The Absolute Configuration of Homocitric Acid (2-Hydroxy-1,2,4-butanetricarboxylic Acid), an Intermediate in Lysine Biosynthesis*

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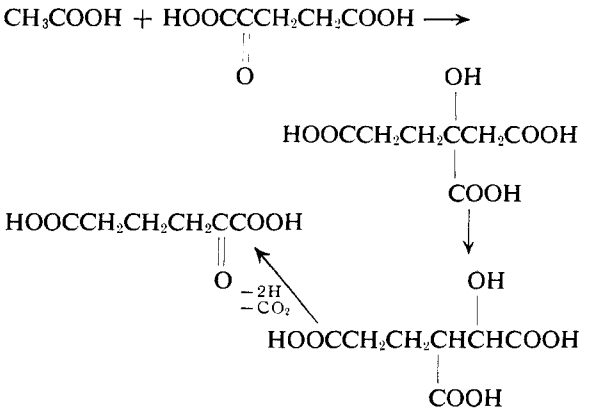
ABSTRACT: (*S*)-2-Hydroxy-1,2,4-butanetricarboxylic acid lactone [(+)-homocitric acid lactone] has been synthesized by oxidation of (−)-quinic acid to (−)-5-dehydroquinic acid, reduction of the product to (−)-5-deoxyquinic acid, and oxidation of the latter with periodate, followed by bromine.

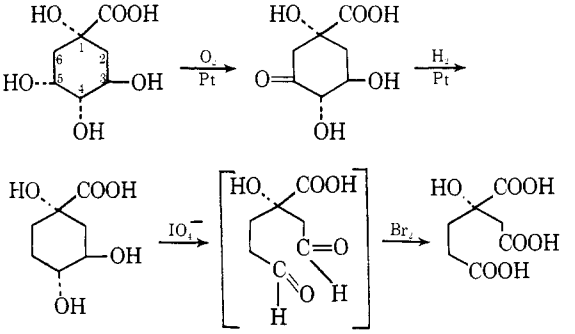
The product obtained was shown to be the enantiomorph of the compound isolated by Maragoudakis and Strassman [*J. Biol. Chem. 241*, 695 (1966)] from a lysine-requiring mutant of yeast. Therefore, the absolute configuration of the intermediate in lysine biosynthesis may be designated as (*R*)-2-hydroxy-1,2,4-butanetricarboxylic acid. In this isomer, the side chain derived from acetate assumes a position opposite to that of the acetate moiety in citrate formed by the action of citrate synthase.

The demonstration by Mitchell and Houlahan (1948) that α-aminoadipic acid supports the growth of certain lysine-requiring mutants of *Neurospora crassa* indicated clearly that the mechanism of biosynthesis of lysine in this fungus must be different from the diaminopimelic acid pathway found subsequently in bacteria. Strassman and Weinhouse (1953) suggested, on the basis of data on the incorporation of ^{14}C-labeled acetate into lysine by *Torulopsis utilis*, that the carbon skeleton of this amino acid might be formed by initial condensation of acetate and α-ketoglutarate to yield homocitrate, which gives rise to homoisocitrate, and α-ketoadipate as intermediates. The proposed conversion of homocitrate to homoisocitrate and α-ketoadipate is apparently analogous to the well-known conversion of citrate to isocitrate and α-ketoglutarate.



Recent work of Strassman *et al.* has provided tracer evidence for the biosynthesis of homocitric acid, or its lactone, from acetate, in homogenates of *Saccharomyces cerevisiae* (Strassman and Ceci, 1964), and for the accumulation of homocitric acid in the culture medium of a lysine-requiring mutant of *S. cerevisiae* (Maragoudakis and Strassman, 1966). The conversion of a synthetic preparation of homoisocitric acid (Yamashita, 1958) into α-ketoadipic acid by a yeast homogenate has also been reported (Strassman *et al.*, 1964; Strassman and Ceci, 1965).

For the elucidation of the stereochemistry and mechanism of the rearrangement involved in the enzymatic conversion of homocitric acid to homoisocitric acid, it is essential to determine the absolute configuration of the compounds involved. In the present work, the configuration of homocitric acid has been established by synthesis of one optical isomer of this compound from the naturally occurring (−)-quinic acid of known configuration (Fischer and Dangschat, 1932; Dangschat and Fischer, 1950; Hanson, 1962) through the sequence of reactions



The final product was compared with the compound isolated by Maragoudakis and Strassman (1966).

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Experimental Results

A sample of homocitric acid lactone, isolated from a culture of a lysine-requiring mutant of *S. cerevisiae*, was kindly supplied by Dr. Murray Strassman. It was recrystallized from ethyl acetate–ligroin.

(±)-Homocitric acid lactone was synthesized from diethyl β-ketoadipate by a procedure similar to that reported subsequently by Strassman and co-workers (Strassman and Ceci, 1964; Maragoudakis and Strassman, 1966). All melting points were taken on a Fischer-Johns melting point apparatus (micro hot stage).

Conversion of (−)-Quinic Acid to (−)-5-Dehydroquinic Acid. The catalytic oxidation procedure of Haslam *et al.* (1963) was employed, using 5 g of commercial (−)-quinic acid. The product was isolated by ion-exchange chromatography on a column (4 × 20 cm) of Amberlite CG-400 (100–200 mesh, in the acetate form). Gradient elution was employed, using a reservoir containing 3000 ml of 6 N acetic acid and a mixing chamber containing 1000 ml of 0.5 N acetic acid. (−)-5-Dehydroquinic acid appeared at approximately 1100–1700 ml. The average yield of white crystalline product was 1.25 g, mp 134–136°, $[\alpha]_{589}^{21}$ −47.8° (*c* 0.626, methanol); lit. (Grewe and Jeschke, 1956) mp 139–140°, $[\alpha]_{D}^{20}$ −44.5 (*c* 0.6, alcohol).

Anal. Calcd for $C_7H_{10}O_6$ (190.15): C, 44.21; H, 5.30. Found: C, 44.19; H, 5.23.

Conversion of (−)-5-Dehydroquinic Acid to (−)-5-Deoxyquinic Acid. The catalytic reduction was carried out as described by Grewe and Jeschke (1956). It was found that the product could be isolated without resort to acetylation and subsequent saponification. The effluent from the Amberlite IR-120 column was evaporated to a syrup *in vacuo* and induced to crystallize by treatment with acetone. Recrystallization from methanol–chloroform yielded a product, mp 155–156°, $[\alpha]_{589}^{21}$ −21.1° (*c* 2.13, water); lit. (Grewe and Jeschke, 1956) 164–165°, $[\alpha]_{D}^{21}$ −20.5° (*c* 2.2, water).

Anal. Calcd for $C_7H_{12}O_5$ (176.17): C, 47.72; H, 6.87. Found: C, 47.50; H, 6.68.

Because of the discrepancy in melting point, this product was converted to the diacetyl lactone as described by Grewe and Jeschke (1956). The resulting compound possessed a melting point of 128–129°, $[\alpha]_{589}^{21}$ −32.3 (*c* 1.01, methanol); lit. (Grewe and Jeschke, 1956) mp 130°, $[\alpha]_{D}^{21}$ −31.2° (*c* 1, methanol).

Anal. Calcd for $C_{11}H_{14}O_8$ (242.22): C, 54.54; H, 5.83. Found: C, 54.48; H, 6.00.

Saponification of the diacetyl lactone yielded (−)-5-deoxyquinic acid with melting point unchanged (155–156°). Paper chromatography suggested slight contamination with quinic acid. However, further purification by chromatography on silicic acid gave a product containing no detectable amount of quinic acid and unchanged melting point. (The general method of Marvel and Rands (1950) was employed, without application of pressure, using 75% *n*-butyl alcohol–25% water-saturated chloroform as solvent.)

Conversion of (−)-5-Deoxyquinic Acid to (+)-Homocitric Acid Lactone. The method employed is a slight modification of the procedure of Hanson and Rose (1963) for the conversion of (−)-quinic acid to citric acid. To a solution of 1.36 g of (−)-5-deoxyquinic acid in 15 ml of water was added 16.5 ml of a 0.54 M aqueous solution of periodic acid. The mixture was allowed to remain in the dark for 1.5 hr at room temperature and then poured on to a column (2 × 50 cm) of Dowex 1-X8 (50–100 mesh, formate form). The column was developed with 3 N formic acid. It turned dark brown and some heat was evolved. Fractions (5 ml) were collected and tested by spotting on filter paper and treatment with sodium nitroprusside and piperazine without prior treatment with sodium periodate (Cartwright and Roberts, 1955). Fractions 25–60, which yielded a brown color in the test, were combined and evaporated *in vacuo* at 35°. The resulting light yellow syrup (1.35 g) was dissolved in 20 ml of water and treated with 1.5 ml of bromine. After standing for 2 days at room temperature, the excess bromine was removed with a current of air, bromide ion was precipitated by addition of aqueous silver acetate, and the slight excess of silver ion was removed by passage through an Amberlite IR-120 column (hydrogen form). The eluent was evaporated *in vacuo*. The resulting syrup crystallized on rubbing with a glass rod (yield 1.26 g), and was recrystallized from ethyl acetate–ligroin (0.885 g; 61% of theory). The melting point of the product was 155–157°, $[\alpha]_{589}^{21}$ +60.0° (*c* 0.55, water).

Anal. Calcd for $C_7H_8O_6$ (188.13): C, 44.70; H, 4.26. Found: C, 44.74; H, 4.30.

The neutralization equivalent by titration at room temperature was 93.3 (calculated for dibasic acid, 94.0); by back-titration after warming with an excess of alkali, 61.9 (calculated for tribasic acid, 62.7). These values agree well with those to be expected for the lactone of homocitric acid.

Comparison of (+)-Homocitric Acid Lactone (Prepared from (−)-Quinic Acid) with (−)-Homocitric Acid Lactone (Isolated from Mutant of S. Cerevisiae). (A) Optical rotations. Optical rotations were determined in aqueous solution on a Durrum-JASCO optical rotatory dispersion spectrometer at a wavelength of 589 mμ and a temperature of 21°. The specific rotation was found to vary markedly with concentration, but agreement between the two isomers is excellent (Table I).

(B) Melting points.[1] Homocitric acid lactone (from (−)-quinic acid), 155–157°; homocitric acid lactone (from mutant), 154–156°; (±)-homocitric acid lactone, 161–162°; 1:1 mixture of the first two

[1] The melting points reported here for the racemic and the optically active forms of homocitric acid lactone are somewhat higher than those reported by Maragoudakis and Strassman (1966). However, under the same conditions of recrystallization and melting point determination, the optically active isomer isolated from yeast and the enantiomorph synthesized from (−)-quinic acid show almost identical melting points.

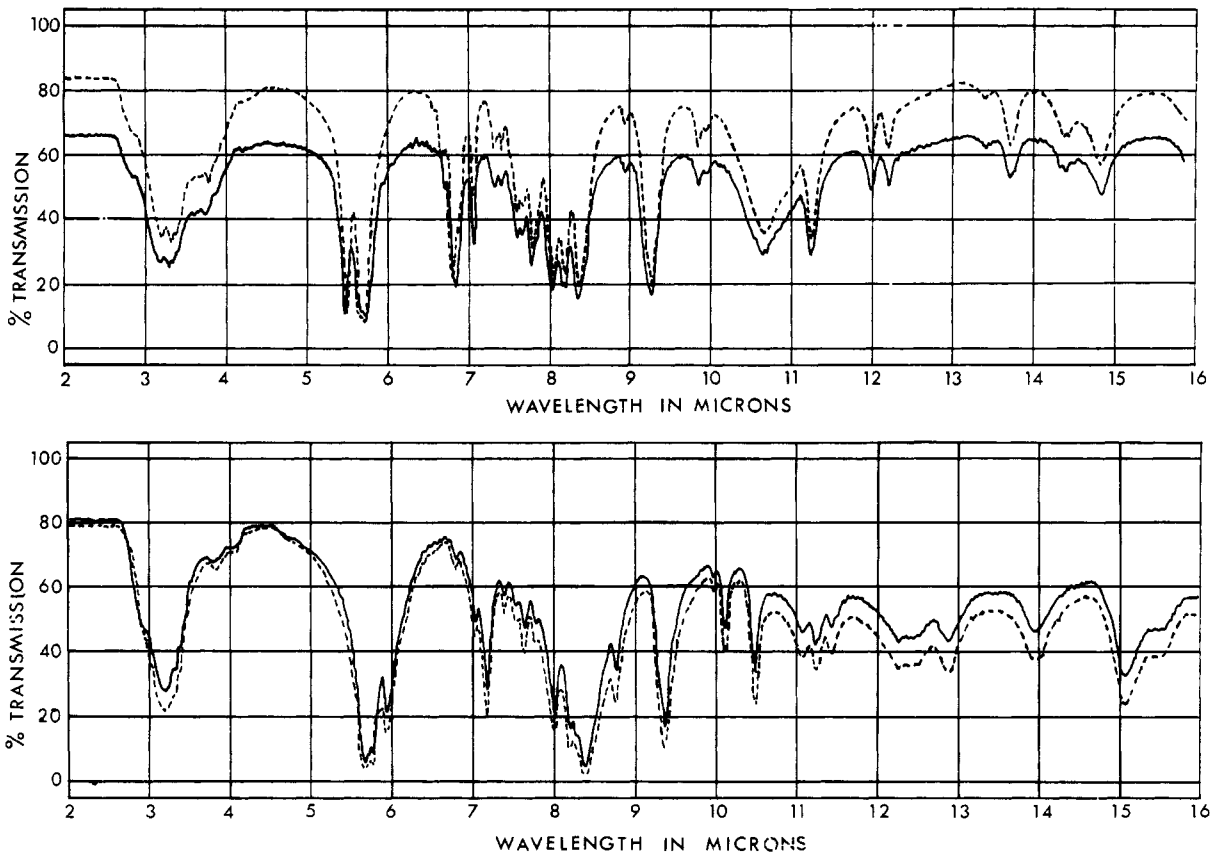


FIGURE 1: Comparison of infrared spectra of natural and synthetic homocitric acid lactone. All samples were run in KBr pellets with a Beckman IR-5 spectrophotometer. Top chart: ———, sample prepared from (−)-quinic acid; - - -, sample isolated from mutant of *S. cerevisiae*. Bottom chart: ———, synthetic (±)-homocitric acid lactone; - - -, 1:1 mixture of the two optically active forms.

TABLE I: Specific Rotations.

	Concn (g/ml)	$[\alpha]_{589}^{21}$ (deg)
Homocitric acid lactone (from (−)-quinic acid)	0.550/100	+60.0
Homocitric acid lactone (from (−)-quinic acid)	0.327/100	+52.6
Homocitric acid lactone (from mutant)	0.557/100	−59.3
Homocitric acid lactone (from mutant)	0.333/100	−50.5

products, recrystallized from ethyl acetate–ligroin,[2] 159–162°.

(C) INFRARED SPECTRA. As indicated in Figure 1, the infrared spectra in KBr pellets of the optically active isomers are virtually identical, as are the spectra of synthetic (±)-homocitric acid lactone and the equal mixture of the two isomers. The differences between the racemic form and either active form may be attributed to intermolecular effects in the crystals, since there were no observable differences between the active and racemic forms when the infrared spectra were determined in acetonitrile solution.

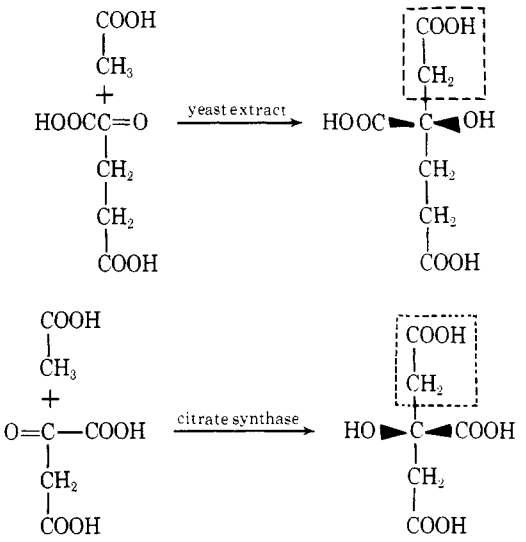
[2] This product resembled the synthetic (±)-homocitric acid lactone, crystallizing in long thin needles as contrasted to the short, thick crystals of the two optically active forms.

Discussion

The results reported above demonstrate that the isomer of homocitric acid accumulated by the lysine-requiring mutant of yeast has the absolute configuration depicted below, and may be designated according to the Cahn–Ingold–Prelog conventions (Cahn, 1964) as (*R*)-2-hydroxy-1,2,4-butanetricarboxylic acid. In the enzymatic synthesis of this compound, the acetate moiety adds to the carbonyl carbon of α-ketoglutarate in a position opposite in configuration to that which has been demonstrated in the analogous synthesis of citrate by citrate synthase (*cf.* Hanson and Rose, 1965).

However, this means that the methylene group which participates in the dehydration and rehydration

to form homoisocitrate is in the same stereochemical position with respect to the carbinol carbon as the participating methylene group in isocitrate formation.



It may prove that the stereochemistry of the rearrangement of homocitrate to homoisocitrate is analogous in all respects with that of citrate to isocitrate. This suggestion can be confirmed only by determining the absolute configuration of homoisocitric acid and the geometric configuration of the unsaturated compound (homoaconitate) indicated by Maragoudakis *et al.* (1966) to be an intermediate in this rearrangement. If elimination and addition of water is *trans*, then it can be predicted that the unsaturated compound will be *cis* and, if the "flip-over" mechanism holds here also, the naturally occurring homoisocitrate will have an absolute configuration corresponding to *threo*-D_s-isocitrate.

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