PREPARATION OF 2-METHYLENEBUTANEDIOIC ACID–4–^{14}C (ITACONIC ACID)

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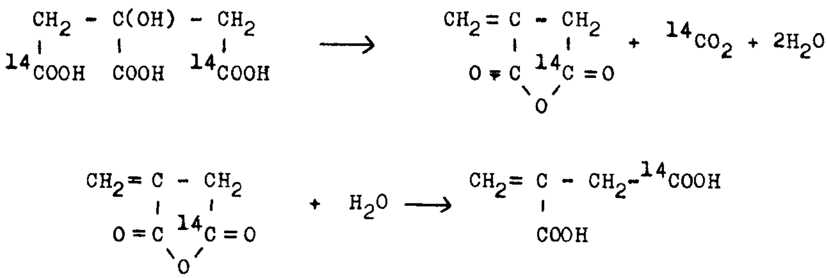
SUMMARY

The pyrolysis of citric acid-1,5-$^{14}C_2$ under controlled conditions and subsequent hydrolysis of the anhydride initially produced yields principally 2-methylenebutanedioic acid-4-^{14}C (itaconic acid-^{14}C), together with a minor amount of cis-2-methylbutenedioic acid-4-^{14}C (citraconic acid-^{14}C). Comparison of the molar specific activities of the precursor and products indicates the absence of a carbon kinetic isotope effect in the decarboxylation-dehydration reaction.

Introduction

The preparation of carboxyl-labeled 2-methylenebutanedioic acid (itaconic acid) by conventional carboxylation methods has been made difficult by lack of suitable precursors. Such methods are especially disadvantageous for synthesis of the acid labeled solely in the carboxyl group most distant from the double bond. Fermentation methods based on glucose and Aspergillus terreus (1-3), although offering yields of up to 40%, require extensive preliminary work to establish labeled sites in the acid derived from specifically-labeled glucose. The pyrolysis of citric acid, on the other hand, proceeds in a known manner (4) and, when citric acid-1,5-$^{14}C_2$ (5) is used, yields the anhydride of 2-methylenebutanedioic acid-4-^{14}C. Thus, only one acid group is labeled. Since polymerization studies involving the acid require that the activity be present in carbons other than those of the double bond, the pyrolytic systhesis gives a labeled product that is particularly desirable for such applications. The present preparative method is designed to yield the acid having a specific activity in the range of intended use, thereby reducing significantly the effect of possible radiochemical impurities on the accuracy of results.



<u>Experimental</u>

Reagent grade citric acid monohydrate (125.0 gm., 0.595 mole) is dissolved in the minimum amount of hot water and the citric acid-1, 5-^{14}C (0.5 mCi at ca. 10 mCi/mmole) is added in water solution. Most of the water is removed by heating on a water bath and the acid is further dried to the anhydrous state in vacuum over phosphorus pentoxide.

Pyrolysis is performed in a 250-ml. long-neck, round-bottom flask, which is attached to a 75°-connector, the other end of which is fitted to the uppermost of two 50-cm. Allihn condensers in series. The tip of the lower condenser is connected to glass tubing that leads to the center of a 2-neck, 1-liter flask, which is attached to a second 1-liter flask by a 75°-connector. Both flasks are surrounded by ice baths. Rubber tubing from the second flask leads to a suitable trap in a hood for collection of evolved carbon dioxide.

The flask containing the citric acid is heated rapidly and uniformly with a Fisher burner at a rate such that melting is complete in seven minutes. The melted acid is then pyrolyzed by continued uniform heating for 5 to 5½ minutes, and distillation is stopped before the vapors in the 250-ml. flask become yellow. The condensers are washed with ca. 100 ml. of hot water, and 25 ml. of water is added to the second 1-liter flask. The contents of both receiving flasks are warmed, combined, and the volume is reduced to 40 to 50 ml. on a water bath. Most of the by-product citraconic acid-^{14}C is removed by steam-distilling the solution until 350 to 400 ml. of distillate is collected. The steam-distilled solution is then evaporated until its weight is ca. 70 gm, and the itaconic acid-^{14}C is isolated by filtration, after cooling the solution several hours in an ice bath. The yield is 10.5 to 11.5 gm. (13.6 to 14.8%); radiochemical yield is one-half the chemical yield. Specific activity of the product is 3.2 to 3.4 microcuries/gm.

The product is assayed in a dioxane-based scintillator solution, together with a specimen of the anhydrous citric acid taken for pyrolysis. Radiochemical and chemical purity are verified by thin layer chromatography on silica gel, with benzene:methanol:acetic acid (45:8:4) or benzene:dioxane:acetic acid (90:24:4), by volume, as solvents (6). Autoradiography is used for detection of labeled components, and alkaline permanganate for chemical development of the chromatograms.

Discussion

The preparative method described differs from the common approach to the preparation of labeled compounds in that the specific activity of the precursor is first reduced to that required in the product. This circumstance is advantageous in this synthesis, since the manner and rate of heating play a critical role in determining yield, and precise control of the pyrolysis on the millimolar scale is tedious and uncertain.

Highest yields were obtained when heat was applied uniformly at a rate that melted the citric acid within about seven minutes, and pyrolyzed it in an additional 5 to 5½ minutes. Lower yields of labeled itaconic acid are the result of rearrangement of its anhydride to the anhydride of cis-2-methylbutenedioic acid-4-^{14}C (citraconic acid). If desired, the latter compound can be prepared by isolating and rapidly distilling (7) the itaconic anhydride obtained from the citric acid. Most of the by-product citraconic acid arising in the preparation of the itaconic acid is removed by steam distillation; the remainder, together with the trace amount of trans-2-methylbutenedioic acid-4-^{14}C (mesaconic acid), is removed by crystallization. Examination of the thin layer chromatograms, both by autoradiography and spraying with alkaline permanganate, showed the presence of only a single component.

A comparison of the specific activities of two preparations of itaconic acid-^{14}C with those of the respective two lots of anhydrous citric acid-1,5-$^{14}C_2$ used is given in Table I.

Table I

Comparison of Specific Activities of Itaconic and Citric Acids

Preparation	Specific Activity, μCi/mmole		Ratio of Specific Act.
	Itaconic Acid	Citric Acid	Itaconic/Citric
A	0.451	0.902	0.500
B	0.415	0.831	0.499

The fact that the molar specific activities differ by a factor of two indicates the absence of a carbon kinetic isotope effect in the decarboxylation reaction.

References

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