acid hydrolysis of peptide B can be shown by the addition of Ba^{++}. Estimation of the amount of sulfate liberated, by a turbidimetric method using Ba^{++}, gave a value of about 0.9 mole per mole of tyrosine. The rate of liberation of inorganic sulfate parallels that of the appearance of the tyrosine absorption peak at 275 mμ.

4. Tyrosine-O-sulfate has been detected in hydrolysates of peptide B prepared by heating in 0.2 *M* $Ba(OH)_2$ for 24 hours at 125°. It was freed of other amino acids and Ba^{++} by passage through a column of Dowex 50 in the H^+ form, and shown to be identical with synthetic tyrosine-O-sulfate by paper chromatography with 3 different solvent systems, and by paper electrophoresis at *p*H 2.4. The amount found, estimated as tyrosine after acid hydrolysis, was 75% of that expected from the tyrosine content of the peptide.

5. The new peptide formed on mild acid hydrolysis of peptide B behaves as a less acidic molecule than the original on paper electrophoresis at *p*H 6.8, 4.1 and 2.4. This is consistent with the loss of a strongly acid group.

The modification in the properties of the phenolic group by conjugation with sulfuric acid may help to account for the assertions of Lorand[3] and Lorand and Middlebrook[4] that the peptide material released from fibrinogen by thrombin is free of tyrosine.

(3) L. Lorand, *Nature*, **167**, 992 (1951).
(4) L. Lorand and W. R. Middlebrook, *Biochim. Biophys. Acta*, **9**, 581 (1952).

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WASHINGTON
SEATTLE,, WASHINGTON AND
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CAMBRIDGE
ENGLAND
F. R. BETTELHEIM

RECEIVED APRIL 12, 1954

ALKALI METAL–AMMONIA SOLUTIONS: GROSS CHEMICAL DIFFERENCES

Sir:

Reactions in liquid ammonia have been reported in which the alkali metals differ from calcium solutions[1]; in which the rates of reaction of the alkalies differ[2]; or in which the alkalies differ in the efficiency with which they bring about reduction of organic compounds.[3] But in the case of liquid ammonia solutions of $BF_3 \cdot NH_3$ our experimental results show a straight-forward difference in the *stoichiometry* of the reactions with different alkali metals. Such inherent differences in the chemical reactivity of these metal solutions have not been demonstrated previously.

Solutions of $BF_3 \cdot NH_3$ were titrated with solutions of the alkali metals in an apparatus[4] which provided for the quantitative recovery of all products. Also, reactions of $BF_3 \cdot NH_3$ solutions with an excess of alkali metal were carried out, followed by back-titration with ammonium iodide solutions to determine the excess metal present.

Similar titrations were carried out with potassium amide, using triphenylmethane as indicator, to investigate the extent of total solvolysis. Results are reported in Table I.

(1) W. M. Burgess and J. W. Eastes, THIS JOURNAL, **63**, 2674 (1941).
(2) G. W. Watt and P. I. Mayfield, *ibid.*, **75**, 1760 (1953).
(3) A. L. Wilds and N. A. Nelson, *ibid.*, **75**, 5360 (1953).
(4) G. W. Watt and C. W. Keenan, *ibid.*, **71**, 3833 (1949).

TABLE I

TITRATION OF $BF_3 \cdot NH_3$–LIQUID NH_3 SOLUTIONS

Titrant	Moles titrant per mole $BF_3 \cdot NH_3$ By forward-titration	By back-titration
Li	2.91	2.97
Na	2.50	2.62
K	1.00	1.02[a]
Cs		1.03
KNH_2	2.6	3.00

[a] Determined by analysis of hydrogen evolved in presence of excess potassium, which was added in form of solid pieces to $BF_3 \cdot NH_3$ solution.

All final end-points were one-drop excesses stable for at least 30 minutes. Of particular interest were the observations of transient end-points in the direct titrations with both sodium and lithium. In the case of sodium there was found a transient end-point at about one equivalent,[5] and in the case of lithium at both one and two and one-half equivalents. These transient end-points were approached sharply and persisted for as long as 40 minutes, at which time the blue color due to excess metal faded suddenly. The subsequent titration reactions were as rapid as normal ionic titrations.

The following over-all equations are in agreement with our findings. Supporting analytical data will be reported in a later paper.

$$6Li + 2BF_3 \cdot NH_3 + 2NH_3 \longrightarrow 6LiF + (NH_2)_2BNHBNH + 3H_2$$

$$5Na + 2BF_3 \cdot NH_3 + 2NH_3 \longrightarrow 5NaF + (NH_2)_2BNHB(F)NH_2 + 5/2H_2$$

$$K + BF_3 \cdot NH_3 \longrightarrow KF + BF_2NH_2 + {}^1/_2H_2{}^6$$

$$3KNH_2 + BF_3 \cdot NH_3 \longrightarrow 3KF + B(NH_2)_3 + NH_3$$

These experimental data suggest a mechanistic explanation based on differences in the alkali metal–oxidant interaction. Such an approach has been defended recently in another case.[3]

(5) For an earlier description of the reaction with sodium see C. A. Kraus and E. H. Brown, *ibid.*, **51**, 2690 (1929).
(6) C. W. Keenan and W. J. McDowell, *ibid.*, **75**, 6348 (1953).

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF TENNESSEE
KNOXVILLE, TENNESSEE
C. W. KEENAN
W. J. MCDOWELL

RECEIVED APRIL 21, 1954

STRUCTURAL STUDIES WITH BACITRACIN A

Sir:

In partial hydrolysis studies with bacitracin A using hydrochloric acid a considerable number of peptides have been isolated as DNP (dinitrophenyl) derivatives which appear to be of satisfactory purity as judged by C.C.D. (countercurrent distribution), two dimensional P.C. (paper chromatography) and P.E. (paper electrophoresis). Each peptide has been completely hydrolyzed and the hydrolysate studied by P.C. and P.E. The DNP amino acid has been extracted from the hydrolysate and identified by a combination of P.C., P.E. and C.C.D. A summary of part of the work is given in Table I. Over-all analytical data, supporting the composition indicated by the results in Table I for many of the peptides, are given in Table II.

TABLE I

Peptide	Phe	Ileu	Leu	Cys	Asp	Glu	His	Lys	Orn	Mol. wt. Found[a]	Mol. wt. Calcd	Interpretation
1	+	DNP	−	−	−	−	−	−	−	408	444	DNP-Ileu-Phe
2[b]	+	+	−	−	−	−	−	−	δ-DNP	560	595	δ-DNP-Orn-Ileu-Phe·HCl
3	+	+	−	−	−	−	i-DNP	−	Di-DNP	826	1027	Di-DNP-Orn-Ileu-Phe(DNP)His
4	DNP	−	−	−	+	−	i-DNP	−	−	800	749	DNP-Phe(DNP)His·Asp
5	−	−	−	−	+	−	Di-DNP	+	−	530	566	DNP-His·Asp·Lys
6	−	+ and DNP	−	−	DNP	−	−	+	δ-DNP	1170	1090	DNP-Asp(DNP-Ileu)Lys(DNP)Orn·Ileu
7	−	+	−	−	DNP	DNP	−	+	−	820	834	DNP-Glu·Ileu(DNP·Asp)Lys
8	−	DNP	−	−	+ and DNP	−	−	+	δ-DNP	1240	1101	DNP·Asp·Asp(DNP·Ileu)Lys(DNP)Orn
9[b]	−	+	−	−	+	+	−	+	−	−	−	Asp·Asp(Glu·Ileu)Lys
10	−	+	DNP	−	+	+	−	+	−	800	782	DNP-Leu·Glu·Ileu(Asp)Lys
11	−	+	−	−	+ and DNP	DNP	−	+	δ-DNP	1450	1230	DNP·Asp·Asp(DNP·Glu·Ileu)Lys(DNP)Orn
12	−	−	DNP	−	−	+	−	−	−	510	426	DNP·Leu·Glu
13	−	DNP	−	DNP	−	−	−	−	−	622	566	DNP-Ileu(DNP)Cys
14	−	DNP	+	DNP	−	−	−	−	−	770	679	DNP-Ileu(DNP)Cys·Leu
15	−	DNP	+	DNP	−	+	−	−	−	740	808	DNP-Ileu(DNP)Cys·Leu·Glu

[a] Calculated from extinction–weight ratio using the molecular extinction coefficient of the DNP amino acid concerned at 350 mμ. [b] From partial hydrolysis of tri-DNP-bacitracin A.

TABLE II

Peptide	Found C	Found H	Found N	Found S	Calcd. C	Calcd. H	Calcd. N	Calcd. S
1	56.63	5.50			56.7	5.45		
2	52.1	6.00			52.3	5.94		
3	51.1	4.37	18.00	None	51.3	4.36	17.8	
4	49.7	3.50	17.14		49.7	3.63	16.8	
13	44.7	4.20	14.42	5.9	44.5	3.90	14.8	5.65
14	48.08	4.68	14.22	4.20	47.8	4.89	14.4	4.71
15	47.89	5.13			47.5	4.99		

The data of Table I and other considerations have forced the conclusion that bacitracin A contains three isoleucine residues rather than two.[1] Direct evidence for racemization of isoleucine during hydrolysis has been found. The unknown band in the methionine position of the amino acid chromatogram[2] is thus D-alloisoleucine as suggested[3] and three residues of isoleucine are indicated. These data and the finding that bacitracin A as prepared retains 1 mole of acetic acid permits an over-all formula of $C_{66}H_{107}O_{18}N_{17}S$ to be written. Found: C, 55.2; H, 7.3; N, 15.8; S, 2.2. Calcd: C, 55.1; H, 7.27; N, 16.05; S, 2.16. This formula results by joining all the known fragments to form two rings.

The results of Table I can be rationalized by the following sequence where → shows a C–N bond

In the tri-DNP derivative[1,4] of bacitracin A the DNP groups have been shown to be attached to ornithine, histidine and isoleucine. However, a poor yield of DNP-isoleucine results on hydrolysis. The yield is improved by oxidation of the sulfur with performic acid. These results, absorption spectrum studies and other observations are consistent with a thiazoline ring system.

The results reported here are in agreement with those of Newton and Abraham[5] for the sequence–Ileu·Cys·Leu·Glu–. However, they do not seem to agree well with most of the sequences of Porath.[6]

Addition of an amide group to the first formula above would give a likely empirical formula. However, more than twice the number of peptide fragments, other than those reported here, have been isolated and studied. Certain of these indicate a complication of cross linkages in addition to those shown above. One of these may involve the nitrogen of the isoleucine next to the cysteine. The pos-

(1) L. C. Craig, W. Hausmann and J .R. Weisiger, *J. Biol. Chem.*, **200**, 765 (1953).
(2) L. C. Craig, W. Hausmann and J. R. Weisiger, *ibid.*, **199**, 865 (1952).
(3) K. A. Piez, *ibid.*, **207**, 77 (1954).
(4) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **53**, 597 (1953).
(5) I. M. Lockhart, G. G. F. Newton and E. P. Abraham, *Nature*, **173**, 536 (1954).
(6) J. Porath, *ibid.*, **172**, 871 (1953).

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

RECEIVED APRIL 12, 1954

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.

RECEIVED MARCH 25, 1954

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).