Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 6, pp. 1571-1572, 1996

0957-4166/96 $15.00 + 0.00

PII: S0957-4166(96)00183-8

Asymmetric Reduction of 2-Oxo-4-Phenylbutanoic Acid Ethyl Ester By *Daucus carota* Cell Cultures

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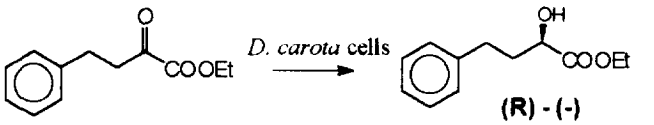
Abstract: *A novel method to produce (R)-(-)-2-hydroxy-4-phenylbutanoic acid ethyl ester* ***1b*** *has been developed. 2-Oxo-4-phenylbutanoic acid ethyl ester* ***1a*** *is reduced to* ***1b*** *by the cell cultures of Daucus carota in high enantiomeric excess and yield.* Copyright © 1996 Elsevier Science Ltd

Introduction: (R)-2-hydroxy-4-phenylbutanoic acid ethyl ester **1b** is a versatile precursor for the production of a variety of Angiotensin Converting Enzyme (ACE) inhibitors[1]. This key intermediate can be obtained by resolution of the racemate, by chemical as well as by biochemical methods [2-6]. In procedures which involve resolution of the racemate, there is always 50% of the unwanted enantiomer. Hence, it is important to develop methods of asymmetric synthesis which do not allow for the formation of the unwanted enantiomer (50%). Reduction of 2-oxo-4-phenylbutanoic acid ethyl ester to give a single enantiomer is one solution to this problem.



1a **1b**

Baker's yeast did not reduce the 2-oxo-4-phenylbutanoic acid ethyl ester. Neither did *Candida parapsilosis*. In our continued attempts to produce (R)-2-hydroxy-4-phenylbutanoic acid in high enantiomeric excess and high yield, we used plant cell cultures for this asymmetric reduction. Plant cell cultures represent an important potential to perform biochemical reactions on organic compounds[7]. Most of these reactions so far, have been confined to the biotransformation of secondary metabolites produced by plant cells[8]. There have been a few examples of the biotransformation of synthetically important foreign substrates [9-11]. Several investigations have been carried out on the biotransformation of monoterpenes [9,12] and aliphatic ketones[13] by using either freely suspended or immobilised cells of *Mentha* and *Nicotiana* species. The biotransformation of aromatic ketones with cell cultures (free and immobilised) of carrot, tobacco and gardenia has been reported[14].

The reduction of 2-oxo-4-phenylbutanoic acid to the corresponding (R)-2-hydroxy compound by a microbial system has been reported[2]. This procedure involves stringent anaerobic conditions. The enyzme, alcohol dehydrogenase has also been used to reduce 2-oxo-4-phenylbutanoic acid[2], but this needs expensive cofactors. The chemical enantioselective hydrogenation methods are elaborate and give an ee of <96%[2]. In this paper, we report the reduction of 2-oxo-4-phenylbutanoic acid ethyl ester by the cells of *Daucus carota* to the corresponding (R)-(-) -2-hydroxy compound in >99% ee and 100% conversion.

Results and Discussions: The biotransformation of **1a** into **1b** by the cells of *Daucus carota* was measured after different incubation periods (Fig.1). Typically, 10 gm of friable callus was suspended in MS medium in a shake flask and shaken at 27°C, 100 rpm. This suspension was sieved weekly to get uniform callus clumps and maintained. To each of these flasks was added 100 mg of the keto ester (bought from Aldrich Chemical Co., USA). The flasks were incubated in a rotary shaker at 27°C, 100 rpm for 10 days. The cell free culture filtrate was extracted with diethyl ether, dried over sodium sulphate, concentrated and analysed on GC (10% Carbowax) for % conversion, (the standard 2-hydroxy compound was prepared by the $NaBH_4$ reduction of the keto ester followed by reesterification using $EtOH/H^+$) while the chiral analysis was done on a Chiracel Column as reported earlier[3]. It turns out that in 10 days, conversion to the hydroxy

compound is maximum (100%) and the ee is >99%; (R)-(-)-2-hydroxy-4-phenylbutanoic acid ethyl ester. $[\alpha]_D^{25}$ = -9.3 (c 1.29, EtOH)[3].The isolated yield is 80%. In the case of acetophenone which gets reduced to the (S)-α-phenethyl alcohol in >99% ee, 100% conversion is seen in 5 days by the cells of *Daucus carota*[14].

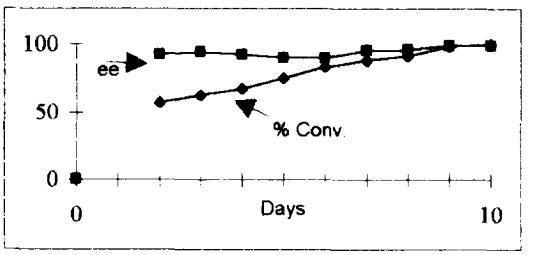


Figure 1. Transformation of **1a** into **1b** using *D. carota* cells

Immobilisation[15] was done with 10 gm of cells suspended in 10 ml of medium mixed with 30 ml of sterile sodium alginate (2%). The homogenous suspension was dropped into a solution of $CaCl_2$ (20 gm/l) by means of a Pasteur pipette. The spherical beads were kept for gelation in $CaCl_2$ for 12h. Immobilised cells in hardened beads were grown in 60 ml MS medium in flasks kept at 27°C and 100 rpm. After one week 100 mg of 2-oxo-4-phenylbutanoic acid ethyl ester was added and the analysis done on the sixth, tenth and fifteenth days. After 10 days of incubation ~90% conversion and >99% ee was seen. These cells were recycled. In our earlier attempt to get the (R)-2-hydroxy-4-phenylbutanoic acid from the racemic acid by the lipase mediated transesterification, the corresponding (S)-2-acetoxy compound was also produced[3]. Ideally, the product from the reduction of the ketone should obviate the problem of recycling the unwanted enantiomer (50% of the yield). This is what is seen.

It is significant to note that the reduction of 2-oxo-4-phenylbutanoic acid was not carried out by the cells of *Daucus carota*, neither was that of the related compounds, 2-oxo-4-phenylbutenoic acid and its ethyl ester.

Acknowledgements: The authors thank Dr. M. D. Nair and Dr. J. Thomas for constant support. Suggestions regarding the manuscript by Dr. N. Kalyanam are gratefully acknowledged.

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(*Received in UK* 29 *March* 1996; *accepted* 9 *May* 1996)