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THE MECHANISTIC PATHWAY OF THE BIOTRANSFORMATION OF ACETOPHENONE BY IMMOBILIZED CELL CULTURES OF *GARDENIA*

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Key Word Index—*Gardenia jasminoides*; Rubiaceae; acetophenone; α-phenethyl alcohol; biotransformation; reduction; oxidation.

Abstract—Immobilized cells of *Gardenia jasminoides* enantioselectively transformed acetophenone into the corresponding (*R*)-alcohol by a two-step pathway involving initial reduction of the aromatic ketone to produce a mixture of (*R*)- and (*S*)-α-phenethyl alcohol followed by stereoselective oxidation of the (*S*)-aromatic alcohol to produce acetophenone.

INTRODUCTION

The study of biotransformations by plant cell cultures is a steadily expanding area of plant biotechnology [1], probably because these cells are capable of transforming enantioselectively not only secondary metabolites but also organic xenobiotics including monoterpenes [2, 3], aliphatic ketones [4] and keto esters [5].

Recently, we prepared immobilized cells of *Daucus carota*, *Nicotiana tabacum* and *Gardenia jasminoides*, and used them to effect the biotransformation of aromatic ketones such as acetophenone (**1**) [6]. It is of interest to find why *G. jasminoides* cells reduced **1** to its corresponding (*R*)-alcohol (**1a**) with an optical purity of 91% ee, whereas the cells of *D. carota* and *N. tabacum* converted the same ketone to the (*S*)-alcohol **1a** with an optical purity of 99% and 88% ee, respectively. The present paper reports on the mechanistic pathway of the biotransformation of **1** into (*R*)-**1a** by immobilized cells of *G. jasminoides*.

RESULTS AND DISCUSSION

The biotransformation of **1** into **1a** by immobilized *G. jasminoides* cells was measured after different incubation periods (Fig. 1). The values of the chemical yields of **1a** varied and a simple relationship between yield and bioconversion was not apparent from the data. However, the value for the optical yield of (*R*)-**1a** increased with increasing bioconversion, reaching a maximum of 91% at *ca* 40% conversion.

The biotransformation of racemic α-phenethyl alcohol (**1a**) was carried out using the same immobilized cells. The reaction ceased at about 45% conversion within one day (Fig. 2.), the oxidation product **1** and the remaining (*R*)-alcohol **1a** with 92% ee being the main products. By contrast, the biotransformation of (*S*)-**1a** with an optical purity of 99% ee stopped at almost 85–90% conversion, yielding the oxidation product **1** in 34% yield and (*S*)-**1a** with 60% ee, which appears to be produced by reduction of the product **1** (Fig. 3).

Although the present biotransformation system has not been optimized, it is reasonable to expect, from these results, that the enantioselective biotransformation of **1** into (*R*)-**1a** with immobilized *G. jasminoides* cells is effected by a two-step pathway. The first step is the low- or non-stereoselective reduction of **1** to give a mixture of (*R*)- and (*S*)-**1a**. The second step is the stereoselective oxidation of (*S*)-**1a** to reproduce **1** (Scheme 1).

EXPERIMENTAL

Each reaction product was analysed by GC (FID) with a PEG-20M 25 m × 0.25 mm WCOT fused silica capillary column (GL Sciences, Tokyo, Japan) at 130° (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 optical purities (% ee) of **1a** were determined by HPLC using a Chiralcel OB 4.6 × 250 mm column as previously described [6].

Cultivation of cells. Suspension cells of *G. jasminoides* were cultivated in B5 medium containing 2 ppm naphthylacetic acid according to the procedure described in refs [5, 6].

Preparation of immobilized cells of G. jasminoides. Suspension cells of *G. jasminoides* (150 g) were immobilized with 5% aq. soln of Na alginate (600 ml) and a 0.6% aq. soln of $CaCl_2$ [7, 8]. The resulting immobilized beads,

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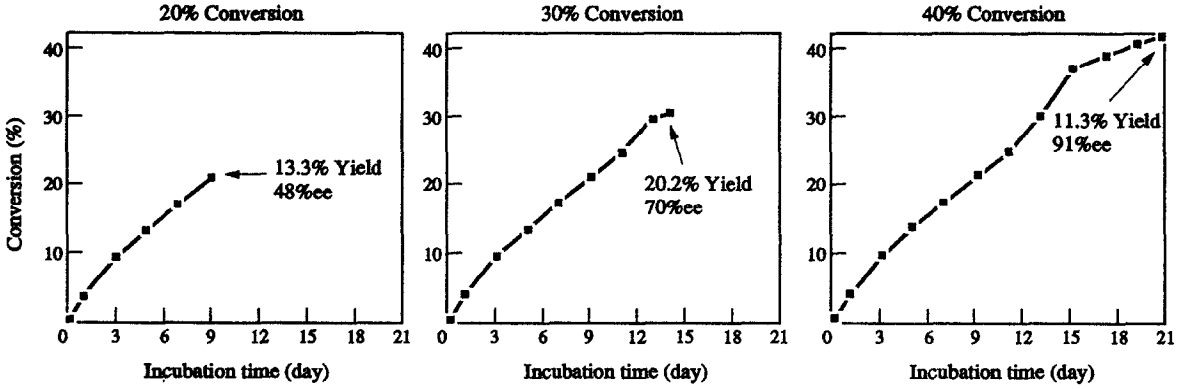


Fig. 1. Biotransformation of **1** to form (*R*)-(+)-**1a** by immobilized cells of *G. jasminoides* at different incubation times.

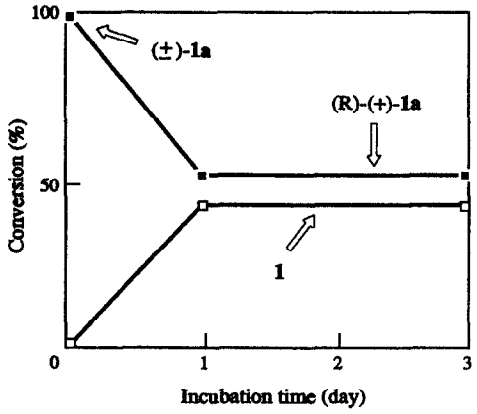
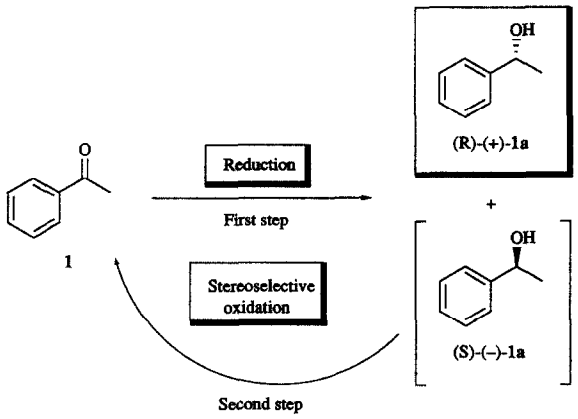


Fig. 2. Biotransformation of (±)-(**1a**) by immobilized cells of *G. jasminoides*.



Scheme 1. A possible pathway of the biotransformation of **1** by immobilized cells of *G. jasminoides*.

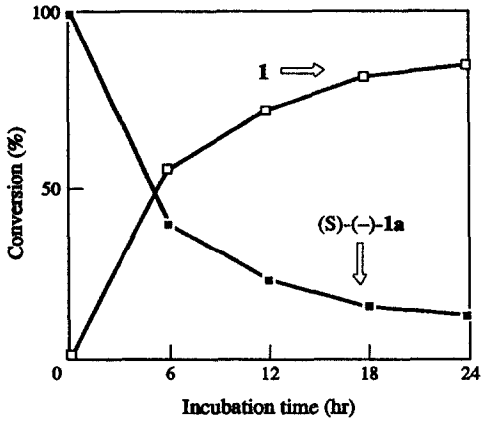


Fig. 3. Biotransformation of (*S*)-(**1a**) by immobilized cells of *G. jasminoides*

ca 4–5 mm diam., were allowed to stand for 30 min and then washed with B5 medium.

Biotransformation of acetophenone (**1**) *with immobilized* G. jasminoides *cells.* Immobilized plant cells prepared from suspension cells of *G. jasminoides* (150 g), as already described, were added to freshly prepared B5 medium (1 l) containing 2 ppm naphthylacetic acid and the suspension shaken for 2 days. Ketone **1** (208, 211 and 210 mg) was administered to the precultured B5 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. filtered and the immobilized cells washed with B5 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 (208, 211 and 210 mg of **1**) at 20, 30 and 40% conversion gave 28 mg (*R*)-**1a** {$[\alpha]_D^{20}$ +28.91° ($CHCl_3$; *c* 2.30)} and 119 mg **1** (57% recovery), 43 mg (*R*)-**1a** {$[\alpha]_D^{20}$ +40.09° ($CHCl_3$; *c* 2.02)} and 78 mg **1** (37% recovery), and 24 mg (*R*)-**1a** {$[\alpha]_D^{20}$ +53.28° ($CHCl_3$; *c* 2.28)} and 36 mg **1** (17% recovery).

Biotransformation of (±)-α-*phenethyl alcohol* [(±)-(**1a**)]. (±)-Alcohol **1a** (210 mg) was administered to the precultured B5 medium containing immobilized cells of

G. jasminoides as described above. The mixt. was incubated at 25° for 1–3 days and worked up as above. Purification of the mixt. by CC gave 40 mg **1** (19% yield) and 48 mg (*R*)-**1a** (23% recovery), $[\alpha]_D^{20} + 53.61°$ ($CHCl_3$; *c* 2.21).

Biotransformation of (S)-**1a** *with an optical purity of* 99% *ee.* A mixt. of (*S*)- **1a** (200 mg) and the precultured B5 medium containing immobilized cells of *G. jasminoides* was incubated at 25° for 24 hr and worked up as described. CC gave 68.5 mg **1** (34% yield) and 10 mg (*S*)-**1a** (5% recovery), $[\alpha]_D^{20} - 33.09°$ ($CHCl_3$; *c* 0.71).

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