Pergamon

Tetrahedron Letters, Vol. 37, No. 48, pp. 8819-8822, 1996
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0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01998-3

Chemoenzymic Resolution and Deracemisation of (±)-1-Methyl-1,2-epoxycyclohexane: the Synthesis of (1-*S* , 2-*S*)-1-Methylcyclohexane-1,2-diol.

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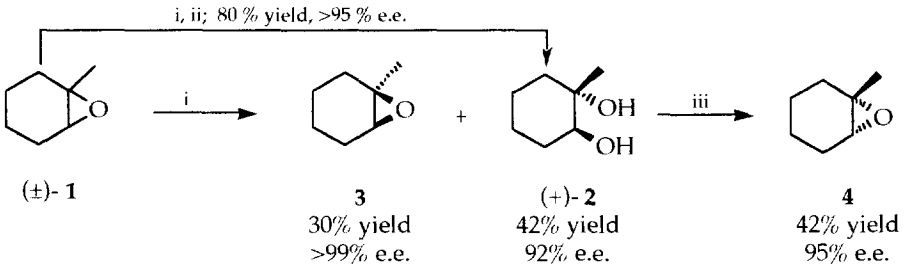
Abstract : *Corynebacterium* C12 epoxide hydrolase transforms (±)-1-methyl-1,2-epoxycyclohexane **1** to the (*1-S, 2-S*)-1-methylcyclohexane-1,2-diol **2** leaving the (*1-S, 2-R*)-epoxide **3** unchanged. The diol **2** is converted to the the (*1-R, 2-S*)-epoxide **4** by sulfonation-ring closure. A one pot combination of *Corynebacterium* C12 epoxide hydrolase and acid catalysed ring opening converts (±)-1-methyl-1,2-epoxycyclohexane **1** to (1-*S* , 2-*S*)-1-methylcyclohexane-1,2-diol **2**.Copyright © 1996 Elsevier Science Ltd

Enantiomerically pure diols and epoxides are valuable intermediates for organic synthesis and in consequence there are many reported chemical and biochemical[1] methods for their synthesis. In the last few years, the chemical approaches have been dominated by the Katsuki-Sharpless[2] and the Jacobsen-Katsuki[3] asymmetric epoxidation and hydroxylation[4] procedures and their variants. These methods have drawbacks and limitations, however, and there is a continued need for the development of new procedures for the generation of enantiopure diols and epoxides.

Mammalian epoxide hydrolases, either microsomal (mEH) or cystolic (cEH), have been studied for many years[5] but their use is restricted by their limited supply and in recent years, microbial sources of epoxide hydrolase activity have been sought. Activity has been reported[6] from a wide range of organisms but in terms of a practical application to the synthesis of enantiopure products, most of these enzymes/organisms proved to be disappointing and high e.e. values for both the diol product and the residual epoxide are rare although regioselectivities can be high. We now report a whole cell system which achieves both of these objectives with 1-methyl-1,2-epoxycyclohexane **1** and because of the regiospecificity of the enzyme, allows the ready application of a simple deracemisation protocol.

A *Corynebacterium* sp. strain C12, previously described by us,[7] exhibits pronounced epoxide hydrolase (EH) activity and the hydrolase enzyme has been purified.[8] Batches of *Corynebacterium* C12 were grown on a mineral salts medium with *trans* -1,2-dihydroxycyclohexane as the sole carbon source in a 20 l fermenter[7] and the cells harvested at the end of the exponential phase and washed with 25 mM phosphate buffer pH 7.0. The resulting concentrated slurry of C12 cells was drop frozen in liquid nitrogen and stored at -70 °C. When required for use, frozen cell pellets were thawed, suspended in 25 mM phosphate buffer (≈10 g wet cell mass : 1.5 l buffer, pH 7.0) at 25 °C and the epoxide added to produce 40 m M concentration.



i. *Corynebacterium* C12, r.t.; ii. 0.02 M $HClO_4$ aq.; iii. $MeSO_2Cl$/NaH

Scheme

Thus 1-methyl-1,2-epoxycyclohexane was incubated with the resuspended resting state cells at room temperature for 140 min. (Scheme). The extent of reaction was assayed by g.l.c. of the total medium on a Tenax TA column at 190°C and the e.e. of the residual epoxide **3** determined by chiral g.c. of an ethereal extract of the medium on a Lipodex C column at 55°C. At 51% conversion, the epoxide **3** (>99% one enantiomer) was isolated in 30% yield (60% recovery).

The diol **2**, 92% e.e. (by chiral g.l.c. on CP Chirasil-dex CB) was isolated in 42% yield (84% recovery). The absolute stereochemistry of **2** was established by comparison with the known (-)-(1*R* , 2 *R*)-isomer,[9] the positive $[\alpha]_D$ established the (1*S* , 2*S*)-configuration depicted in **2**.

The absolute stereochemistry of **3** ($[\alpha]_D$ -28) was determined by the perchloric acid catalysed ring opening of the epoxide **3** to the same diol **2** (81% e.e. by chiral g.l.c. analysis). Since this diol would be formed by breaking of the C_1– O bond,[10] it follows that the enzymic ring opening must have occurred by attack of oxygen nucleophile at the C_2 centre of the epoxide. Epoxide **3**, therefore, has the 1*R*, 2*S* -configuration.[11]

These two reactions could be run in tandem to convert the (±)-epoxide **1** directly to the (*1-S*, 2-*S*)-1-methylcyclohexane-1,2-diol **2** in 80% yield and 95% e.e. (>99% after one recrystallisation).[12]

With the diol **2** available, simple cyclisation with methanesulfonyl chloride and sodium hydride produced the enantiomeric (1*S*, 2*R*)-epoxide **4** (42% yield, 95% e.e., $[\alpha]_D$ +25) and completed the kinetic resolution of the epoxide.

Overall, the use of the robust *Corynebacterium* C12 whole cell system gives rise to a simple and effective procedure for the deracemisation of the epoxide **1** and ready and highly efficient access to the enantiomerically pure diol **2**.

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12. *Typical Procedure.* (±)-1-Methyl-1,2-epoxycyclohexane **1** (3.0 g, 27 mmol) was added to a suspension of C12 whole cells (≈4.5 g wet cell paste) in phosphate buffer (675 ml, 25 mM, pH 7.0) to give a starting epoxide concentration of 40 mM. The heterogeneous mixture was stirred at room temperature and the epoxide concentration was followed by packed column G.C. (direct injection, Tenax TA column, N_2 carrier, oven temperature 190 °C). The e.e. was determined at each stage by chiral capillary g.c. (Lipodex C column, split ratio 100:1, He carrier, oven temperature 55 °C) of a dried (Na_2SO_4) ether extract. When a single

enantiomer of epoxide remained (≈ 1 – 2 h) perchloric acid (≈4 ml, 60%w/v aqueous solution) was added to pH ≈ 1. The reaction was stirred for a further 5 min. until no more epoxide was detectable. The cells were removed by centrifugation (Sorvall RC-5B plus, GS-3 rotor, 5,000 rpm, 15 min). The supernatant saturated with sodium chloride and extracted with ethyl acetate (4 x 400 ml) The combined organic phases were dried (Na_2SO_4) and the solvent removed under reduced pressure and the residual product (2.9 g, 83% yield, 84% e.e.) was recrystallised from diethyl ether/100-120°C petroleum ether to yield pure (1*S* , 2*S*)-1,2-dihydroxy-1-methylcyclohexane m.p. 84-85 °C (lit.[9] 84-5 °C); $[\alpha]_D$ + 16.5 (c = 1, ethanol) (lit.[9] –7.5 for the enantiomer of **2** of 50% e.e.); >99%e.e. (by chiral g.c.); ν_{max} (film)/ cm^{-1} 3337, 2949, 2932, 1169, 997; δ_H / ppm (270 Mhz, $CDCl_3$) 3.47 (1H, dd, *j* 10.3, 4.6 Hz, C*H* OH), 2.32 (1H, br s, CHO*H*), 2.06 (1H, br s, CHO*H*), 1.2 - 2.0 (8H, complex), 1.18 (3H, s, CH_3); δ_C / ppm (67.5 MHz, $CDCl_3$) 77.4, 74.1, 38.6, 31.1, 24.1, 23.2, 19.4; *m* / z (CI, NH_3) 148 (*M* + NH_4^+). [Found: 148.1331; calc. for $C_7H_{18}NO_2$, 148.1338].

(*Received in UK* 30 *September* 1996; *accepted* 11 *October* 1996)