sibility of rearrangement during breakdown is not being overlooked.

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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 (H. L. Dryden, Jr., and B. E. Burgert, unpublished work).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°.

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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	*p*H 7.2	*p*H 9.5	*p*H 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).