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BIFUNCTIONAL CHIRAL SYNTHONS VIA BIOCHEMICAL METHODS. 5. PREPARATION OF (S)-ETHYL HYDROGEN-3-HYDROXYGLUTARATE, KEY INTERMEDIATE TO (R)-4-AMINO-3-HYDROXYBUTYRIC ACID AND L-CARNITINE.[1]

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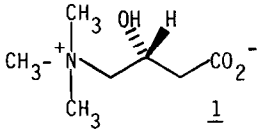
Microbial enantioselective hydrolysis of diethyl-3-hydroxyglutarate afforded (S)-ethyl hydrogen-3-hydroxyglutarate, which was transformed into (R)-4-amino-3-hydroxybutyric acid and L-carnitine, via a Curtius and Hunsdiecker rearrangement, respectively.

The effectiveness of L-carnitine (1) in the treatment of systemic and myopathic deficiencies is now well recognized.[2] (R)-4-Amino-3-hydroxybutyric acid (2), a chiral precursor of 1[3], is itself a useful antiepileptic drug.[4] As tedious kinetic resolution methods are currently employed in their preparation[5], interest in developing improved asymmetric syntheses of these substances has risen steadily. Thus far, a few asymmetric syntheses of 1 and 2 using carbohydrates as chiral starting materials have been reported.[6] Recently, we reported a synthesis of L-carnitine[7] based on stereochemical control of yeast reductions of γ-chloro-β-keto esters. As part of our continuing interest in the application of biochemical systems to asymmetric synthesis, we herein describe a novel synthesis of 1 and 2 from the chiral precursor, (S)-ethyl hydrogen-3-hydroxyglutarate (3), which is readily derived via microbial enantioselective hydrolysis of diethyl-3-hydroxyglutarate (4).[8]



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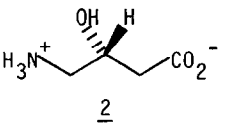


2

Asymmetric hydrolysis of 4 by α-chymotrypsin to give (R)-ethyl hydrogen-3-hydroxyglutarate (5) had been recorded many years ago.[9] The reaction is believed to be highly enantioselective. However, the rate of hydrolysis is very slow. Hence, a substantial quantity of α-chymotrypsin is required to complete the reaction (substrate to enzyme weight ratio was 2:1). In contrast, pig liver esterase[10] catalyzed rapid hydrolysis of 4 to give (S)-ethyl hydrogen-3-hydroxyglutarate (3) of low optical purity. Further, while both (+) and (-)-methyl hydrogen β-acetoxyglutarate can be prepared by chemical resolution methods[8], these processes are tedious and give low yield of the chiral product. Although 3 is a useful chiron[10], until now it has not been readily accessible.

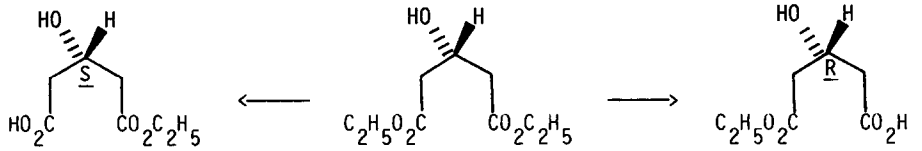
The above disadvantages prompted us to examine the enantioselective hydrolysis of 4 by various microorganisms and the results are tabulated in Table 1. Whereas most microorganisms hydrolyzed the pro-R ester grouping of 4 to yield 3 of high optical purity in good yields, Acinetobacter lowfii preferentially cleaved the pro-S ester grouping of 4 selectively to afford 5. Similar results were obtained using dimethyl-3-hydroxyglutarate as the substrate.

Table 1. Microbial enantioselective hydrolysis of 4.

Microorganism	Yield %	Stereochemical preference	Product	ee[a]
Arthrobacter sp (ATCC 19140)	38	Pro-R	S	>0.69
Corynebacterium equi (IFO-3730)	70	Pro-R	S	>0.97
Acinetobacter lowfii	70	Pro-S	R	>0.80
Soil isolate S-29	54	Pro-S	R	0.77

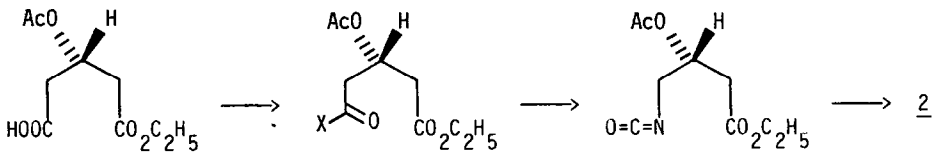
Each of the microorganisms[11] was exposed to 2 g/L of 4 for 48 hours.

[a]Enantiomeric excess was determined by comparison with the optical rotation of 5 reported in the literature[9].



3 4 5

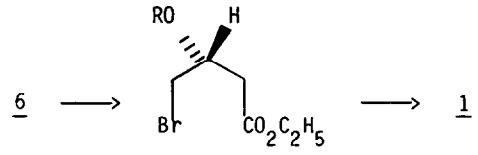
A sample of (S)-ethyl hydrogen-3-hydroxyglutarate, $[\alpha]_D^{23}$ +1.24° (c, 9.7 in acetone), obtained from the fermentation of diester 4 with Arthrobacter sp (ATCC 19140) was acetylated (Ac_2O, pyr.) to give 6. Treatment of 6 with oxalyl chloride in benzene at 6°C afforded the acid



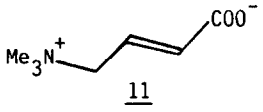
6 7 X = Cl 8 X = N_3 9 2

chloride 7. Reaction of 7 with sodium azide in aqueous acetone at 0°C yielded 8. On refluxing with benzene for 70 hours, the acid azide 8 underwent slow Curtius rearrangement to give the isocyanate 9, as a brown oil. Hydrolysis of 9 (18% HCl, 100°-110°C, 4 hrs) afforded 2, isolated by chromatographing the crude residue over a Dowex (1-X4, ^-OH) column (2 x 7 cm). Elution of the column with 15% NH_4OH gave 2 as a white crystalline solid (36% from 3, $[\alpha]_D^{23}$ -12.56°, H_2O, mp 205-207°C). The easy conversion of 2 into L-carnitine 1 by methylation is already well established.[3]

A sample of (S)-monoacid, 3, $[\alpha]_D$ +1.75° (c, 7.9 in acetone), obtained from fermentation of the diester 4 with Corynebacterium equi (IFO-3730) was directly converted to L-carnitine (1) in the following manner. Acetylation (Ac_2O, pyr.) gave the acetate 6, $[\alpha]_D^{23}$ -6.23° (c, 2.36 in $CHCl_3$), which was then subjected to the Hunsdiecker rearrangement[12] (HgO, CCl_4, Br_2). The



6 ⟶ 10 R = Ac; 12 R = H ⟶ 1



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bromide 10 was isolated in 46% yield after chromatography ($[\alpha]_D$ +16.22°; c, 4.58 in $CHCl_3$). Direct coupling of the bromoacetate 10 with excess aqueous trimethylamine afforded L-carnitine (1) accompanied by a considerable quantity of crotonylbetaine (11). Hence, it was necessary to deprotect the acetate prior to successful coupling. Deprotection of the acetate 11 was readily achieved by means of an exchange reaction (EtOH, cat. HCl) to give the bromohydrin 12, $[\alpha]_D$ +13.8° (c, 1.22 in $CHCl_3$), in 50% yield. The enantiomeric purity of 12 was proved to be 96:4 by means of its MTPA-ester analysis.[13] Coupling of the bromohydrin 12 with excess trimethylamine and subsequent Dowex-$^-$OH chromatography, gave L-carnitine (1), $[\alpha]_D^{23}$ -27.1° (H_2O), in 50% yield.

Acknowledgment

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References and Notes

1) For part 4 of this series, see: Y. F. Wang and C. J. Sih, Tetrahedron Lett., submitted for publication.
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11) Conditions for microbial conversions were carried out as follows: The surface growth from an agar slant of the bacterium was transferred to a 125 ml Erlenmeyer flask containing 25 ml of Difco nutrient broth. After incubation at 25°C on a rotary shaker (250 cycles/min - 2" radius) for 24 hours, the contents were transferred to a 2 L Erlenmeyer flask containing 500 ml of Difco nutrient broth. After incubation for a further 24 hours, the diester 4 was added as an emulsion consisting of 1 g of substrate suspended in 10 ml of 3% Tween 80 solution. After 48 hours, the incubation mixture was acidified to pH 3 with 6 N HCl and extracted several times with ethyl acetate. Chromatography of the crude residue on a silica gel column gave the desired chiral monoacid 3 or 5.
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