## [76] Synthesis of *threo*- and *erythro*-Isocitric Acids[1]

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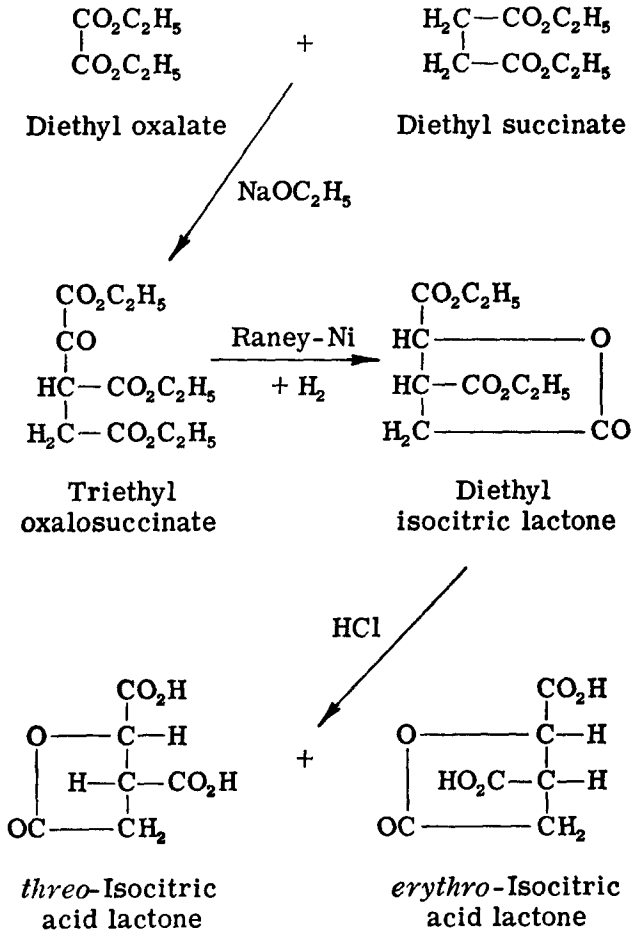


### Triethyl Oxalosuccinate[2]

A 500 ml three-necked flask is fitted with a reflux condenser, a dropping funnel, and stirrer. The opening of the dropping funnel is fitted with a drying tube filled with anhydrous calcium chloride. Absolute ethanol (180 ml) is placed in the flask, followed by 12.5 g (0.5 g atom)

[1] H. Katsura, *Nippon Kagaku Zasshi* (*J. Chem. Soc. Japan Pure Chem. Sect.*) **82**, 91 (1961).

[2] This procedure is based upon a private communication from Setsuji Sakurai.

of metallic sodium, added in small portions and dissolved by gentle heating. Ethyl oxalate, 81 g (0.5 mole), is then added dropwise at 60°, and the solution is stirred continuously for a few minutes after the addition is complete. This is followed by 87 g (0.55 mole) of ethyl succinate, added in one portion. The reaction mixture is set aside at 10–30° overnight. After ethanol is removed *in vacuo*, the reddish residue is dissolved in 500 ml of water and extracted to remove impurities with 100 ml portions of ether until the ether extract is colorless. The aqueous layer is acidified with 100 ml of concentrated hydrochloric acid, using Congo-red paper as an indicator.

An oily substance separates, which is extracted with 100 ml portions of ether, until the aqueous layer is almost colorless. The ether layer is washed with 100 ml portions of 0.1 *N* sodium hydroxide until the combined washings are no longer acidic to litmus paper. This is followed by one extraction with 100 ml of water. The ether layer is then dried with anhydrous sodium sulfate. The solvent is removed *in vacuo*. The residue consists of almost pure ethyl oxalosuccinate.

The yield is 102–113 g (72–83% of calculated value). This substance may be used in the subsequent steps without further purification.

### Diethyl Isocitric Lactones

*Preparation of Catalyst.* To a solution of 26 g of sodium hydroxide in 100 ml of water, 20 g of Raney-alloy (nickel content about 50% by weight) is added in small portions to permit a continuous, vigorous evolution of hydrogen gas. After all alloy is added, the reaction mixture is heated on a boiling water bath until gas evolution stops. The resulting nickel catalyst is washed repeatedly with distilled water until the washings are free of alkali.

*Chloroplatinate Solution.* One gram of chloroplatinic acid is dissolved in 10 ml of 1 *N* hydrochloric acid. It is neutralized with 1 *N* sodium hydroxide solution just before use.

*Method A.* To a solution of 6.3 g (0.023 mole) of ethyl oxalosuccinate in 120 ml of methanol, add the catalyst (prepared from 20 g of alloy) and 1.5 ml of the chloroplatinate solution. Shake the mixture in an atmosphere of hydrogen gas at room temperature. Hydrogen absorption occurs immediately and 368 ml of hydrogen (about 80% of the theoretical amount) is absorbed within 1 hour; thereafter hydrogen absorption is slow. Continue the reduction another 8 hours until the ferric chloride test is negative. The hydrogen uptake reaches 448 ml (almost the theoretical amount). Filter the catalyst from the reaction mixture and wash it with methanol several times. Combine the filtrate and washings and concentrate them *in vacuo*. Distill the residue under reduced

pressure (5 mm Hg), discarding a small amount of the early distillate. A mixture of the lactones of ethyl isocitrate distills at 145–165°. The yield is 4.8 g (90% of theoretical value).

*Method B.* To a solution of 60 g (0.22 mole) of ethyl oxalosuccinate in a 500 ml autoclave, add the catalyst (prepared from 80 g of alloy), 3 ml of the chloroplatinate solution, and 150 ml of methanol. Shake the mixture with hydrogen at a pressure of about 50 atmospheres at room temperature. Although the hydrogen absorption ceases within 1 hour, the reduction must be continued for another 2 hours. The lactones of ethyl isocitrate are obtained as described in Method A. The yield is 45 g (90% of theoretical value; $B.P._2$ 135–140°).

### *threo-* and *erythro*-Isocitric Lactones

Heat diethyl isocitrate (70 g) for 6 hours under reflux with 700 ml of 3 *N* hydrochloric acid. Concentrate the reaction mixture *in vacuo* and heat the residue in a boiling water bath under reduced pressure. A mixture of *threo-* and *erythro*-isocitric lactones is obtained in a quantitative yield.

### Separation of *threo-* and *erythro*-Isocitric Lactones

Dissolve the mixture of lactones (4.3 g, 0.025 mole) in 43 ml of water. To this solution, add 8.6 ml (8.4 g, 0.106 mole) of pyridine and 43 ml of 94% ethanol. Refrigerate the solution overnight. Filter off crystals of the pyridine salt of the *erythro* isomer; these are recrystallized from 94% ethanol. The yield is 1.4 g (22.4%). The pure salt melts at 139.5–141°.[3]

Dissolve the above salt (1.8 g) in 1.5 *N* aqueous sodium hydroxide solution and concentrate it to dryness *in vacuo*. Acidify the residue with concentrated hydrochloric acid, approximately 3.2 ml, concentrate the mixture to dryness *in vacuo*, and heat it at 100° for 2 hours *in vacuo*. The resulting residue is extracted ten times with 30 ml portions of hot ethyl acetate. Evaporate the combined extracts at 40–50° *in vacuo* to obtain pure *erythro*-isocitric lactone. The yield is 1.0 g (80%).

The filtrate obtained from the pyridine salt of the *erythro* isomer contains *threo*-isocitric lactone. It is made alkaline by adding 25 ml of 1.5 *N* sodium hydroxide and is then concentrated to dryness *in vacuo*. The residue is acidified with about 10 ml of concentrated hydrochloric acid, the mixture is concentrated to dryness *in vacuo*, and is heated *in vacuo* at 100° for 2 hours. The resulting residue is extracted ten times

[3] A. F. Senear, [*J. Am. Chem. Soc.* 77, 2564 (1955)] reported a melting point of 138–140°.

with 100 ml portions of hot ethyl acetate. The combined extracts are evaporated to dryness *in vacuo* at 40–50° to yield pure *threo*-isocitric lactone. The yield is 2.9 g (67.5%; B. P. 159.5–161°).

The *threo*- and *erythro*-isocitric lactones can be identified by means of paper chromatography in the solvent system butanol–formic acid–water (4:1:2). The spots are detected by spraying with 0.05% thymol-blue in 94% ethanol. The $R_f$ values are 0.55 and 0.60 for the *threo* and *erythro* isomers, respectively.

### Addendum

Isocitric acid was synthesized from sodium succinate and chloral,[4] and by the reduction of ethyl oxalosuccinate with sodium amalgam.[5] *threo*-$D_s$-(−)-Isocitric lactone was separated in considerable amounts from leaves of *Bryophyllum calycinum* by Pucher and Vickery,[6] and *erythro*-$L_s$-(+)-isocitric lactone was separated from a culture of *Penicillium purpulogenum* var. *rubrisclerotium* Thom. No. 1148 by Sakaguchi.[7]

[4] R. Fittig and H. E. Miller, *Ann. Chem.* **255**, 43 (1889); H. A. Krebs and L. V. Eggleston, *Biochem. J.* **38**, 426 (1944); G. W. Pucher and H. B. Vickery, *J. Biol. Chem.* **163**, 169 (1946); H. P. Kato and S. R. Dickmann, *Biochem. Prep.* 3, 52 (1953).

[5] W. Wislecenus and N. Nassauer, *Ann. Chem.* **285**, 1 (1895); G. W. Pucher and H. B. Vickery, *J. Biol. Chem.* **163**, 169 (1946).

[6] G. W. Pucher, *J. Biol. Chem.* **145**, 511 (1942); G. W. Pucher, M. D. Abrahams, and H. B. Vickery, *ibid.* **172**, 579 (1948).

[7] T. Beppu, S. Abe, and K. Sakaguchi, *Bull. Agr. Chem. Soc. Japan* **21**, 263 (1957); K. Sakaguchi and T. Beppu, *Arch. Biochem. Biophys.* **83**, 131 (1959).

## [77] Isolation and Properties of Hydroxycitric Acid

*By* Y. S. Lewis

Hydroxycitric acid (1,2-dihydroxypropane-1,2,3-tricarboxylic acid) has two asymmetric centers, hence two pairs of diastereoisomers or four different isomers (I, II, III, and IV) are possible.[1] Being a γ-hydroxy acid, it cyclizes readily to the corresponding lactone. The relative rates of cyclization of the different isomers to the lactones are not known.

[1] The nomenclature for the stereoisomers of hydroxycitric acid was kindly suggested by Dr. H. B. Vickery, Connecticut Agricultural Experimental Research Station, New Haven, Connecticut.