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SMALL SCALE PREPARATION OF C¹⁴ LABELED

SUCCINIC, MALIC, FUMARIC and TARTARIC ACIDS

E. C. Jorgensen, J. A. Bassham, M. Calvin and B. M. Tolbert

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ABSTRACT

The directions for preparing the following acids from labeled acetic acid on a 1 to 20 millimole scale is presented: Succinic acid, malic acid, fumaric acid and tartaric acid. Two methods for preparing the succinic acid are detailed.

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(*) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

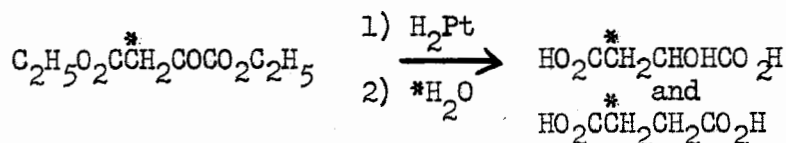
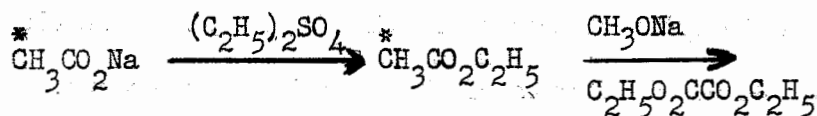
SMALL SCALE PREPARATION OF C¹⁴ LABELED
 SUCCINIC, MALIC, FUMARIC AND TARTARIC ACIDS (*)

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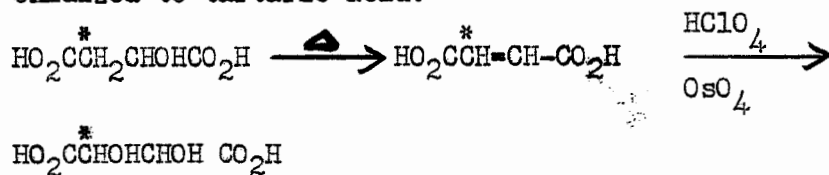
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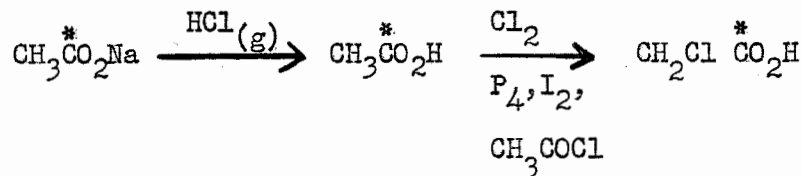
On a 20 millimole scale, succinic and malic acid labeled in either the β or the γ position with carbon-¹⁴ were prepared from the corresponding labeled acetic acids as follows.



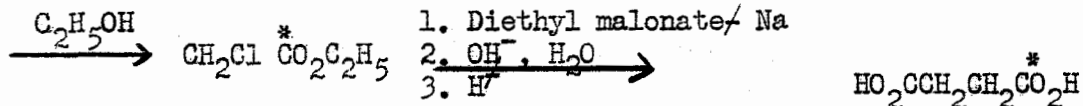
The malic and succinic acids were separated by distribution between ether and water and 1 millimole malic acid dehydrated to fumaric acid which was then oxidized to tartaric acid:



In an alternate preparation of succinic acid, the following steps were used.



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This method is superior to the oxalacetic procedure in yield, purity of product and reliability for the preparation of succinic acid from acetic acid.

The purity of all products was checked by two-dimensional paper chromatography and radioautographs of the papers. The succinic acid prepared via chloroacetic acid was pure, i.e. it contained only one radioactive compound. In the separation of malic and succinic acid prepared via oxalacetic acid, distribution of the acids was continued until less than one per cent of the other labeled compound remained in the desired acid. The fumaric and tartaric acids were radioactively pure within the limits of the analytical procedure.

EXPERIMENTAL

Chloroacetic-1-C¹⁴ acid: Sodium acetate-1-C¹⁴ (1.15 g., 14 millimoles, specific activity 10 μ c/mg) was converted to free acetic acid and chlorinated as previously described^(1,2).

Ethyl Chloroacetate-1-C¹⁴: The crude chloroacetic acid from the previous steps was transferred with a small amount of water (5 ml.) to a liquid-liquid extractor and extracted with ether for 15 hours. At the end of this time the aqueous residue was found to contain only 0.3% of the original activity. The ether solution was dried with sodium sulfate and the ether fractionally distilled off.

The residual chloroacetic acid was washed into the pot of a 50 ml. Soxhlet extractor with 15 ml. of ethanol (dried by distillation from sodium and diethyl phthalate). Five μ of 95% sulfuric acid was added. The cup of the extractor was filled with 4 mesh anhydrous calcium sulfate and the reaction mixture heated for six hours under dry reflux.

Succinic-1-C¹⁴-Acid: To a three necked 120 ml. pear-shaped flask equipped with a water condenser, dropping funnel and protected from moisture with calcium sulfate, were added 20 ml. anhydrous ethanol and 1.15 g. (50 millimoles) of sodium. After the sodium had dissolved, 8 g. (50 millimoles) diethyl malonate was added and the reaction mixture refluxed for a few minutes.

The solution of labeled ethyl chloroacetate was transferred to the dropping funnel. The Soxhlet extractor was then washed by refluxing anhydrous ethanol in the system. This wash, which contained considerable activity, was added to the dropping funnel.

The ethyl chloroacetate-1-C¹⁴ was added to the refluxing solution of sodium malonic ester over a one-hour period, then held under reflux for an additional

(1) R. Ostwald, J. Biol. Chem. 173, 207 (1948).

(2) D. M. Hughes, R. Ostwald and B. M. Tolbert, submitted for publication.

half hour. Twenty-five ml. of 4 N sodium hydroxide was added to the reaction mixture and refluxing continued over night (10-15 hours).

The ethanol was distilled off and the aqueous solution washed into a liquid-liquid extractor, carefully acidified with 10 ml. 18 N sulfuric acid, and extracted with ether for 20 hours. The ether was distilled off and the residue heated at 150-160° for two hours to decarboxylate the residue of malonic and substituted malonic acid. During this heating most of the acetic acid from the excess malonic acid distilled off. The final heating was done under a water pump vacuum. The evolved CO₂ was inactive. The crude succinic acid was dissolved in 15 ml. hot water, treated with charcoal (Nuchar 00), and the filtered solution evaporated to a few ml. Three crops of pure crystals (mp 188-190°) were removed to give 1.47 g. succinic acid-1-C¹⁴ (59.4% based on acetic acid used) with a specific activity of 5.0 μ c/mg. (calculated specific activity 4.8 μ c/mg.)

Succinic-2-C¹⁴ acid: This acid was prepared from 21 millimoles sodium acetate-2-C¹⁴ following the above method. The yield of succinic acid was 1.46 g. (59.2%).

Ethyl acetate-2-C¹⁴: Sodium acetate-2-C¹⁴ (1.42 g., 17.4 millimoles, 8.71 mc) was converted to ethyl acetate using diethyl sulfate as previously described⁽³⁾.

Ethyl oxalacetate-C¹⁴: To a 120 ml. pear-shaped flask equipped with a water solid condenser that could be attached to the vacuum line, were added 2.16 g. (40 millimoles) sodium methoxide, 50 ml. ether dried over sodium and 4.38 ml. (30 millimoles) diethyl oxalate. The condenser was attached to the vacuum line, the mixture frozen in liquid nitrogen and the labeled ethyl acetate prepared previously distilled in under vacuum. The reaction unit was removed from the vacuum line, protected with a calcium sulfate drying tube and refluxed overnight.

(3) B. M. Tolbert, et al., J. Org. Chem., 14, 525 (1949).

The reaction mixture was cooled, acidified with 3.5 ml. 18 N sulfuric acid and filtered with an ether wash directly into a hydrogenating flask through a sintered glass funnel which was covered with anhydrous calcium sulfate. The ether was evaporated off in a dry air stream.

Malic- β -C¹⁴ acid and succinic-2-C¹⁴ acid: Fifty ml. absolute ethanol and 500 mg. platinum oxide and a small covered bar magnet stirrer were added to the flask containing the diethyl oxalacetate-2-C¹⁴ and hydrogenation carried out at room temperature and pressure with stirring. At the end of 3 hours 100 mg. more platinum oxide was added and reaction continued 2 more hours to 75% reduction.

The catalyst was filtered off through a layer of filter aid into a 500 ml. round bottom flask and the filtration was followed by numerous ethanol washes. To the filtrate 175 ml. of 0.5 M sodium hydroxide was added, and after 10 hours of stirring, the mixture was evaporated under vacuum at room temperature to a small volume and neutralized with cold 6 N sulfuric acid. The excess oxalic acid was precipitated with 15 ml. of 1 M calcium chloride and the calcium oxalate filtered off. The filtrate was acidified with sulfuric acid and steam distilled to recover any unreacted sodium acetate-2-C¹⁴ (202 mg., 14% of starting material).

The residue was concentrated to 15 ml. on the steam bath, and extracted with ether in liquid-liquid extractor for 50 hours. The ether was evaporated off, the residue taken up in 3 ml. 0.1 N hydrochloric acid and the malic and succinic acids repeatedly distributed between ether and water until the combined aqueous solutions contained over 90% of the malic acid and the ether solution over 90% of the succinic acid. The distribution coefficients between ether and water are 0.15 for succinic acid and 0.016 for malic acid.

The combined solutions were evaporated to dryness and recrystallized from ethylacetate-hexane. Based on unrecovered sodium acetate the yield of malic- ~~β~~ -C¹⁴ acid was 512 mg. (25.8%) with a specific activity of 3.2 μ c/mg. For the succinic-2-C¹⁴ acid 98 mg. (5.6%) with a specific activity of 3.9 μ c/mg was obtained. In another high specific activity run 39% malic and 24% succinic acid were obtained, but no labeled acetic acid was recovered.

Fumaric-2-C¹⁴ acid: Approximately one millimole (150 mg. of malic- ~~β~~ -C¹⁴ acid was weighed into a small (about 2 ml.) ignition tube, which was sealed and heated 2.5 hours at 160-170°. The contents of the tube was washed with a total of 0.5 ml. hot water into a 1 ml. centrifuge tube, dissolved by warming, and allowed to cool. The crystals were centrifuged down and washed with a little cold water. The washes were combined in an ignition tube, evaporated to dryness, and the tube sealed and reheated. This process was repeated three times to give a total of 105 mg. of fumaric-2-C¹⁴ acid (81% yield based on malic acid).

dl-Tartaric- ~~α~~ -C¹⁴ acid: The fumaric-2-C¹⁴ acid from the preceding preparation, 105 mg. (0.905 millimoles), was dissolved in 15 ml. hot water in a 40 ml. graduated centrifuge tube. To this solution was added 143 mg. (1.17 millimoles) potassium chlorate dissolved in a few ml. water and 0.050 ml. of a freshly prepared 1% solution of osmic acid. The tube was stoppered and heated 12 hours at 50° C. The cooled solution was extracted with benzene to remove excess osmic acid, concentrated on the steam bath to 7 ml. neutralized with dilute sodium hydroxide solution and 3 ml. saturated barium chloride solution added. The tube was cooled overnight in the refrigerator and the barium tartrate filtered off and washed with cold water.

Excess dilute sulfuric acid was added to the precipitate and the solution ether extracted overnight. The ether was evaporated off and the crystals washed with a little cold water. Yield of dl-tartaric- ~~α~~ -C¹⁴ acid

was 2.9 ~~mc~~/mg. Subsequent work has shown that barium tartrate may be more easily converted to the free acid by use of Dowex 50 cation exchange resins. By this method the yield of dl-tartaric acid from 570 mg. of barium tartrate was 98%.