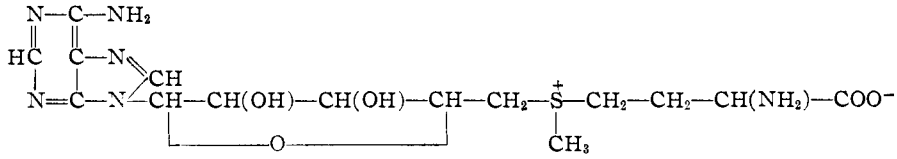
containing compound, presumably thiolribose or thiomethylribose.

On the basis of this evidence active methionine has been assigned the structure shown below.



Active methionine can be considered as an addition product of methionine and the adenosine portion of ATP, with the elimination of the inorganic tripolyphosphate chain. It should be noted also that the sulfur of methionine acquires an additional covalent bond and it is thought that formation of the positively charged sulfonium compound confers lability upon the methyl group. It is suggested that the compound formed from L-methionine and ATP by the action of the methionine-activating enzyme, which has been designated heretofore as active methionine, might more properly be referred to as S-adenosyl-methionine. On the basis of the data given above the preparation obtained by paper chromatography is at least 80% pure with relation to adenine compounds.

DEPARTMENT OF PHARMACOLOGY
SCHOOL OF MEDICINE
WESTERN RESERVE UNIVERSITY
CLEVELAND, OHIO
G. L. CANTONI

RECEIVED MAY 9, 1952

IDENTIFICATION OF DROSOPHILIN A AS *p*-METHOXYTETRACHLOROPHENOL[1]

Sir:

Drosophilin A, an antibiotic compound recently isolated in this Laboratory[2] has been identified as *p*-methoxytetrachlorophenol.

Drosophilin A was isolated as previously described, and further purified by sublimation under reduced pressure. Analytical values[3] for a sample resublimed four times agreed with the expected for a compound of formula $C_7H_4O_2Cl_4$. Found: C, 32.19; H, 1.50; Cl, 54.05; OCH_3, 11.88. Calcd. for $C_7H_4O_2Cl_4$ (261.93) C, 32.10; H, 1.54; Cl, 54.15; OCH_3, 11.85.

Demethylation of Drosophilin A with boiling 70% hydriodic acid yielded a crystalline compound which melted at 232°,[4] and gave no melting point depression on admixture with an authentic sample of tetrachlorohydroquinone.

Methylation of Drosophilin A with ethereal diazomethane yielded a crystalline product which melted at 160° and gave no depression on admixture with an authentic sample of 1,4-dimethoxytetrachlorobenzene prepared by methylation of the Eastman-Kodak preparation of tetrachlorohydroquinone.

(1) This investigation was supported in part by a research grant from the National Microbiological Institute of the National Institutes of Health, Public Health Service.

(2) F. Kavanagh, A. Hervey and W. J. Robbins, *Proc. Nat. Acad. Sci.*, in press.

(3) Microanalyses were performed by the Huffman Microanalytical Laboratories, Denver, Colorado.

(4) All melting points are uncorrected.

The melting point of the dimethyl ether agrees with that reported in the literature.[5] The monomethyl ether, as far as can be ascertained, has been described only once in the literature,[6] and the melting point reported is 103°, instead of 116° as found for Drosophilin A. Difference in the state of purity of the two samples may account for this discrepancy.

A sample of the monomethyl ether was prepared by removal of one methyl group from the dimethyl ether by treatment with warm concentrated sulfuric acid and melted at 114° alone or when mixed with Drosophilin A.

Drosophilin A is believed to be the first antibiotic compound isolated, which contains a halogenated benzene ring. The chlorine atoms of chloramphenicol, the first halogenated antibiotic compound reported, are in a side chain.

(5) A. Binz and C. Räth, *Ber.*, **58**, 309 (1925).

(6) E. Burès and J. Hutter, *Časopis Českoslov. Lékárnictva*, **11**, 29, 57 (1931) (*C. A.*, **25**, 5153 (1931)).

THE NEW YORK BOTANICAL GARDEN
BRONX PARK
NEW YORK 58, N. Y.
MARJORIE ANCHEL

RECEIVED MAY 9, 1952

DISSOCIATION MECHANISM FOR THE AQUATION OF SOME COBALT(III) COMPLEX IONS[1]

Sir:

Based on the published observations on substitution reactions of complex ions, it has not been possible to designate whether any of these reactions proceed by a displacement (S_N2) or dissociation (S_N1) mechanism. It may appear that these reactions of cobalt(III) complexes should proceed by a dissociation mechanism since the complex has an invert gas configuration which means there are no low-lying orbitals available for attack by the incoming group. However displacement reactions are known to occur with carbon compounds which likewise have an inert gas configuration.

The aquation rates of several substituted ethylenediamine complexes of the type $[Co(AA)_2Cl_2]^{+1}$ have recently been determined by us and some preliminary results are shown in Table I. It is apparent that the complex ions which contain C-substituted ethylenediamine aquate more rapidly than the corresponding ethylenediamine ion. The fact that increased crowding around the central ion does not decrease the rate suggests that these reactions

TABLE I

RATES OF AQUATION OF SOME *trans*-$[Co(AA)_2Cl_2]^{+1}$ IONS
First chlorine only, temperature 25°, *p*H 1

(AA) Diamine	$k \times 10^3$ (min.$^{-1}$)
$NH_2—CH_2—CH_2—NH_2$	1.9
$NH_2—CH_2—CH(CH_3)NH_2$	3.7
dl-$NH_2—CH(CH_3)—CH(CH_3)—NH_2$	8.4
meso	250
$NH_2—C(CH_3)_2—C(CH_3)_2—NH_2$	Very rapid

(1) This investigation was supported by a grant from the United States Atomic Energy Commission under contract AT(11-1)-89-Project No. 2.

do not proceed through a seven coördinated activated complex, that is, by an S_N2 mechanism.

The results are in good agreement with those which would be expected if the intermediate is an activated complex which is penta coördinated such as in S_N1 mechanism. They seem in fact to constitute another example of steric acceleration of reaction velocity such as has been noted in the solvolysis of highly branched tertiary halides.[2]

A significant point is the thirty-fold difference in aquation rates between the *dl*- and *meso*-butylenediamine complexes. It can be seen by the use of molecular models that in the case of the *meso* butylenediamine complex where both methyl groups are on the same side of the five-membered ring there is considerable crowding of these two adjacent groups. This repulsion would not be lessened if the activated complex has a coördination number of seven but could be considerably decreased if this were five. That steric factors are important in accounting for the rapid rate of reaction is further substantiated by the almost instantaneous reaction of the complex which contains the tetramethylethylenediamine.

Other complexes of this type are being studied and in addition activation energies are being determined. These findings will be reported in more detail in the near future.

(2) H. C. Brown, and R. S. Fletcher, THIS JOURNAL, **71**, 1845 (1949).

DEPARTMENT OF CHEMISTRY
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS

RALPH G. PEARSON
CHARLES R. BOSTON
FRED BASOLO

RECEIVED APRIL 18, 1952

THE USE OF CARBOXYPEPTIDASE FOR THE IDENTIFICATION OF TERMINAL CARBOXYL GROUPS IN POLYPEPTIDES AND PROTEINS. ASPARAGINE AS A C-TERMINAL RESIDUE IN INSULIN[1]

Sir:

A study of the action of carboxypeptidase on ACTH protein and peptide preparations[2] has led to a reinvestigation of its use as a general method for the identification of C-terminal groups in polypeptides and proteins; results obtained with insulin, fractions A and B derived from insulin by the method of Sanger,[3] and lysozyme, are summarized in Table I.

According to Sanger[4] the insulin molecule (m.w. 12,000) is composed of two pairs of identical polypeptide chains joined together through six S–S linkages; on this basis the insulin molecule should contain four free α COOH groups. Lens[5] reported the liberation of three moles of alanine by the action of carboxypeptidase and concluded that at least one, and possibly three, of the constituent peptide chains in insulin had alanine C-terminal groups. Other investigators using chemical methods found evidence for the presence of both alanine[6,7,8] and glycine[6,7] C-terminal groups in insulin.

TABLE I

ACTION OF CARBOXYPEPTIDASE ON INSULIN AND ITS FRACTIONS, AND LYSOZYME

Substance	Products of carboxypeptidase digestion: C-Terminal groups	Adjacent amino acids
Insulin	Alanine, asparagine	Lysine, leucine, glutamic acid, tyrosine
Fraction B	Alanine	Lysine
Fraction A	Asparagine	Cysteic acid,[a] leucine, tyrosine, glutamic acid
Lysozyme	Leucine	.....

[a] Cystine was not detected from insulin. This is of interest since it suggests that the enzyme proceeded to split peptide bonds further down the A chain, leaving the cystine residue attached only through its –S–S– group to one of the B chains.

In a typical experiment, insulin (Eli Lilly Lot No. 200-18-15; 80 mg., 0.5% solution, *p*H 7.8) was treated with carboxypeptidase[9] (160 μg. N) at 25°; equal aliquots were removed from the digestion mixture at intervals during an eight-hour digestion period. The addition of trichloroacetic acid (TCA) to a final concentration of 5% by volume served to terminate enzyme action, and to precipitate residual protein which was then removed by centrifugation; formation of free amino groups in supernatant fractions was followed by the ninhydrin reaction. For paper chromatographic studies, aliquots of the same supernatant fractions were passed through Amberlite (IR 4B) resin columns to remove TCA. Chromatography in butanol/acetic acid/water (4:1:5) revealed the presence of alanine, asparagine and aspartic acid after only two minutes digestion with the enzyme; after 8 hours of digestion, lysine, glutamic acid, tyrosine and leucine could also be detected. Starch column analysis of the fraction soluble at *p*H 5.4, after a four-hour digestion of insulin with carboxypeptidase under the conditions described above, confirmed the presence of alanine and asparagine.[10] Liberation of alanine was found to be complete after four hours, and amounted to two moles of alanine per mole of insulin. Preliminary studies indicate that proteolysis follows first order kinetics and that the rate of formation of alanine is about eight times that of the asparagine.

Digestion of Fraction B with carboxypeptidase confirmed that alanine[11] is in fact the C-terminal group of the phenylalanine chains in insulin. When Fraction A was treated with the enzyme, asparagine was found to be the initial product of digestion; further digestion led to the formation of cysteic acid, leucine, tyrosine and glutamic acid. It is concluded that, contrary to previous results,[6] asparagine occurs at the carboxyl end of the glycyl chains in the insulin molecule and that the other

(1) This work was supported in part by a grant to Dr. C. H. Li from the Rockefeller Foundation, New York. The author wishes to acknowledge the able assistance of Ning G. Pon.

(2) J. I. Harris and C. H. Li, Abstracts XIIth International Congress of Chemistry, 68 (September, 1951).

(3) F. Sanger, *Biochem. J.*, **44**, 126 (1949).

(4) F. Sanger, *ibid.*, **45**, 563 (1949).

(5) J. Lens, *Biochem. et Biophys. Acta*, **3**, 367 (1949).

(6) C. Fromageot, M. Justisz, D. Meyer and L. Penasse, *ibid.*, **6**, 283 (1950).

(7) A. C. Chibnall and M. W. Rees, *Biochem. J.*, **48**, xlvii (1951).

(8) S. G. Whaley and J. Watson, *J. Chem. Soc.*, 2394 (1951).

(9) The crystalline enzyme (8× recrystallized) used in this work was obtained through the generosity of Prof. H. Neurath.

(10) Some decomposition of asparagine to give aspartic acid was found to occur during the recovery and subsequent chromatography of TCA soluble fractions.

(11) F. Sanger, and H. Tuppy, *Biochem. J.*, **49**, 481 (1951).