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

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 C. W. KEENAN
KNOXVILLE, TENNESSEE 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.