

**THE STABILITY OF THE KETO ACID FROM
METHIONINE¹**

Sir:

The metabolic path of most amino acids seems to be preponderantly through the corresponding keto acids. Hence a thorough knowledge of the behavior of these keto acids is essential for the understanding of amino acid metabolism. In a study of the mechanism of methylation processes in the animal organism we were interested, therefore, in the behavior of the keto acid from methionine. This amino acid is one of the few physiological substances which may yield methyl groups. According to the prevailing view methionine is demethylated to homocysteine during metabolism. We have obtained evidence by studying the behavior of the corresponding keto acid that a second reaction—splitting off of the $-\text{SCH}_3$ group—is also possible.

Since the keto acid from methionine has as yet not been synthesized, we prepared it biologically by the deamination of methionine in the presence of kidney slices according to the technique developed by Krebs.² A dinitrophenylhydrazone of the keto acid melting at 149° was obtained in about 20% yield.³

Anal. Calcd. for $\text{C}_{11}\text{H}_{12}\text{O}_6\text{N}_4\text{S}$: C, 40.24; H, 3.68; N, 17.07; S, 9.77. Found: C, 40.40; H, 3.81; N, 16.57; S, 9.80.

Solutions of methionine which had been similarly incubated with kidney slices were deproteinized and the filtrates containing the free keto acid and unchanged methionine were digested with acid and with alkali. Under both conditions methyl mercaptan was produced copiously. The liberated methyl mercaptan was identified by its yellow silver compound and by the melting point and analysis of the mercury mercaptide. In one experiment 400 mg. of *dl*-methionine was metabolized by kidney slices. After deproteinization the keto acid was determined in an aliquot of the solution as the dinitrophenylhydrazone (yield, calculated to the total sample, 210 mg. of the hydrazone). To another aliquot sufficient 10 *N* sodium hydroxide was added to make the solution 2 *N*. The solution was refluxed under nitrogen for one hour and the liberated mercaptan was absorbed in a

solution of mercuric cyanide. Most of the methyl mercaptan was liberated during the first twenty minutes. The mercaptan weighed as mercury mercaptide corresponded to 72% of the keto acid in the solution (68 mg. $\text{Hg}(\text{SCH}_3)_2$). Since the original solution contained negligible amounts of mercaptan and as methionine does not yield mercaptan under these conditions, the reaction is ascribed to the decomposition of the keto acid. This finding may be explained on the basis of the instability of β -keto sulfides investigated by Nicolet.⁴

To establish the possible physiological significance of our findings we are continuing our studies with other sulfur-containing compounds.

(4) B. H. Nicolet, *THIS JOURNAL*, **53**, 3066 (1931).

FROM THE DEPARTMENT OF BIOCHEMISTRY
NEW YORK STATE PSYCHIATRIC INSTITUTE
AND HOSPITAL AND THE DEPARTMENT OF
NEUROLOGY, COLUMBIA UNIVERSITY HEINRICH WAELSCH
NEW YORK, N. Y. ERNEST BOREK

RECEIVED JULY 19, 1939

**THE VERATRINE ALKALOIDS. VI. THE OXIDA-
TION OF CEVINE**

Sir:

In previous work on the degradation of cevine, high temperature pyrolytic procedures (soda lime distillation or selenium dehydrogenation) have been rewarding in the search for degradation products. These, however, have been limited in number and their nature has made them difficult to relate to the parent substance with the exception of those derived from its basic portion. More recently it has been possible, for the first time, to achieve a crystalline oxidation product. Chromic acid in dilute sulfuric acid has given a mixture from which an acid fraction in good yield has been separated. This fraction, still a mixture, could not be directly crystallized. However, when heated to 180° evolution of carbon dioxide occurred, with production in good yield of a crystalline product which was non-nitrogenous (m. p. $273\text{--}278^\circ$), $[\alpha]^{25}_D +47.6^\circ$ ($c = 0.925$ in pyridine). Analysis indicated a formula $\text{C}_{14}\text{H}_{14}\text{O}_6$. Calculated: C, 60.41; H, 5.07. Found: C, 60.51; H, 5.20.

Diazomethane gave a product which crystallized readily from acetone (m. p. $165\text{--}166^\circ$). Analysis indicated 2 methoxyl groups. Calcd. for $\text{C}_{16}\text{H}_{18}\text{O}_6$: C, 62.75; H, 5.92; OCH_3 , 20.26. Found: C, 62.96; H, 6.07; OCH_3 , 19.90. The

(1) This work was made possible through a grant from the Friedsam Fund donated to the Division of Child Neurology, Neurological Institute, New York, N. Y.

(2) Krebs, *Z. physiol. Chem.*, **217**, 216 (1933).

(3) Bernheim, *J. Biol. Chem.*, **114**, 657 (1936), obtained a phenylhydrazone by incubating methionine with a kidney extract.

molecular weight by the Rast method was found to be 326. Calculated for $C_{16}H_{18}O_6$, 306.14.

15.06 mg. of the acid in 2.5 cc. of alcohol was titrated against phenolphthalein. Found 1.014 cc. of 0.1 *N* NaOH; calculated for 2 equivalents, 1.082 cc. No additional alkali was consumed after boiling with excess 0.1 *N* sodium hydroxide.

14.96 mg. of the ester required 0.478 cc. of 0.1 *N* NaOH for direct titration in the cold. Calculated for one equivalent: 0.488 cc. After boiling for two hours an additional 0.437 cc. of alkali was consumed.

From these data it appears likely that the substance contained a labile lactone group and that one of the two methyl groups introduced with diazomethane involved a phenolic or enolic hydroxyl. Presence of a phenolic or enolic group was indicated by a prompt, deep red-purple color obtained with ferric chloride. The acid as well as the ester coupled with diazotized sulfanilic acid. On hydrogenation with platinum approximately 3 moles of hydrogen was absorbed, but the product could not be crystallized. It no longer gave the original color reactions.

These properties suggest that the substance

contained a benzene ring and is possibly the lactone of a substituted tetrahydronaphthalene. However, the substance when treated with alkali gave a solution from which carbon dioxide was liberated on acidification. From this solution crystalline degradation products could be isolated which suggested that the original acid is capable of undergoing decomposition similar to that of β -ketonic acids. The phenolic or enolic character may therefore be due to such a grouping and thus the unsaturation of the substance to the presence of ketonic linkages rather than to a benzene ring. The details of this further degradation must be left to a later communication.

Our previous studies [*J. Biol. Chem.*, **119**, 141 (1937); **120**, 447 (1937)] have indicated that the basic portion of the cevine molecule is a substituted octahydropyridocholine. The substance, $C_{14}H_{14}O_6$, must be derived from that portion of the molecule all or in part distinct from the basic portion.

THE LABORATORIES OF
THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH
NEW YORK, N. Y.

LYMAN C. CRAIG
WALTER A. JACOBS

RECEIVED JULY 5, 1939

NEW BOOKS

The Principles of Electrochemistry. By DUNCAN A. MACINNES, Associate Member, Rockefeller Institute for Medical Research. Reinhold Publishing Corporation, 330 West 42d Street, New York, N. Y., 1939. 478 pp. Price, \$6.00.

This book treats the conductive and thermodynamic properties of electrolytic solutions and is not concerned with metallic and gaseous conduction, which the author considers a branch of electrophysics. After a brief historical introduction, there follows an excellent treatment of transference phenomena embodying the masterly researches of the author and his collaborators on the moving boundary method and precision conductance determinations; several well written chapters on the thermodynamics of galvanic cells, standard electrode potentials and the ramifications of Debye-Hückel theory. The vexing problem of liquid junction potentials receives a critical and modern treatment. The practical determination and meaning of *pH*, conductance methods, oxidation potentials, potentiometric titrations with the glass and other electrodes which have found wide application, are adequately presented. Conductance in non-aqueous and mixed solvents is treated in an up-to-date fashion. The book closes with chapters on ionization constants

and structure, effects of centrifugal forces, dielectric constants, passivity and overvoltage, and the latest accepted developments in electrokinetic phenomena.

A comparison of the tables with those of previous texts gives ample evidence of the discrimination that has been exercised in assembling these data. The expert will notice the omission or correction of many of the time-honored values of the pioneers but he will be gratified to learn that these omissions and corrections are the result of painstaking recomputation and critical evaluation of the data from the original sources. Misprints and errors of statement are rare. On page 142, eq. 19, "extremely dilute" instead of "infinitely dilute" would be a more concrete expression. On page 143, *kappa* is to be held constant in the Güntelberg-Müller charging process. The reviewer has not found a single meaningless sentence. The format and printing are in keeping with the high standard of presentation.

Readers interested in locating the very latest theoretical contributions or commercial applications will be disappointed. The author consistently refrains from including theories which have not been adequately verified by experiment, and makes no pretense of dealing with the patent literature.