Indian Journal of Chemistry
Vol. 36 A & B, June 1997, pp.530 - 532

Biotransformations by plant cell cultures

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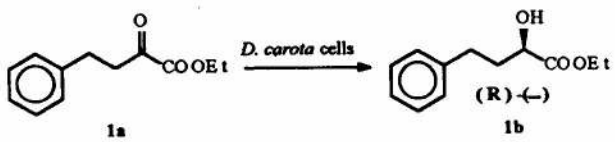
Received 22 April 1997

Plant cell cultures of carrot have been used to effect the asymmetric reduction of 2-oxo-4-phenylbutanoic acid ethyl ester to (*R*)-2-hydroxy-4-phenylbutanoic acid ethyl ester in high enantiomeric excess and yield.

Fermentation technology utilizing fungal and bacterial cultures has made enormous progress particularly in the pharmaceutical industry, but similar technology with plant cells has not reached this level of application as yet. Nevertheless, plant cell cultures represent an important potential to perform biochemical reactions on organic compounds[1]. Most of these reactions so far, have been confined to the biotransformation of secondary metabolites produced by plant cells[2]. There have been a few examples of the biotransformation of synthetically important foreign substrates[3-5]. Several investigations have been carried out on the biotransformation of monoterpenes[3,6] and aliphatic ketones[7] by using either freely suspended or immobilised cells of *Mentha* and *Nicotiana* species. The biotransformation of aromatic ketones to optically pure hydroxy compounds with cell cultures (free and immobilised) of carrot, tobacco and gardenia has been reported[8]. Thus, it is clear that plant cells provide a good source of enzymes for straightforward biotransformations and also for the synthesis of optically active molecules.

(*R*)-2-hydroxy-4-phenylbutanoic acid ethyl ester **1b** is a key intermediate in the production of a variety of Angiotensin Converting Enzyme (ACE) inhibitors[9]. This versatile molecule can be obtained by resolution of the racemate, by chemical as well as by biochemical methods[10-14]. 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. Reduction of 2-oxo-4-phenylbutanoic acid ethyl ester **1a** to give a single enantiomer is one



1a **1b**

solution to this problem. 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.

Materials and Methods

(a) Plant cell cultures: (i) *Carrot*-Carrot seeds were germinated aseptically on MS[15] basal medium (D). Hypocotyls of 12-day old *in vitro* seedings were used for callus induction on MS basal salts medium with 2% sucrose containing 2,4- dichlorophenoxyacetic acid (0.5mg/L; D_{116}) Calli appeared in 30 days when the tissues were incubated in the dark at 27°C. These yellow calli (10g) were suspended in MS basal salts medium (60 mL) amended with 2% sucrose and kinetin and a-naphthalene acetic acid (D_3)[8]. These flasks were shaken at 100 rpm, 27°C in diffuse light for 30 days before being used for the biotransformation.

(ii)*Horseradish.* Leaf bits of *in vitro* maintained horseradish plants were regenerated on MS(9)[17] agar medium (MS basal salts, 3% sucrose, kinetin and α-naphthalene acetic acid). Regenerated shoot and root tissues were obtained within 30 days of incubation in light. These plant clumps were transferred to liquid MS(9) medium and cultured for 7 days before being used for biotransformation.

(iii) *Orchids*. Protocorms of the orchid *Dendrobium* were grown in liquid KCM[18] medium for 15 days at 100 rpm, 27°C in light. The cell-free liquid

medium was withdrawn periodically to assess the biotransformation of the keto ester as reported earlier[19].

(b) Biotransformation. Typically, 10g of friable callus was suspended in MS medium in a shake flask and shaken at 27°C, 100 rpm. The cells were maintained as per the earlier reported procedure[16]. To each of these flasks was added 100 mg of the keto ester (bought from Aldrich Chemical Co., USA) and the biotransformation was done as reported[19] following which the chiral analysis was done on a chiracel Column[11].

(c) Immobilisation of carrot cells. Immobilisation[20] was done with 10g 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 g/L) by means of a Pasteur pipette. The spherical beads were kept for gelation in $CaCl_2$ for 12hr. 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.

Results and Discussion

The conversion of **1a** into **1b** by the cells of *Daucus carota* was measured after different incubation periods (Figure 1). In 10 days, the conversion to the hydroxy compounds 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)[11].

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].

When cells of horseradish were used, after 10 days the conversion was 95% while the ee had to be determined. However, the orchid cell cultures did not show the desired reduction after 10 days.

In our earlier attempt to get (*R*)-2-hydroxy-4-phenylbutanoic acid from the racemic acid by the lipase-mediated transesterification, the corresponding (*S*)-2-acetoxy compound was also produced[11]. However the present biocatalytic reduction using plant cell culture produced exclusively the (*R*)-2-hydroxy-4-phenylbutanoic acid ethyl ester in >99% ee carrot cells showing the best results so far.

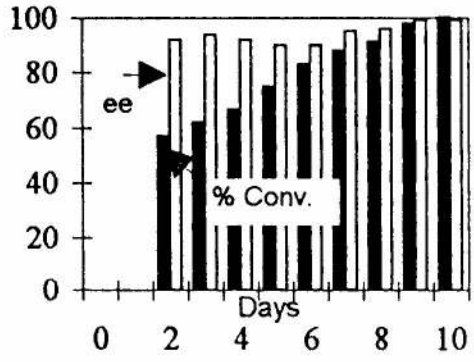


Fig. 1 – Asymmetric reduction of **1a** into **1b** using *D. carota* cells

Significantly, the reduction of 2-oxo-4-phenylbutanoic acid was not affected by the cells of *Daucus carota* neither was that of the related compounds, 2-oxo-4-phenylbutenoic acid and its ethyl ester.

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