line calcium salts ($CaC_6H_4O_7 \cdot 4\ H_2O$). The lactones give a positive test with hydroxylamine and can be assayed (0.1–1 μM) as their hydroxamates by a slight modification[13] of the Lipmann-Tuttle procedure.[14] Hydroxycitric acid (IV) can be determined by a modification[13] of the metavanadate procedure.[15] Some preliminary work on the microbial metabolism of hydroxycitric acid (II) has recently been reported.[16]

The structures predicted for the *Hibiscus* and *Garcinia* acids[8] have now been verified and found correct by X-ray diffraction studies carried out by Dr. Jenny Glusker, The Institute for Cancer Research, Philadelphia, Pennsylvania (private communication).

Acknowledgment

The author is indebted to Dr. P. R. Krishnaswamy and Dr. D. Rajagopala Rao for their assistance in the preparation of this article.

[13] Unpublished procedure of D. R. Rao, 1965.
[14] F. Lipmann and L. C. Tuttle, *J. Biol. Chem.* **159**, 21 (1945).
[15] J. R. Matchett, R. R. Legavit, C. C. Nimmo, and G. K. Notter, *Ind. Eng. Chem.* **36**, 851 (1944).
[16] D. Rajagopal Rao and M. Ramakrishna, *Biochem. Z.* **344**, 399 (1966).

[78] Preparation of Homocitric, Homoaconitic, and Homoisocitric Acids

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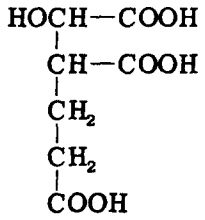
Principle

The methods for the preparation of homocitric and homoaconitic acids are based on the work of Strassman and co-workers.[1,2] The synthesis of homoisocitric acid is based on the method of Yamashita.[3] Homoisocitric acid (I) is obtained by reduction of triethyl 2-oxaloglutarate, prepared by condensation of diethyl glutarate with diethyl oxalate in the presence of sodium ethoxide. The free acid is obtained from the triester of homoisocitric acid by hydrolysis. Homoaconitic acids (II) are prepared by dehydrating triethyl homoisocitrate with acetyl chloride, followed by hydrolysis to the free acids. The *cis* and *trans* isomers of homoaconitic acid may be separated by anion-exchange chromatography; *trans*-homo-

[1] M. Maragoudakis and M. Strassman, *J. Biol. Chem.* **241**, 695 (1966).
[2] M. Strassman and L. N. Ceci, *J. Biol. Chem.* **241**, 5401 (1966).
[3] M. Yamashita, *J. Org. Chem.* **23**, 835 (1958).

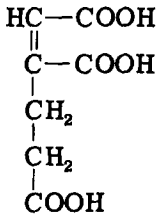
aconitic acid is eluted first from a column of Dowex 1-formate according to the method of Busch *et al.*[4]

Homocitric acid (III) is prepared by treating diethyl β-ketoadipate with hydrogen cyanide, followed by hydrolysis of the cyanohydrin to the free acid. The intermediate diethyl β-ketoadipate is prepared by condensing magnesium malonic ester with β-carbethoxypropionyl chloride followed by thermal decomposition of diethyl β-keto-α-carbethoxyadipate in the presence of β-naphthalenesulfonic acid.



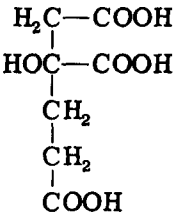
Homoisocitric acid

(I)



Homoaconitic acid

(II)



Homocitric acid

(III)

Procedure

Homocitric Acid

Preparation of Diethyl β-Ketoadipate.[5] The preparation of diethyl β-keto-α-carbethoxyadipate via the condensation of magnesium malonic ester with β-carbethoxypropionyl chloride is described in Vol. III [88]. To 220 g of diethyl β-keto-α-carbethoxyadipate is added 14 g of β-naphthalene sulfonic acid monohydrate. The reaction mixture is heated slowly; gas evolution occurs at 130–140°, and heating is continued to 190–200°, until effervescence ceases. The mixture is cooled, and ether is added (100 ml). The ether solution is washed with four portions of cold 10% sodium carbonate solution. The combined aqueous washes are extracted once with ether. The combined ether solutions are washed successively with water, *M* sulfuric acid, and water, and then dried with anhydrous sodium sulfate. The carbonate solution is acidified and extracted with ether. This ether extract contains approximately 50 g of unreacted diethyl β-keto-α-carbethoxyadipate, which may be treated again with 5 g of β-naphthalene

[4] H. Busch, R. B. Hurlbert, and V. R. Potter, *J. Biol. Chem.* **196**, 717 (1952); and H. Busch, Vol. III [70].

[5] B. Riegel and W. M. Lilienfeld, *J. Am. Chem. Soc.* **67**, 1273 (1945).

sulfonic acid and worked up as described above. The ether layers are combined and fractionated through a 30 cm Vigreux column at a pressure of 0.5 mm Hg after removal of ether. The forerun, b.p. 80–90°, consists of diethyl succinate and diethyl malonate and is discarded. The main fraction distills at 122–126° at 0.5 mm Hg and is relatively pure (95%) diethyl β-ketoadipate (86 g, 40% yield).

Preparation of Homocitric Acid. The diethyl β-ketoadipate obtained above is dissolved in 300 ml of ether to which is added 30 ml of water and 45 g of finely powdered potassium cyanide. The mixture is stoppered and cooled in an ice bath for 10 minutes. Then 60 ml of concentrated hydrochloric acid is added in 5 ml portions with vigorous shaking over a period of 2 hours. The reaction mixture is kept at room temperature overnight, the ether layer is separated, and the aqueous layer extracted 4 times with 50 ml portions of ether. The combined ether solutions are dried with anhydrous sodium sulfate and evaporated. The residue is dissolved in 125 ml of concentrated hydrochloric acid and heated on a steam bath for 6 hours. After cooling, the mixture is filtered to remove ammonium chloride. The filtrate is evaporated to dryness under reduced pressure and the residue is dissolved in 100 ml of hot ethyl acetate. The remaining insoluble ammonium chloride is filtered off. Evaporation of the ethyl acetate yields a viscous residue of homocitric acid (72 g). To crystallize, the residue is dissolved in water, the pH is adjusted to 1 with concentrated sulfuric acid and continuously extracted with ether for 2 days, fresh ether being added after 24 hours. The combined ether extracts are evaporated to dryness, and the residue is crystallized from hot ethyl acetate, yielding homocitric lactone, m.p. 160–162° (70 g, 93% yield). The salt of the free tricarboxylic acid is obtained by heating the lactone in excess base.

Homoisocitric acid

Preparation of Triethyl β-Oxaloglutarate. To an ice-cooled stirred suspension of freshly made sodium ethoxide (34 g) in 370 ml of anhydrous ether is added 73 g of diethyl oxalate; when nearly all the ethoxide is dissolved, 94 g of diethyl glutarate is added over 5 minutes with continued stirring and cooling until the solution becomes clear (the color turns from yellow to red). The mixture is kept at 0–5° for 3 days, then poured onto ice. The ether solution is separated, and the aqueous layer is washed with ether. The aqueous solution is acidified with cold *M* sulfuric acid, and then extracted with ether. The ether solution is shaken with 1 g of barium carbonate, dried with anhydrous sodium sulfate, and filtered. Evaporation of the ether filtrate yields 120 g (81% yield) of a viscous, light yellow residue of triethyl β-oxaloglutarate.

Preparation of Homoisocitric Acid. Thirty grams of triethyl β-oxaloglutarate, 500 ml of 95% ethanol, and 180 mg of platinum dioxide are shaken with hydrogen at 45 psi pressure for 16 hours. The mixture is filtered, and the filtrate is evaporated under reduced pressure. The residue is dissolved in 500 ml of ether, washed 3 times with 50 ml portions of 10% potassium carbonate, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to yield 28 g of a residual oil having a fruitlike odor. On distillation, the colorless product (b.p. 140–141° at 0.005 mm Hg) triethyl homoisocitrate is collected. The triethyl homoisocitrate is refluxed for 2 hours with 262 ml of 0.9 *N* sodium hydroxide (about an equivalent amount). Then the solution is acidified with hydrochloric acid to pH 1 and continuously extracted with ether for 5 days; the ether extract is evaporated to dryness under reduced pressure. The residue is dissolved in water and evaporated to dryness again to remove traces of hydrochloric acid. The residual sirup, stored over phosphorus pentoxide *in vacuo,* solidifies. Homoisocitric acid is crystallized from acetone–benzene (m.p. 127–129°, 81% yield).

Homoaconitic Acid

Triethyl homoisocitrate (140 g, 0.48 mole), obtained as described above, is placed in a flask immersed in ice. One hundred milliliters of freshly distilled acetyl chloride is added, the flask is stoppered and shaken vigorously, a condenser fitted with a drying tube is inserted, and the flask is heated to reflux for 3 hours. The reaction mixture is rapidly cooled to 20°, an additional 75 ml of acetyl chloride is added, and refluxing resumed for an additional 3 hours. The reaction mixture is cooled, and volatile material removed by evaporation under reduced pressure, followed by distillation at a pressure of 0.5 mm Hg at 70°, yielding a viscous yellow residue.

The mixture of triesters is hydrolyzed by refluxing with 400 ml of 5 *N* sodium hydroxide for 1 hour. The solution is neutralized by the addition of 6 *N* hydrochloric acid and treated repeatedly with 5 g portions of activated charcoal. After removal of the charcoal, the solution is evaporated to dryness, leaving a light yellow glassy residue. The residue is dissolved in 300 ml of water with warming, the solution adjusted to pH 1 with concentrated hydrochloric acid, and is continuously extracted with ether for 24 hours. The extraction is interrupted after 3 hours and again after 10 hours. The 0–3 hour ether extract contains 18 g of homoaconitic acid (predominantly the trans isomer) and the 3–10 hour extract contains 15 g of a mixture of *cis*-homoaconitic acid (approximately 3 g), *trans*-homoaconitic acid, and approximately 11 g of homoisocitric acid. The last ether extract and the aqueous phase contains predominantly unreacted homoisocitric acid.

cis-Homoaconitic acid can be obtained in pure form by chromatography of the 3–10 hour ether extract on a column of Dowex 1-formate.[4] Five grams of the residue are dissolved in 10 ml of water and neutralized to pH 7 by addition of 5 *N* sodium hydroxide. The solution is placed on a Dowex 1-formate column (3.5 × 40 cm) and the column is eluted by gradient elution with 6 *N* formic acid flowing into a mixing flask containing 400 ml of water. Fractions of 10 ml are collected. Homoisocitric acid is eluted in fractions 90–130, *trans*-homoaconitic acid in fractions 225–275, and *cis*-homoaconitic acid in fractions 350–450. Both *cis*- and *trans*-homoaconitic acids can be crystallized from ethyl acetate–benzene. *trans*-Homoaconitic acid melts at 148–149°, *cis*-homoaconitic acid at 80–81°. *cis*-Homoaconitic acid forms an anhydride more readily than *trans*-homoaconitic acid and may be separated from *trans*-homoaconitic acid by sublimation of the anhydride at 80° at a pressure of 1 μ Hg.

Alternative Procedures

Optically active homocitric acid has been isolated from the culture medium of a lysine-requiring yeast mutant[1]; its enantiomorph, (+)-homocitric acid lactone, has been synthesized by oxidation of (−)-quinic acid to (−)-5-dehydroquinic acid, reduction of the product to (−)-5-deoxyquinic acid, and oxidation of the latter with periodate followed by bromine.[6] Natural isomers of all three acids, homocitric, *cis*-homoaconitic, and homoisocitric acids have been isolated from the culture medium of a single lysine-requiring yeast mutant.[7]

[6] U. Thomas, M. G. Kalyanpur, and C. M. Stevens, *Biochemistry* **5**, 2513 (1966).
[7] J. K. Bhattacharjee and M. Strassman, *J. Biol. Chem.* **242**, 2542 (1967).

[79] Chemical Properties and Synthesis of Fluoro Analogs of Compounds Related to Substrates of the Citric Acid Cycle[1]

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