY
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS
HUGH L. DRYDEN, JR.

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ON THE MECHANISM OF ACTION OF ISONICOTINIC ACID HYDRAZIDE

Sir:

The mechanism of the antituberculous activity of isonicotinic acid hydrazide (INAH) has been under investigation in this laboratory. Experiments were designed to study the chemical and enzymatic activities of the INAH analog of diphosphopyridine nucleotide (DPN). Zatman, *et al.*,[1] have recently described the isolation of this analog in which the nicotinamide moiety of DPN has been replaced by INAH yielding D-INAH-N.

Although Zatman, *et al.*,[1,2] have reported that the beef spleen DPN-ase is inhibited by both INAH and D-INAH-N, we have taken advantage of the fact that this inhibition, while marked, is incomplete. The analog, accordingly, was prepared by incubating 100 μ moles of DPN (sigma "90"), 180 mg. of beef spleen DPN-ase,[3] and a large excess of INAH[4] (10 mmoles) for 4 hours at 38° in 0.015 *M* phosphate buffer (*p*H 7.4). Under these conditions, this amount of enzyme would normally catalyze the cleavage of as much as 7,200 μmoles of DPN. The slow rate of reaction indicates a very strong inhibition of the enzyme, a finding which is in complete agreement with that of Zatman and coworkers.

The analog, after isolation by charcoal adsorption, pyridine elution, and precipitation of the nucleotides with cold acetone, contained about 10% DPN which was removed by incubating the isolated nucleotide mixture with DPN-ase and INAH. D-INAH-N isolated from the second exchange reaction is essentially free of DPN. Molar ratios of the analog are shown in Table I. Extinction coefficients for D-INAH-N at several *p*H's are shown in Table II. The increase in the 260 mμ absorption of D-INAH-N at *p*H 2 corresponds closely to that observed for INAH itself.[5] The yellow color formed on exposing preparations of D-INAH-N to alkali[1] shows an absorption peak at 360 mμ. Crude preparations of D-INAH-N show approximately the same absorption spectra with the exception that the 360 mμ peak is shifted to about 385 mμ.

TABLE I

	Moles per mole of D-INAH-N			
	INAH[a]	Ribose[b]	P[c]	DPN[d]
Theory	1.00	2.00	2.00	0.00
Found	1.00	1.95	2.28	<0.02

[a] J. M. Kelly and R. B. Poet, *Am. Rev. Tuberc.*, **65**, 484 (1952). [b] A. H. Brown, *Arch. Biochem.*, 11, 269 (1946). [c] E. J. King, *Biochem. J.*, **26**, 292 (1932). [d] Assayed by the alcohol–alcohol dehydrogenase reaction. Detection limits under assay conditions are 1–2% DPN.

TABLE II

EXTINCTION COEFFICIENT FOR D-INAH-N[a]

Wave length, mμ	*p*H 2	7.2	9.5	12
260	27.8	25.9	25.0	25.1
360	0.6	4.0	5.8	6.4

[a] All values expressed as $\epsilon \times 10^6$ cm.^{2} mole^{-1}.

The following results have been obtained for the chemical and enzymatic activities of D-INAH-N: (a) D-INAH-N, in contrast to DPN, is not reduced by hydrosulfite to a dihydro form and it does not form a cyanide complex.[6] (b) The activity of D-INAH-N as an electron acceptor has been investi-

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

(2) L. J. Zatman, S. P. Colowick, N. O. Kaplan and M. M. Ciotti, *Bull. Johns Hopkins Hosp.*, **91**, 211 (1952).

(3) L. J. Zatman, N. O. Kaplan and S. P. Colowick, *J. Biol. Chem.*, **200**, 197 (1953).

(4) Pure INAH was generously supplied by Hoffmann-LaRoche, Inc., Nutley, N. J.

(5) D. S. Goldman, *Science*, in press.

(6) S. P. Colowick, N. O. Kaplan and M. M. Ciotti, *J. Biol. Chem.*, **191**, 447 (1951).

gated by the substitution of this analog for DPN or triphosphopyridine nucleotide (TPN) in the specific assay systems for the following pyridine nucleotide-requiring oxidative enzymes[7]: alcohol dehydrogenase (DPN), α-keto-glutaric dehydrogenase (DPN), malic dehydrogenase (DPN), β-hydroxy-acyl CoA dehydrogenase (DPN), isocitric dehydrogenase (TPN) and malic enzyme (TPN). D-INAH-N is not reduced in these reactions. Furthermore, the reduction by their specific dehydrogenases of either DPN or TPN is not inhibited by D-INAH-N. The oxidation of DPNH by the specific DPN-cytochrome *c* reductase[7] of pig heart muscle is not inhibited by D-INAH-N. These several pyridine nucleotide-requiring dehydrogenases were tested in order to obtain as broad a picture as possible of the potential inhibiting nature of this analog.

This anomalous behavior may be interpreted as evidence that DPN is normally reduced by the addition of a proton to the 4-position of the pyridine ring; this position is apparently unavailable in D-INAH-N where the pyridine ring is substituted in the *para* position. This confirms the recent report by Pullman, *et al.*,[8] who investigated the stereospecificity of DPN reduction. These workers concluded from isotope experiments that DPN was reduced in the *para* position of the pyridine ring. The antituberculous action of INAH may accordingly be due to the intracellular formation of an inactive pyridine nucleotide analog with a concomitant reduction in cellular oxidative metabolism.

(7) Generously supplied by Drs. H. R. Mahler, D. R. Sanadi and S. Wakil, Enzyme Institute, University of Wisconsin.

(8) M. E. Pullman, A. San Pietro and S. P. Colowick, *J. Biol. Chem.*, **206**, 129 (1954).

RESEARCH DIVISION
VETERANS ADMINISTRATION HOSPITAL DEXTER S. GOLDMAN
MADISON, WISCONSIN

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URIDINE- AND INOSINE-TRIPHOSPHATES AS PHOSPHATE DONORS FOR PHOSPHOHEXOKINASE[1]

Sir:

The widespread natural occurrence of the 5'-triphosphates of uridine[2-6] (UTP), inosine[6] (ITP) and other nucleosides raises the question of whether they participate directly in phosphorylating reactions[3,7] in addition to their possible role in nucleic acid synthesis.

Kleinzeller[8] reported ITP to be inactive in the phosphorylation of fructose-6-phosphate with muscle extract as the enzyme source, but Muntz[9] reported this nucleotide to be half as active as ATP when a partially purified phosphofructokinase from brain was employed. The slow rate of glucose phosphorylation by UTP and ITP may, according to Berg and Joklik,[10] result from enzymatic transphosphorylation between these respective nucleotides and ADP, followed by utilization of the formed ATP for the hexokinase reaction. This raises the question whether other cases, where ITP has been found active as a phosphate donor, might have involved a similar transphosphorylation to adenine nucleotide.

A direct utilization of both ITP and UTP for the phosphorylation of fructose-6-phosphate to fructose-1,6-diphosphate has been found to be catalyzed by a highly purified phosphofructokinase (Table I).

TABLE I

PHOSPHORYLATION OF FRUCTOSE-6-PHOSPHATE BY VARIOUS NUCLEOSIDE 5'-TRIPHOSPHATES

The reaction mixture contained 2 μmoles of fructose-6-phosphate, 1 μmole of diphosphopyridine nucleotide, 20 μmoles of cysteine, 10 μmoles of arsenate, 300 μg. of triosephosphate dehydrogenase, 100 μg. of aldolase, 100 μmoles of tris-(hydroxymethyl)-aminomethane HCl buffer (*p*H 8.3), nucleotides as indicated and $MgCl_2$ at a molar concentration equal to that of the nucleotides. Final volume 3.0 ml. The reaction was initiated by addition of 0.2 μg. of phosphofructokinase. The rate of DPN reduction is zero without added nucleotide or without any of the other reaction constituents save cysteine and arsenate. Stock solutions of all enzymes (kept in ammonium sulfate solutions) were diluted with 0.5% serum albumin in 0.01 *M* tris-buffer. ATP and UTP were chromatographed samples from Pabst; ITP obtained from Sigma Chemical Co.

Prof. Herman M. Kalckar kindly supplied a sample of highly purified UTP known to contain less than 0.01% of ATP. It gave similar results.

Phosphate donor (μmoles)	$\Delta \log I_0/I$ at 340 mμ per minute — Minutes 1st	2nd	3rd	4th	5th
ATP, 6	0.035	0.040	0.045	0.047	0.048
UTP, 4.3	.033	.037	.043	.043	.044
UTP, 0.1	.027	.025	.032	.026	.028
UTP, 0.1 + ADP, 0.44	.023	.026	.031	.030	.031
ITP, 3.9	.025	.040	.041	.041	.041
ITP, 0.1[a]	.010	.012	.010	.010	.010

[a] Purified by the method of Cohn and Carter.[11]

The enzyme was extracted from homogenized muscle of magnesium-anesthetized[12] rabbits with 0.03 *M* KOH[13] and was purified by fractionation with alcohol and with ammonium sulfate. The procedure results in a 200-fold purification of the enzyme from the original extract. At this stage of purity 100,000 g. of enzyme phosphorylates about 10,400 moles of fructose-6-phosphate per minute at 26°. It is free from detectable myokinase, triosephosphate isomerase, aldolase, triosephosphate dehydrogenase, α-glycerophosphate dehydrogenase and phosphohexoisomerase. The activity is determined by the rate of reduction of diphosphopyridine nucleotide in a system[14] containing recrystallized aldolase[15] and triosephosphate dehydrogenase,[16] DPN, fructose-6-phosphate, Mg^{++} and a

(1) Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

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