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Chemistry-General

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Chemical Degradation of Isotopic Succinic and Malic Acids

by

A. A. Benson and J. A. Bassham

25 May 1948

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Chemical Degradation of Isotopic Succinic and Malic Acids

by

A. A. Benson and J. A. Bassham

From the Radiation Laboratory and Department
of Chemistry, University of California,
Berkeley, California*

25 May 1948

Abstract

A chemical degradation of isotopic succinic acid using the Curtius reaction has been adapted for small quantities of material and accurately gives the distribution of C^{14} in the methylene groups and carboxyl groups. The methylene groups, isolated as ethylenediamine dihydrochloride are obtained entirely free from other radioactivity.

An oxidation of isotopic malic acid is also reported and is capable of separating the alpha, beta and the carboxyl carbon atoms. This method is also applicable to the degradation of isotopic aspartic acid.

* This paper is based on work performed under contract No. W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, California.

Chemical Degradation of Isotopic Succinic and Malic Acids

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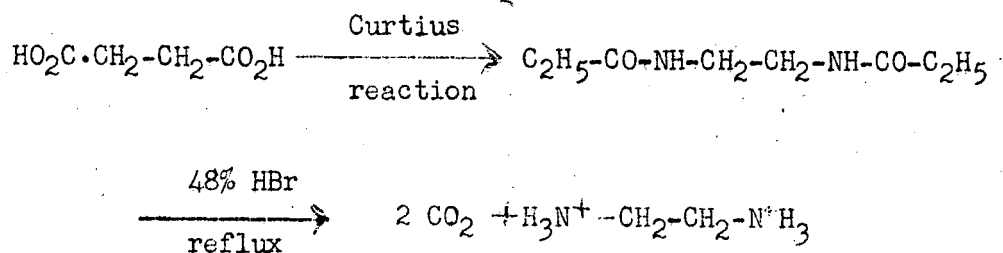
From the Radiation Laboratory and Department
of Chemistry, University of California,
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The path of carbon in photosynthesis (1) may be traced by determination of the positions of labeled carbon atoms in the intermediates involved. Enzymatic degradation of succinic and malic acids have been reported (2) but require pure enzyme preparations. Chemical degradations of these intermediates which are unequivocal and capable of detecting small fractions of isotopic carbon are reported in this note. Succinic and malic acids containing C^{14} were isolated from plant extracts using silica gel partition chromatography (3) and cocrystallized with suitable amounts of carrier acids.

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- (1) M. Calvin and A. A. Benson, *Science* 105, 648 (1947); 107, 476 (1948)
 - (2) H. G. Wood, G. H. Werkman, A. Hemingway and A. O. Nier, *J. Biol. Chem.*, 135, 7890 (1940)
 - (3) F. A. Isherwood, *Biochem. J.*, 40, 688 (1946)

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The Curtius (4) degradation of succinic acid has been modified to give good yields on small amounts. Methyl succinate prepared using diazomethane, is converted to the diazide through the dihydrazide. (4, 5). Rearrangement of the diazide in ethanol gives ethylenediurethane which was hydrolyzed to give carbon dioxide from the carboxyl groups and ethylene diamine from the methylene groups of the original succinic acid.



With this method it is possible to determine accurately as little as one micromicrocurie of C^{14} per milligram of succinic acid in the methylene groups in the presence of any amount of carboxyl activity.

Malic acid has been oxidized with chromic acid to yield two molecules of carbon dioxide from the carboxyl groups and one molecule of acetic acid from the alpha and beta carbon atom. The same procedure has been used to determine radioactive carbon fixed in the alpha and beta carbon atoms of aspartic acid. Degradation of the acetic acid may then be performed by decarboxylation (6).

- (4) T. Curtius, J. prakt. chem., [2] 52, 222 (1895)
 (5) Shöfer and Schwan, J. prakt. chem., [2] 51, 190 (1895)
 (6) S. A. Aronoff, V. A. Haas and B. A. Fries, To be published.

Experimental

Ethylenediurethane.--With a tracer amount of unequally C^{14} labeled succinic acid synthesized in the dark by preilluminated *Chlorella* was crystallized 300 mg. of succinic acid. The 280 mg. yield of crystalline acid was converted to methyl succinate in the usual manner with diazomethane and distilled quantitatively into a conical reaction flask. The hydrazide prepared from the ester in 90% yield, was converted to the diurethane using the methods of Shöfer and Schwan (5) and Curtius (4). The product was recrystallized from water and sublimed in vacuo to give a yield of 145 mg. (30%). The specific activity of a thin (less than 0.2 mg/cm^2) sample was accurately ($\pm 2\%$) determined with a Geiger counter.

Hydrolysis of Ethylenediurethane.--In a 30 ml. two-neck flask equipped with a reflux condenser and nitrogen inlet tube was refluxed a solution of 100 mg. of ethylene diurethane in 5 ml. of 48% hydrobromic acid for two hours. A slow stream of nitrogen gas during this period carried the evolved carbon dioxide through the condenser into a sodium hydroxide bubbler from which it was quantitatively recovered as barium carbonate (100% yield). The ethylenediamine was obtained upon evaporation of excess acid in vacuo and adding excess methanolic potassium hydroxide. After removal of the methanol, ethylenediamine was distilled in vacuo and converted to the dihydrochloride by addition of methanolic hydrogen chloride. After recrystallization from methanol-water, the specific activity of the pure ethylenediamine dihydrochloride was determined.

Oxidation of malic Acid.--To a solution of 100 mg. of C^{14} labeled malic acid in 10 ml. of 1.0 N sulfuric acid in a 100 ml. flask equipped

with nitrogen inlet bubbler, reflux condenser and dropping funnel was added 20 ml. of 0.15 M chromic acid solution during 2 hours on the steam bath.

The CO_2 evolved was collected in a sodium hydroxide trap and precipitated with barium chloride to give 294 mg. (100%) of barium carbonate. Acetic acid was obtained from the residual solution upon steam distillation. It was converted to barium acetate (80% yield) and its specific activity was determined after recrystallization from water.

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