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BIOTRANSFORMATION OF ACETOPHENONE WITH IMMOBILIZED CELLS OF CARROT, TOBACCO AND *GARDENIA*

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Key Word Index—*Daucus carota*; Umbelliferae; *Nicotiana tabacum*; Solanaceae; *Gardenia jasminoides*; Rubiaceae; acetophenone; α-phenethyl alcohol; organic xenobiotic; biotransformation; reduction; oxidation.

Abstract—Immobilized cells of *Daucus carota* transformed acetophenone into (*S*)-α-phenethyl alcohol by a stereoselective reduction pathway. Immobilized cells of *Nicotiana tabacum* also transformed the same substrate into the (*S*)-alcohol, but neither stereoselective reduction nor stereoselective oxidation was observed in the biotransformation process. (*R*)- and (*S*)-α-phenethyl alcohol of a high enantiomeric purity of >99% ee can be prepared by the biotransformation of acetophenone with immobilized cells of *Gardenia jasminoides* and *Daucus carota*.

INTRODUCTION

Plant cell cultures will transform enantioselectively important foreign synthetic substrates, as well as secondary metabolites [1–4]. We have been interested in the biotransformation of organic xenobiotics e.g. β-keto esters and aromatic ketones by immobilized plant cell cultures, in order to learn about the stereoselectivity and the mechanistic pathway in terms of synthetic chemistry [3–5].

Immobilized cells of *Daucus carota*, *Nicotiana tabacum* and *Gardenia jasminoides* are effective biocatalysts for the preparation of the enantiomers of aromatic alcohols [5]. Very recently we showed that the biotransformation of acetophenone (**1**) into (*R*)-α-phenethyl alcohol (**1a**) by immobilized *G. jasminoides* cells was effected by a two-step pathway involving initial non-stereoselective reduction of **1** to give a mixture of (*R*)- and (*S*)-**1a**, followed by stereoselective oxidation of (*S*)-**1a** to produce **1** [6]. In this work the mechanistic pathway for the biotransformation of the ketone **1** into the alcohol (*S*)-**1a** with immobilized cells of *D. carota* and *N. tabacum* has been studied and both the enantiomers of **1a** have been prepared in high enantiomerically pure forms by the biotransformation of the ketone **1** with immobilized cells of *G. jasminoides* and *D. carota*, respectively.

RESULTS AND DISCUSSION

The biotransformation of **1** with immobilized *D. carota* cells, unlike the analogous transformation with immobilized *G. jasminoides* cells, proceeded primarily by stereoselective reduction to give (*S*)-**1a** with a high enantiomeric purity. The bioreduction of **1** was measured after different incubation periods (Fig. 1). The value of the chemical yield of **1a** increased with increasing bioconversion, while the value of the optical yield of (*S*)-**1a** remained constant throughout the incubation period. An attempt to oxidize racemic alcohol **1a** by immobilized *D. carota* cells was unsuccessful. No oxidation product was observed and 51% of the alcohol was recovered. These observations indicate that the bioreduction of **1** by *D. carota* cells can take place stereoselectively, leading to (*S*)-**1a** with an enantiomeric purity of >99% ee.

The biotransformation of **1** carried out by immobilized *N. tabacum* cells stopped before reaching 100% conversion (Fig. 2). Although a simple relationship between the chemical yield of **1a** and bioconversion was not apparent from the data, the value of the optical yield of (*S*)-**1a**

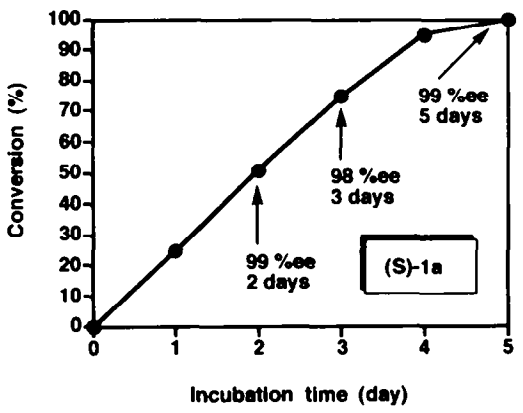

Fig. 1. Biotransformation of **1** to form (*S*)-**1a** by immobilized cells of *D. carota* at different incubation times.

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increased with conversion, reaching a maximum of 88% ee at *ca* 50% conversion. The biotransformation of racemic alcohol **1a** was examined after different incubation periods using the same immobilized cells (Fig. 3). The reaction showed a very low conversion (12%, 24 days), the yield of the oxidation product **1** being only 3%; (*S*)-**1a** with an enantiomeric purity of 40% ee was obtained as the main product. The present data tell us that the biotransformation by *N. tabacum* cells can be neither a stereoselective reduction nor a stereoselective oxidation, and that other metabolic sequences than these two reactions may participate in the formation of (*S*)-**1a**. However, the exact mechanism for the biotransformation of **1** into (*S*)-**1a** is still not elucidated.

The present biotransformation by *D. carota* cells and the previous one by *G. jasminoides* cells [6] were applicable to the preparation of both the chiral alcohols (*R*)- and (*S*)-**1a** with a high enantiomeric purity. The alcohol (*R*)-**1a** of *ca* 90% ee, which was prepared by either the biotransformation of the ketone **1** or the stereoselective oxidation of racemic alcohol **1a** by using immobilized *G. jasminoides* cells [6], was reincubated with *Gardenia* cells as shown in Scheme 1. The resulting (*R*)-**1a** showed an enantiomeric purity of >99% ee. On the other hand, the present biotransformation of **1** with carrot cells exclusively gave (*S*)-**1a** with an enantiomeric purity of >99% ee as described above.

A survey of the present and previous results indicates that the immobilized cells of *D. carota*, *N. tabacum* and *G. jasminoides* differ from one another in stereoselectivity and mechanistic pathway in regard to the biotransformation of the ketone **1** into the alcohol **1a** (Scheme 2).

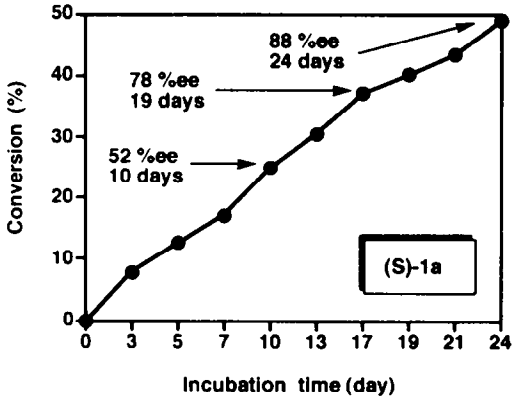


Fig. 2. Biotransformation of **1** to form (*S*)-**1a** by immobilized cells of *N. tabacum* at different incubation times.

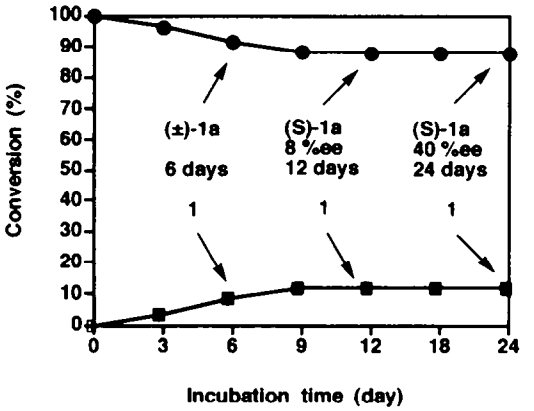
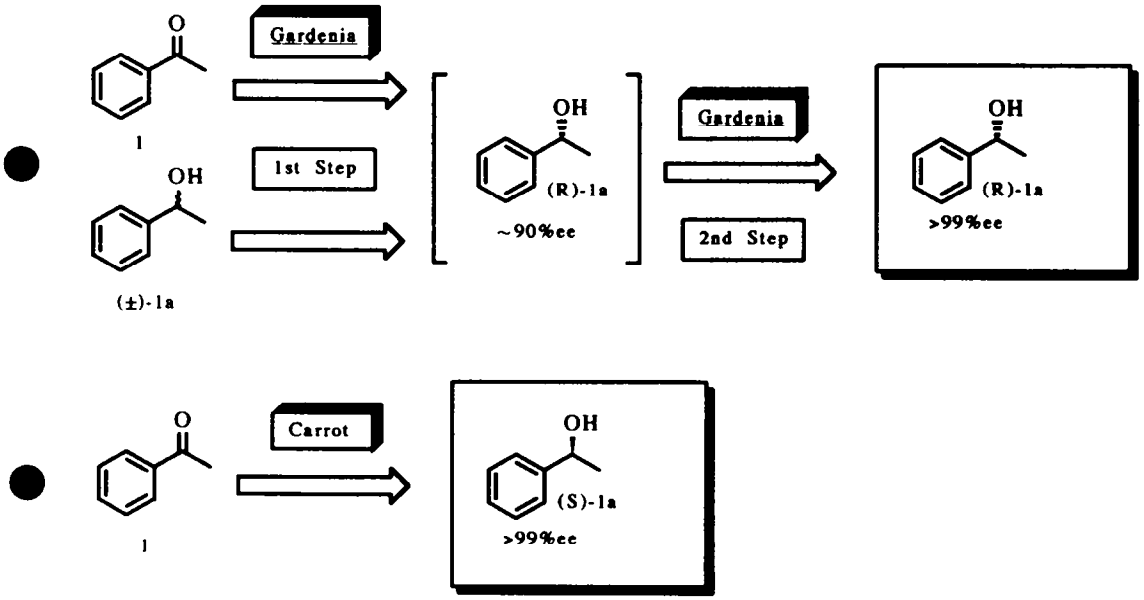
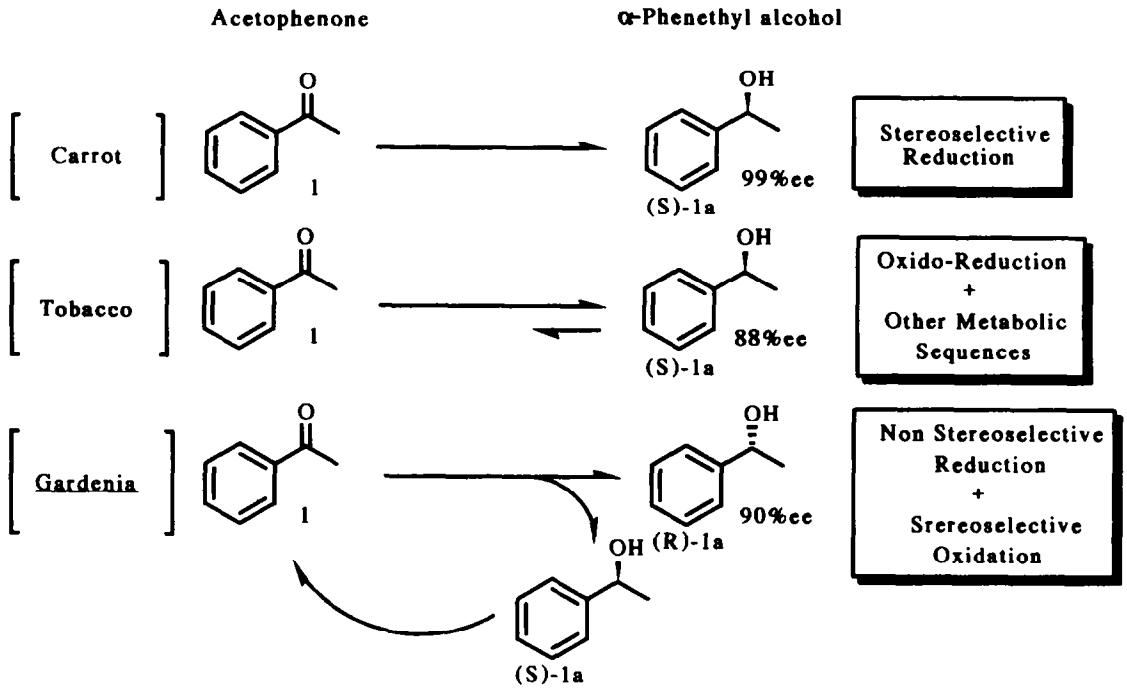


Fig. 3. Biotransformation of (±)-**1a** by immobilized cells of *N. tabacum* at different incubation times.



Scheme 1. Preparation of (*R*)- and (*S*)-**1a** with high enantiomeric purities by immobilized cells of *G. jasminoides* and *D. carota*.



Scheme 2. Survey of the mechanistic pathway for the biotransformation of **1** by immobilized cells of *D. carota*, *N. tabacum* and *G. jasminoides*.

EXPERIMENTAL

Each reaction product was analysed by GC (FID) with a PEG-20M 25 m × 0.25 mm WCOT fused silica capillary column (GL Science, Tokyo, Japan) at 170° (carrier gas, He 0.48 ml min^{-1}). The conversion ratios were determined on the basis of the peak areas of **1** and **1a**.

All products were fully characterized by comparing their spectra with those of authentic samples. Chemical yields refer to compounds purified by CC on silica gel. The enantiomeric purities (% ee) of **1a** were determined by HPLC using a Chiralcel OB 4.6 × 250 mm column as previously described [5].

Cultivation of cells. Suspension cells of *D. carota* were cultivated in MS medium (free from NH_4NO_3) containing 2 ppm naphthylacetic acid and 0.1 ppm kinetin (pH 5.8) and those of *N. tabacum* and *G. jasminoides* were cultivated according to the procedures described in refs [4, 5].

Preparation of immobilized cells. Suspension cells of *D. carota* (150 g), *N. tabacum* (60 g) and *G. jasminoides* (150 g) were immobilized with a 5% aq. soln of Na alginate (600 ml) and a 0.6% aq. soln of $CaCl_2$ as previously described [3, 5].

Biotransformation of acetophenone (**1**) *with immobilized* D. carota *cells.* Immobilized plant cells prepd from suspension cells of *D. carota* (150 g) were added to freshly prepd MS medium (1 l) containing 2 ppm naphthylacetic acid and 0.1 ppm kinetin and the suspension shaken for 2 days. Ketone **1** (219, 209 and 205 mg) was administered to the precultured MS medium containing the immobilized cells and the mixt. incubated at 25° on a rotary shaker (95 rpm) in the dark. Each transformation was stopped after a different incubation period (different conversion, Fig. 1), the mixt. was filtered and the immobilized cells washed with MS medium. The filtrate (the cultured medium from the immobilized beads) and washings were combined and extracted with Et_2O. Work-up of the extracts gave a crude product, which was purified by CC. The transformations (219, 209 and 205 mg of **1**) at 50, 75 and 100% conversion gave 67 mg (*S*)-**1a** $[\alpha]_D^{20}$ −57.11° ($CHCl_3$; *c* 2.88) and 62 mg **1** (28% recovery), 73 mg (*S*)-**1a** $[\alpha]_D^{20}$ −56.74° ($CHCl_3$; *c* 2.89) and 23 mg **1** (13% recovery), and 107 mg (*S*)-**1a** $[\alpha]_D^{20}$ −57.23° ($CHCl_3$; *c* 2.21).

Biotransformation of (±)-α-*phenethyl alcohol* [(±)-**1a**] *with immobilized* D. carota *cells.* (±)-Alcohol **1a** (219 mg) was administered to the precultured MS medium containing immobilized cells of *D. carota* as described above. The mixt. was incubated at 25° for 5 days and worked-up as above. Purification of the mixt. by CC gave 112 mg (±)-**1a** (51% recovery).

Biotransformation of **1** *with immobilized* N. tabacum *cells.* Immobilized cells prepd from suspension cells of *N. tabacum* (60 g) were added to freshly prepd MS medium (1 l) containing 2 ppm 2,4-dichlorophenoxyacetic acid and the suspension shaken for 2 days. Ketone **1** (223, 213 and 214 mg) was administered to the precultured MS medium containing the immobilized cells. Each transformation was stopped at a different incubation period (Fig. 2). Work-up of the mixt. gave a crude product, which was purified by CC. The transformations (223, 213 and 214 mg of **1**) at 25, 40 and 50% conversion

gave 46 mg (*S*)-**1a** $[\alpha]_D^{20}$ −30.56° ($CHCl_3$; *c* 2.29) and 82 mg **1** (37% recovery), 42 mg (*S*)-**1a** $[\alpha]_D^{20}$ −46.3° ($CHCl_3$; *c* 2.17) and 67 mg **1** (31% recovery), and 41 mg (*S*)-**1a** $[\alpha]_D^{20}$ −52.86° ($CHCl_3$; *c* 2.16) and 47 mg **1** (22% recovery).

Biotransformation of (±)-**1a** *with immobilized* N. tabacum *cells.* (±)-Alcohol **1a** (204, 213 and 224 mg) was administered to the precultured MS medium containing immobilized cells of *N. tabacum* as described above. The transformations (204, 213 and 224 mg of racemic **1a**) at 8, 12 and 12% conversion gave 113 mg (±)-**1a** (53% recovery), 18 mg **1** (8% yield) and 97 mg (*S*)-**1a** (44% recovery), $[\alpha]_D^{20}$ −4.38° ($CHCl_3$; *c* 2.51), and 7 mg **1** (3% yield) and 47 mg (*S*)-**1a** (23% recovery), $[\alpha]_D^{20}$ −24.44° ($CHCl_3$; *c* 2.25).

Biotransformation of (R)-**1a** *with an enantiomeric purity of* 90% *ee with immobilized* G. jasminoides *cells.* (*R*)-Alcohol **1a** (202 mg) of *ca* 90% ee, which was prepd from the ketone **1** or racemic alcohol **1a** according to the procedure described in ref. [6], was treated with immobilized *G. jasminoides* cells [prepd from suspension cells of *Gardenia* (150 g)] as previously described for (*S*)-**1a** [6]. Work-up of the mixt. and then CC gave 9 mg **1** (5% yield) and 102 mg (*R*)-**1a** (51% recovery), $[\alpha]_D^{20}$ +56.55° ($CHCl_3$; *c* 2.06).

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