

An Improved Synthesis of Trisodium (*R*)-Homocitrate from Citric Acid

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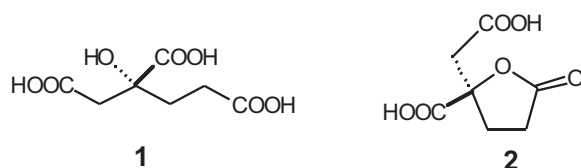
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A method of synthesis of trisodium (*R*)-homocitrate starting from citric acid is reported. The procedure affords the final product of high optical purity with satisfactory yield.

Key words: homocitric acid, synthesis, Arndt-Eistert reaction

(*R*)-Homocitric acid (2-hydroxy-1,2,4-butanetricarboxylic acid) **1**, is a natural product, known as an intermediate in the L- α -aminoadipate pathway of the L-lysine biosynthesis in some yeast, fungi and archa [1,2]. The compound is formed upon the condensation of α -oxoglutarate and AcetylSCoA. The condensation in the first step of the α -aminoadipate pathway is catalyzed by homocitrate synthase (EC 4.1.3.21). Since lysine is an essential amino acid for animals and the α -aminoadipate pathway is absent in these organisms, the enzymes catalyzing the unique steps of the fungal lysine biosynthesis pathway are considered potential targets for antifungal chemotherapy [1,2]. Inhibitors of these enzymes, including some structural analogues of (*R*)-homocitric acid may thus become effective antifungals. (*R*)-Homocitric acid is also an important component of the iron-molybdenum cofactor of bacterial nitrogenase [3]. The compound is therefore a subject of interest as a tool for biochemical studies. The acid is not available commercially and the known methods of its synthesis afford either a racemic product or after multi-step procedures the optically active homocitric acid γ -lactone **2**.



Most of the methods described in the literature have been developed to synthesize the homocitric acid γ -lactone **2**, known to be more stable than homocitric acid **1**. Some of these methods afforded a racemic product by hydrolysis of cyanohydrin of diethyl β -oxoadipate [4–7] or by indium metal-mediated allylation/oxidative cleavage procedure of α -oxoglutarate [8]. The enantiomers of the homocitric acid

γ -lactone were obtained by either an enantioselective synthesis starting from various optically active natural compounds or by homologation of an asymmetric citric acid derivative [9]. Most of the reported asymmetric syntheses use Seebach's self-regeneration of the stereo centers methodology [10] with L-serine or L-lactic acid [11], D- or L-malic acid [12,13], and L-phenylalanine [14] as starting materials. In alternative approaches, D-quinic acid [15,16], ephedrine-derived morpholinedione [17] or spiro- γ -dilactone [18] was used to give the optically active **2** upon oxidative ring cleavage.

In this paper we propose a method leading to the formation of chiral trisodium (*R*)-homocitrate from citric acid, in which the chiral resolution was applied in the preparation of one of the early intermediates.

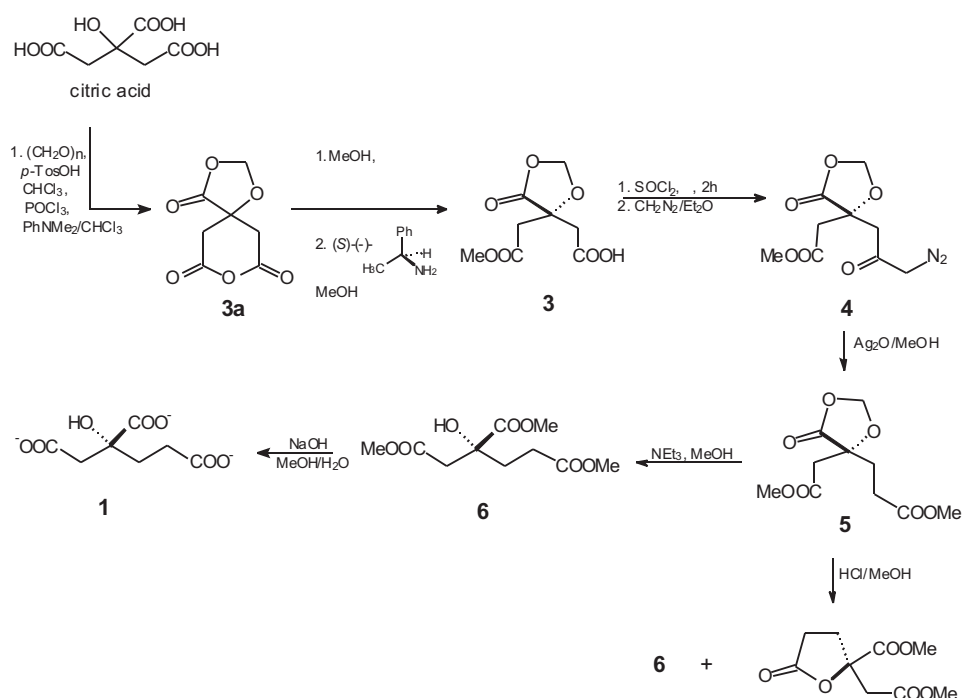
RESULTS AND DISCUSSION

Our approach to (*R*)-homocitric acid shown in Scheme 1 is based on the initial formation of an optically active derivative of citric acid, obtained in a way similar to that described by Ancliff *et al.* [9]. Although the citric acid itself is not a chiral compound, some of its derivatives are chiral. In our case, the stereogenic center was generated upon the conversion of citric acid into an asymmetric monoester of methylenecitric acid. The compound, *rac*-**3**, was prepared in three steps, according to the previously described procedure of Nau *et al.* [19], with some modifications as proposed in one of our previous publications [20]. The chiral resolution was achieved upon the formation of diastereoisomeric salts with (*S*)- α -methylbenzylamine, previously applied for the separation of methylenecitric acid [9] and homocitric acid γ -lactone [21]. It is worth mentioning that the (*S*)-enantiomer of methyl methylenecitrate can be isolated only if (*R*)- α -methylbenzylamine is used for the purpose of the chiral resolution (details are not shown). Unlike the previously published procedures, we used methanol as solvent preventing the possibility of transesterification and giving rise to the high optical purity of the (*R*)-**3** (*ee* \geq 95%) product. Extension of the $-\text{CH}_2\text{COOH}$ unit was achieved by the Arndt-Eistert method. The diazoketone **4**, which was formed from the acyl chloride previously obtained in a standard way [22], was subjected to the Wolff rearrangement in the presence of Ag_2O in methanol. Such conditions ensured the formation of dimethyl methylenehomocitrate **5** in one step, thus facilitating the product isolation.

Moreover, a possible side product, the corresponding α -chloroketone, was not formed. The cleavage of the 4-oxo-1,3-dioxolane ring is usually acid [9,12,14,20] or base catalyzed [9]. However, under acid catalyzed conditions one could expect rapid cyclization of homocitric acid to its γ -lactone. In fact, when HCl/MeOH was used, a mixture of the dimethyl γ -lactone and trimethyl homocitrate **6** was obtained. Therefore, we found more convenient and effective to obtain trimethyl homocitrate **6** in good yield by applying triethylamine in methanol. Finally, the optically active trisodium homocitrate **1** was obtained after base catalyzed hydrolysis of **6**.



Scheme 1



Since no literature data on optical activity of **1** are available, we converted a sample of it into the well characterized homocitric acid γ -lactone **2**. Physical and spectral properties of this product matched those previously reported [15,17], thus indirectly confirming an optical purity of **1**.

The described procedure is advantageous in some aspects, as compared to the previously proposed methods. Similarly as Ancliff *et al.* [9] we used citric acid, as an easily accessible and inexpensive substrate but in our case, the final product was almost optically pure, stable trisodium (*R*)-homocitrate instead of the respective lactone. Moreover, most of the intermediates **3–6** may be used for the formation of many different homocitric acid derivatives, potential inhibitors of homocitrate synthase and homoaconitase. Trimethyl homocitrate **6** is a lipophilic derivative of homocitric acid, which is able to pass through the biological membranes by free diffusion. It can subsequently be hydrolyzed by the intracellular esterases to give homocitric acid. Our method may be therefore used for the efficient formation of the optically pure homocitric acid and its derivatives.



EXPERIMENTAL

^1H and ^{13}C NMR spectra were obtained with a Varian Unity Plus spectrometer operating at 500 and 125 MHz, respectively. The deuterated solvents were used as an internal lock for ^1H and ^{13}C NMR. Optical rotations were measured on Autopol II (Rudolph Research Flanders) polarimeter. Mass spectra were recorded on Applied Biosystems MD; Sciex 4000 Q Trap LC/MS system. Melting points (m.p.) were uncorrected. Flash column chromatography was carried out on silica gel (300–400 mesh).

4-Oxo-1,3-dioxolan-5,5-diacetic acid [20]. Citric acid **1** (63 g, 0.33 mol), paraformaldehyde (12 g, 0.39 mol) and *p*-toluenesulphonic acid (0.3 g) were refluxed in chloroform (300 ml) with azeotropic trapping of H_2O for 10 h. The solvent was evaporated and the residue was crystallized from hot water. The methylenecitric acid 51.5 g (75% yield) was obtained as white crystals, m.p. 202–204°C [lit. [23] m.p. 205°C, 46% yield}.

Methylenecitric acid anhydride 3a. This compound was synthesized by procedure of Nau *et al.* [19]. The anhydride **3a** was recrystallized from ethyl acetate; yield: 87%; m.p. 154–155°C [lit. [19] m.p. 153°C}.

Monomethyl methylenecitrate rac-3. The methylenecitric acid anhydride (10 g, 0.053 mol) was refluxed in anhydrous methanol (300 ml) for 30 minutes. The excess of methanol was evaporated, water (150 ml) was added, and the mixture was extracted with diethyl ether (4×80ml). The organic phase was dried (MgSO_4) and the solvent was evaporated to dryness. The product **3** was recrystallized from H_2O ; yield: 90% (10.5 g); m.p. 126–127°C [lit. [9] m.p. 126–127°C}.

Monomethyl (R)-methylenecitrate (R)-3. The *rac*-**3** (8.1 g, 37 mmol) was dissolved in hot anhydrous methanol (32 ml) and (*S*)-(-)-benzyl methylamine (4.78 ml, 4.49 g, 37 mmol) was added dropwise. The solution was then cooled to room temperature, and anhydrous diethyl ether (55 ml) was added. The white crystals of the salt {3.6 g; m.p. 136–138°C; $[\alpha]_D^{20} + 7$ (*c* 1, CHCl_3)} were dissolved in 0.5M HCl, and the solution was extracted with diethyl ether (5×35 ml). The organic phase was washed with brine (1×15 ml) and dried over MgSO_4 . The solvent was evaporated to give (*R*)-**3** 2.2 g (27% yield) as colorless oil $[\alpha]_D^{20} + 8.9$ (*c* 4.48, MeOH) [lit. [24] *ee* 90–92%, $[\alpha]_D^{20} + 8.4$ (*c* 2.46, MeOH)}. ^1H NMR (CDCl_3) δ [ppm]: 2.91 (s, 2H, CH_2); 2.96 (s, 2H, CH_2); 3.72 (s, 3H, OCH_3); 5.56 (s, 2H, OCH_2O). ^{13}C NMR (CDCl_3) δ [ppm]: 41.24; 41.43; 52.77; 75.75; 95.53; 169.34; 173.18; 173.74.

(-)-Diazoketone 4. Methyl methylenecitrate (*R*)-**3** (3.6 g, 0.016 mol) was refluxed in SOCl_2 (10 ml) for 1 h. Excess of SOCl_2 was evaporated, CCl_4 (10 ml) was added, and the mixture was evaporated to dryness. The last step was repeated twice to remove traces of SOCl_2 . The product, 3.8 g (100% yield) was obtained as colorless oil. The oil in ether (10 ml) was treated portionwise with diazomethane in ether until development of the firm yellow color of the reaction mixture. The mixture was then stirred at room temperature for 15 h under argon. The resulting mixture was evaporated and the yellow solid residue was dissolved in ethyl acetate (30 ml). The solution was washed with 10% aqueous sodium carbonate. The water layers were back-extracted with ethyl acetate (3×30 ml). The combined organic layers were washed with water, 0.5M HCl, brine and the solution was dried over anhydrous magnesium sulfate. After being concentrated under vacuum, the residue was crystallized from hexanes to give the diazoketone (-)-**4** as yellow crystals (3.67 g, 95% yield); m.p. 53–6°C, $[\alpha]_D^{20} - 3.66$ (*c* 4.1, MeOH).

rac-Diazaketone **4**. This compound was also obtained and after flash chromatography (eluent: ethyl acetate) was isolated as pale yellow oil.

Dimethyl (R)-methylenhomocitrate 5. Ag_2O (8.75 g, 0.038 mol) was added to the solution of diazoketone **4** (3.37 g, 0.015 mol) in anhydrous methanol (190 ml) of and the mixture was stirred for 72 h at room temperature in dark. The resulting mixture was filtered to remove silver compounds and the solvent was evaporated to give the crude product, which was purified by ethyl acetate. The product **5** was obtained as pale yellow oil (2.5 g, 70%); $[\alpha]_D^{20} - 40.7$ (*c* 2.67, MeOH); *m/z* (CI): found 246.2184; $[\text{C}_{10}\text{H}_{14}\text{O}_7+\text{H}]$ requires 247.2201; ^1H NMR (CDCl_3) δ [ppm]: 2.15 (t, 2H, $\text{CH}_2\text{CH}_2\text{CO}$); 2.35–2.49 (m, 2H, $\text{CH}_2\text{CH}_2\text{CO}$); 2.90 (AB, $J = 17.1$ Hz, 2H, CH_2CO); 3.70 (s, 3H, OCH_3); 3.72 (s, 3H, OCH_3); 5.50 (s, 1H) and 5.55 (s, 1H) (both from OCH_2O); ^{13}C NMR (CDCl_3) δ [ppm]: 28.48; 32.23; 40.78; 52.19; 52.48; 77.61; 94.83; 169.82; 172.74; 173.51.

Trimethyl (*R*)-homocitrate 6. To the solution of dimethyl (*R*)-methylenhomocitrate (**5**) (285.3 mg, 1.16 mmol) in anhydrous methanol (20 ml), triethylamine (0.16 ml, 1.16 mmol) was added and the mixture was refluxed for 10 h. The reaction progress was followed by TLC. After completion of the reaction, the solvent was evaporated, the residue was dissolved in chloroform (10 ml) and solution was washed with: water, 5% KHSO₄, and water (2 ml). The organic phase was dried (MgSO₄), and the solvent was evaporated, to give (*R*)-**6** as yellow oil (200 mg, 70%) [α]_D²⁰ -10.26 (*c* 0.78, flash chromatography (eluent: CHCl₃); [α]_D²⁰ -6.5 (*c* 1.23, MeOH) {lit. [16] [α]_D²⁰ -10.2 (*c* 1.46, CHCl₃)}; *m/z* (CI): found 248.2343; [C₁₀H₁₆O₇+H] requires 249.2340; ¹H NMR (CDCl₃) δ [ppm]: 2.01–2.09 (m, 2H, CH₂CH₂CO₂CH₃); 2.27–2.31 (m, 1H, CH₂CH₂CO₂CH₃); 2.43–2.63 (m, 1H, CH₂CH₂CO₂CH₃); 2.74, 2.92 (AB, *J* = 16.4 Hz, 2H, CH₂CO₂CH₃); 3.67 (s, 3H, OCH₃); 3.68 (s, 3H, OCH₃); 3.81 (s, 3H, OCH₃); ¹³C NMR (CDCl₃) δ [ppm]: 28.58; 34.32; 43.74; 52.23; 52.38; 53.54; 74.77; 171.47; 173.74; 175.48.

Trisodium (*R*)-homocitrate 1. To a solution of trimethyl (*R*)-homocitrate (**6**) (160.8 mg, 0.65 mmol) in methanol (1.95 ml) 1M aqueous NaOH (1.95 ml) was added and the reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated and the residue was precipitated with diethyl ether to obtain (*R*)-**1**×3Na⁺ as white solid (170 mg, 96%) [α]_D²⁰ -4.79 (*c* 1.7, H₂O); *m/z* (CI): found 272.0994 [C₇H₇O₇Na₃+H] requires 273.0525.

(*R*)-Homocitric acid γ -lactone 2. A solution of trisodium (*R*)-homocitrate (**1**) (90 mg, 0.33 mmol) in TFA (2 ml) was refluxed for 8 h. The solvent was evaporated and the residue was purified on Dowex 50X8-100[H⁺] to give after solvent evaporation the final product as white solid (52 mg, 84%) [α]_D²⁰ -52.88 (*c* 1.04, H₂O) {lit. [17] [α]_D²⁰ -57 (*c* 1, H₂O), [15] [α]_D²⁰ -50 (*c* 1, H₂O)}; ¹H NMR (*d*₆-acetone) δ [ppm]: 2.0–2.7 (m, 4H); 3.0 (d, *J* = 16 Hz, 1H); 3.16 (d, *J* = 16 Hz, 1H); ¹³C NMR (CDCl₃) δ [ppm]: 28.32; 31.92; 41.74; 83.51; 170.47; 172.5; 176.48.

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REFERENCES

1. Strassman M. and Ceci L.N., *Biochem. Biophys. Res. Commun.*, **14**, 262 (1964); Strassman M. and Ceci L.N., *J. Biol. Chem.*, **240**, 4357 (1965).
2. Zabrieski T.M. and Jackson M.D., *Nat. Prod. Rep.*, **17**, 85 (2000).
3. Shah V.K. and Brill W.J., *Nat. Proc. Acad. USA*, **74**, 3249 (1977); Hoover T.R., Imperial J., Ludden P.W. and Shah V.K., *Biochemistry*, **28**, 2768 (1989).
4. Maragoudakis M.E. and Strassman M., *J. Biol. Chem.*, **241**, 695 (1966).
5. Tucci A.F., Ceci L.N. and Bhattacharjee J.K., *Methods Enzymol.*, **13**, 619 (1969).
6. Li Z.C. and Xu J.Q., *Molecules*, **3**, 31 (1998).
7. Zhou Z.H., Hou S.Y., Cao Z.X., Tai K.R. and Chow Y.L., *Inorg. Chem.*, **45**, 8447 (2006).
8. Chen H.B., Chen L.Y., Huang P.Q., Hang H.K., Zhou Z.H. and Tai K.R., *Tetrahedron*, **63**, 2148 (2007).
9. Ancliff R.A., Russell A.T. and Sanderson A.J., *Tetrahedron: Asymmetry*, **8**, 3379 (1997).
10. Seebach D., Imwinkelried R. and Weber T., *Modern Synthetic Methods*, Springer: Berlin 1986, vol. 4, p. 125.
11. Rodriguez G.H.R. and Biellmann J.F., *J. Org. Chem.*, **61**, 1822 (1996).
12. Ma G. and Palmer D.R.J., *Tetrahedron Lett.*, **41**, 9209 (2000).
13. Xu P.F., Matsumoto T., Ohki Y. and Tatsumi K., *ibid*, **46**, 3815 (2005).
14. Huang P.Q. and Li Z.Y., *Tetrahedron: Asymmetry*, **16**, 3367 (2005).
15. Thomas U., Kalyanpur M.G. and Stevens C.M., *Biochemistry*, **5**, 2513 (1966).
16. Tavassoli A., Duffy J.E.S. and Young D.W., *Tetrahedron Lett.*, **46**, 2093 (2005); Tavassoli A., Duffy J.E.S. and Young D.W., *Org. Biomol. Chem.*, **4**, 569 (2006).
17. Pansare S.V. and Adsool V.A., *Tetrahedron Lett.*, **48**, 7099 (2007).
18. Paju A., Kanger T., Pehk T., Eek M. and Lopp M., *Tetrahedron*, **60**, 9081 (2004).



19. Nau C.A., Brown E.B. and Bailey J.R., *J. Am. Chem. Soc.*, **47**, 2596 (1925).
20. Milewska M.J. and Połowski T., *Synthesis*, 475 (1988).
21. Jia Y., Palmer D.R.J. and Quail J.W., *Acta Cryst.*, **E61**, 4034 (2005).
22. Meier H. and Zeller K.P., *Angew. Chem. Int. Ed.*, **14**, 32 (1975).
23. Sternberg W., *Chem. Zentr.*, **I**, 300 (1902).
24. Chênevert R., Ngatcha B.T., Rose Y.S. and Goupil D., *Tetrahedron: Asymmetry*, **9**, 4325 (1998).