[CONTRIBUTION FROM THE RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY][1]

Small Scale Preparation of Carbon-14 Labeled Succinic, Malic, Fumaric and Tartaric Acids

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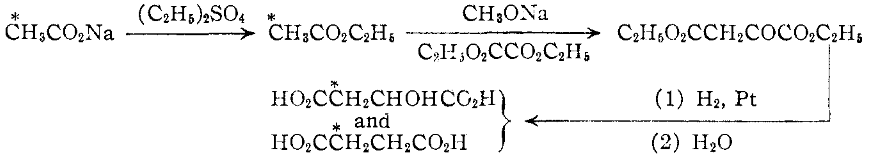
The directions for preparing the following acids from labeled acetic acid on a twenty-millimole scale are presented: succinic acid, malic acid, fumaric acid and tartaric acid. Two methods for preparing the succinic acid are detailed.

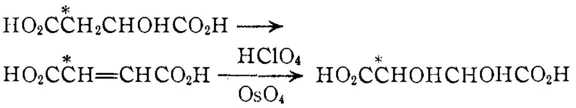
On a twenty-millimole scale, succinic acid, labeled in either the one or two position, and malic acid, labeled in either the three or four position, with carbon-14 were prepared from the corresponding labeled acetic acids as[2,3] shown:



$$\overset{*}{C}H_3CO_2Na \xrightarrow{(C_2H_5)_2SO_4} \overset{*}{C}H_3CO_2C_2H_5 \xrightarrow[C_2H_5O_2CCO_2C_2H_5]{CH_3ONa} C_2H_5O_2CCH_2COCO_2C_2H_5$$

$$\left.\begin{matrix} HO_2C\overset{*}{C}H_2CHOHCO_2H \\ \text{and} \\ HO_2C\overset{*}{C}H_2CH_2CO_2H \end{matrix}\right\} \xleftarrow[(2)\ H_2O]{(1)\ H_2,\ Pt}$$

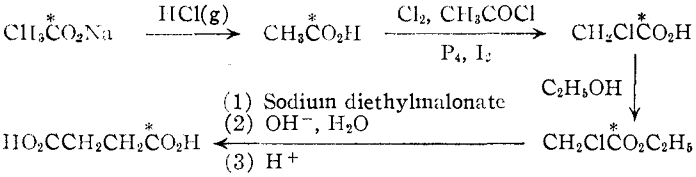
The malic and succinic acids were separated by distribution between ether and water and one millimole of malic acid dehydrated to fumaric acid[4] which was then oxidized to tartaric acid[5]



$$HO_2C\overset{*}{C}H_2CHOHCO_2H \longrightarrow$$

$$HO_2C\overset{*}{C}H{=}CHCO_2H \xrightarrow[OsO_4]{HClO_4} HO_2C\overset{*}{C}HOHCHOHCO_2H$$

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



$$CH_3\overset{*}{C}O_2Na \xrightarrow{HCl(g)} CH_3\overset{*}{C}O_2H \xrightarrow[P_4,\ I_2]{Cl_2,\ CH_3COCl} CH_2Cl\overset{*}{C}O_2H$$

$$CH_2Cl\overset{*}{C}O_2H \xrightarrow{C_2H_5OH} CH_2Cl\overset{*}{C}O_2C_2H_5$$

$$HO_2CCH_2CH_2\overset{*}{C}O_2H \xleftarrow[(3)\ H^+]{(1)\ \text{Sodium diethylmalonate}\quad (2)\ OH^-,\ H_2O} CH_2Cl\overset{*}{C}O_2C_2H_5$$

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 using butanol–propionic acid–water (2:1:1.4 ratio) in one direction and phenol saturated with water in the other.[6] Radioautographs of the papers were then made and the location of the spots (*i.e.*, the R_f values) were compared with chromatograms of authentic materials.

In the separation of the malic and succinic acid prepared *via* oxalacetic acid, distribution of the acids was continued until less than 1% of the other labeled compound remained in the desired acid. This was accurately determined by paper chromatographic separation of the products and counting of the radioactive spots.

Although it has been reported that heating malic acid for various periods at temperatures above 150° will give a mixture of products[7,8] including maleic acid and maleic anhydride in addition to the desired fumaric acid, these products were not observed in the chromatograms and radioautographs of the recrystallized fumaric acid. Tests with inactive material showed these likely impurities would readily separate with the solvents used and were not lost by volatilization of the product from the paper.

The osmium tetraoxide oxidation of malic acid has been shown to give at least 99% pure tartaric acid.[5] As expected the paper chromatograms and radioautographs showed only one radioactive compound in the purified product.

Experimental

Chloroacetic-1-C^{14} Acid.—Sodium acetate-1-C^{14} (1.15 g., 14 millimoles, specific activity 10 μc./mg.) was converted to free acid and chlorinated as previously described.[9,10]

Ethyl Chloroacetate-1-C^{14}.—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 λ of 95% sulfuric acid was added. The cup of the extractor was filled with four-mesh anhydrous calcium sulfate and the reaction mixture heated for six hours under dry reflux.

Succinic-1-C^{14} 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) of 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-C^{14} was added to the refluxing solution of sodiomalonic ester over a one-hour period, then held under reflux for an additional half-hour. Twenty-five ml. of 4 N sodium hydroxide was added to the reaction mixture and refluxing continued overnight (10–15 hours).

The ethanol was distilled off and the aqueous solution washed into a liquid–liquid extractor, carefully acidified

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

(2) F. Adickes and G. Andreson, *Ann.*, **555**, 41 (1943).

(3) M. Fallebin, *Ann. chim.*, [10] **4**, 156 (1925).

(4) A. Baeyer, *Ber.*, **18**, 676 (1885).

(5) N. A. Milas and E. M. Terry, THIS JOURNAL, **47**, 1412 (1925).

(6) A. A. Benson, *et al.*, *ibid.*, **72**, 1710 (1950).

(7) J. Pelouze, *Ann.*, **11**, 263 (1834).

(8) J. Wislicenus, *ibid.*, **246**, 91 (1888).

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

(10) D. M. Hughes, R. Ostwald and B. M. Tolbert, THIS JOURNAL, **74**, 2434 (1952).

with 10 ml. of 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 acid 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 carbon dioxide 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 (m.p. 188–190°) were removed to give 1.47 g. of succinic-1-C^{14} acid (59.4% based on acetic acid used) with a specific activity of 5.0 μc./mg. (calculated specific activity 4.8 μc./mg.).

Succinic-2-C^{14} Acid.—This acid was prepared from 21 millimoles of sodium acetate following the above method. The yield of succinic acid was 1.46 g. (59.2%).

Ethyl Acetate-2-C^{14}.—Sodium acetate-2-C^{14} (1.42 g., 17.4 millimoles, 8.71 mc.) was converted to ethyl acetate using diethyl sulfate as previously described.[11]

Ethyl Oxalacetate-3-C^{14}.—To a 120-ml. pear-shaped flask equipped with a water-cooled condenser that could be attached to the vacuum line were added 2.16 g. (40 millimoles) of sodium methoxide, 50 ml. of ether dried over sodium and 4.38 ml. (30 millimoles) of diethyl oxalate. The condenser was attached to the vacuum line, the mixture frozen in liquid nitrogen and the labeled ethyl acetate distilled under vacuum. The reaction unit was removed from the vacuum line, protected with a calcium sulfate drying tube opened to the air, and refluxed overnight.

The reaction mixture was cooled, acidified with 3.5 ml. of 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-3-C^{14} Acid and Succinic-2-C^{14} Acid.—Fifty ml. of absolute ethanol, 500 mg. of platinum oxide and a small covered bar magnet stirrer were added to the flask containing the diethyl oxalate-2-C^{14} and hydrogenation carried out at room temperature and pressure with stirring. At the end of three hours 100 mg. more of platinum oxide was added and reaction continued two more hours to 75% reduction.

The catalyst was filtered off through a layer of filter aid into a 500-ml. round bottom flask. The filtration was followed by numerous ethanol washes. To the filtrate 175 ml. of 0.5 *M* sodium hydroxide was added, and after ten 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 the unreacted sodium acetate-2-C^{14} (202 mg., 14% of starting material).

The residue was concentrated to 15 ml. on the steam-bath and extracted with ether in a 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 ethyl acetate–hexane. Based on unrecovered sodium acetate the yield of malic-3-C^{14} acid was 512 mg. (25.8%) with a specific activity of 3.2 μc./mg. For the succinic-2-C^{14} acid 98 mg. (5.6%) with a specific activity of 3.9 μc./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-C^{14} Acid.—Approximately one millimole (150 mg.) of malic-3-C^{14} 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. of 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-C^{14} acid (81% yield based on malic acid).

***dl*-Tartaric-2-C^{14} Acid.**—The fumaric-2-C^{14} acid from the preceding preparation, 105 mg. (0.905 millimole), was dissolved in 15 ml. of hot water in a 40-ml. graduated centrifuge tube. To this solution was added 143 mg. (1.17 millimoles) of potassium chlorate dissolved in a few ml. of water and 0.050 ml. of freshly prepared 1% solution of osmic acid. The tube was stoppered and heated for 12 hours at 50°. 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 and 3 ml. of 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. The yield of *dl*-tartaric-2-C^{14} acid was 56.5 mg. of 42% based on fumaric acid. The observed and calculated specific activity was 2.9 μc./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%.

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[11] B. M. Tolbert, *et al.*, *J. Org. Chem.*, **14**, 525 (1949).