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Chemical Degradation of Isotopic Succinic and Malic Acids

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Publication Date

1948-08-16

UCRL 118 (Revised)
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Radiation Laboratory

Contract No. W-7405-Eng-48

Chemical Degradation of Isotopic Succinic and Malic Acids

by

A. A. Benson and J. A. Bassham

16 August 1948

Berkeley, California

Special Review of Declassified Reports

Authorized by USDOE JK Bratton

Unclassified TWX P182206Z May 79

REPORT PROPERLY DECLASSIFIED

<u>JN Green</u>	<u>8/16/79</u>
Authorized Derivative Classifier	Date
<u>ROK Hunt</u>	<u>8/17/79</u>
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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*

16 August 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.

For publication in the Journal of the American Chemical Society.

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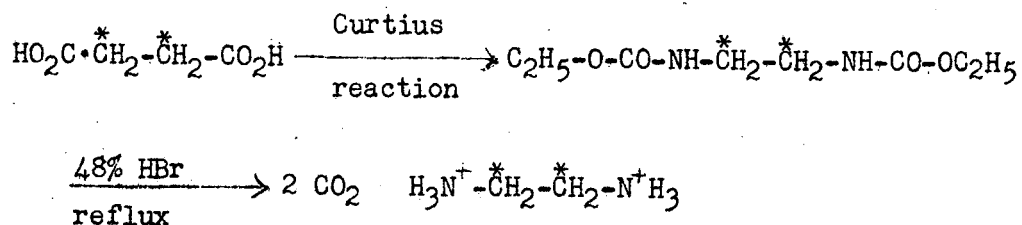
The path of carbon in photosynthesis (1) may be traced by determination of the positions of labeled carbon atoms in the intermediates involved. Enzymatic degradation of succinic and malic acids have been reported (2) but require pure enzyme preparations. Unequivocal chemical degradations of these intermediates are reported in this note. Succinic and malic acids containing C¹⁴ were isolated from plant extracts using silica gel partition chromatography (3) and cocrystallized with convenient amounts of carrier acids.

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).

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- (1). Calvin and Benson, *Science*, 105, 648 (1947); 107, 476 (1948).
 - (2). Wood, Werkman, Hemingway and Nier, *J. Biol. Chem.*, 135, 7890 (1940).
 - (3). Isherwood, *Biochem. J.*, 40, 688 (1946).
 - (4). Curtius, *J. prakt. chem.*, [2] 52, 222 (1895).
 - (5). Sh8fer and Schwan, *J. prakt. chem.*, [2] 51, 190 (1895).

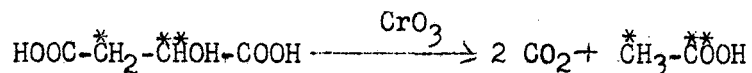
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Rearrangement of the diazide in ethanol gives ethylenediurethane which was hydrolyzed to give carbon dioxide from the carboxyl groups and ethylenediamine from the methylene groups of the original succinic acid.



With this method it is possible to determine the activity of the methylene carbon atoms (6) without interference from any amount of carboxyl carbon 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 (7).

Experimental

Ethylenediurethane.--A tracer amount of unequally C¹⁴-labeled succinic acid synthesized in the dark by preilluminated Chlorella was diluted by crystallizing with 300 mg. of succinic acid. The 280 mg. yield of crystalline acid was converted to methyl succinate in the usual manner with diazo-

- (6). An activity of one count per minute determined on a 1 mg. sample of ethylenediamine dihydrochloride by the Nucleometer corresponds to 0.89 c./m./mg. or $8 \cdot 10^{-10}$ millicurie/mg. for the succinic acid.
- (7). Aronoff, Haas and Fries, to be published.

methane and distilled quantitatively into a conical reaction flask. The hydrazide was prepared from the ester in 90% yield and was converted to the diurethane using the methods of Shöfer and Schwan (6) and Curtius (5). The product was recrystallized from water and sublimed in vacuo to give a yield of 117 mg. (36%). The specific activity of a thin (less than 0.2 mg./cm.²) sample was determined with a Geiger counter and found to be 21.7 ± 1 c./m./mg.

Hydrolysis of Ethylenediurethane.---In a 30 ml. two-neck flask equipped with a reflux condenser and nitrogen inlet tube was refluxed a solution of 104 mg. of ethylenediurethane 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 recovered as barium carbonate, 210 mg., specific activity 10.0 c./m./mg. The ethylenediamine was obtained upon evaporating excess acid in vacuo and adding excess methanolic potassium hydroxide. After evaporating the methanol, ethylenediamine was distilled in vacuo and converted to the dihydrochloride, 52 mg. (theoretical yield 68 mg.) by addition of methanolic hydrogen chloride. After recrystallization from methanol-water, the specific activity of the pure ethylenediamine dihydrochloride was found to be 0.86 c./m./mg. This indicates that 2.6% of the succinic acid radioactivity was in the methylene carbon atoms.

Oxidation of Malic Acid.---To a solution of 109.7 mg. of C¹⁴-labeled malic acid (275 c./m./mg. of 30,100 c./m. total.) 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.

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The CO_2 evolved was collected in carbonate-free sodium hydroxide solution and precipitated with barium chloride to give 326 mg. (101%) of barium carbonate. The specific activity was found to be 81.5 c./m./mg. or 26,600 c./m. total. Acetic acid was obtained from the residual solution upon steam distillation. It was converted to barium acetate (80% yield based on titre of steam distillate) which was recrystallized from water. From the specific activity (2.0 c./m./mg.) and the theoretical yield (112 mg.), the activity in the α and β carbon atoms of the malic acid, 224 c./m., can be determined accurately without interference from carboxyl activity. Incomplete recovery of the carbon dioxide and over-oxidation of the malic acid is assumed to have caused the loss of carboxyl activity.

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Sent for publication: _____

Received for publication: _____