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BIOTRANSFORMATION OF AROMATIC KETONES WITH CELL CULTURES OF CARROT, TOBACCO AND *GARDENIA*

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Key Word Index—*Daucus carota*; Umbelliferae; *Nicotiana tabacum*; Solanaceae; *Gardenia jasminoides*; Rubiaceae; aromatic ketones; biotransformation; reduction.

Abstract—Immobilized cells of *Daucus carota* enantioselectively transformed aromatic ketones such as acetophenone and propiophenone, into the corresponding (*S*)-alcohols with an optical purity of 89–99% ee in a chemical yield of 54–70%. Immobilized *Gardenia jasminoides* cells yielded the (*R*)-alcohols with a 63–91% ee.

INTRODUCTION

The use of plant cells in biotechnology has been steadily increasing over the past decade, because of their ability to produce secondary metabolites and to transform foreign substrates [1, 2]. Much attention has also been paid to immobilized plant cell cultures [3, 4] and in recent years we have systematically investigated the possibility of using immobilized plant cell cultures as potential biocatalysts for the transformation of synthetically important foreign substrates. Immobilized *Nicotiana tabacum* cells stereoselectively reduced some 3-oxobutanoates, selected as an organic foreign substrate, leading to the corresponding (*S*)-3-hydroxy-butanoates in relatively high optical yields [5, 6]. Several investigations have been carried out on the biotransformation of foreign substrates such as monoterpenes [7, 8] and aliphatic ketones [9] by using either freely suspended or immobilized cells of *Mentha* and *Nicotiana* species. Most of these reports, however, seemed to be unsatisfactory because of the difficulties in obtaining a high chemical or optical yield of the products.

As part of a continuing effort toward the transformation of organic foreign substrates with immobilized plant cell cultures, we have prepared the three different immobilized cells (*Daucus carota*, *N. tabacum* and *Gardenia jasminoides*), all of which were entrapped in calcium alginate beads, and examined the first bioreduction of the four aromatic ketones **1**–**4** by these immobilized cells.

RESULTS AND DISCUSSION

The bioreduction of acetophenone (**1**) with immobilized *D. carota* cells (IDCC) was complete within five days and the (*S*)-aromatic alcohol **1a**, having a high optical purity of 99% ee, was obtained in a chemical yield of 54% (Fig. 1). A similar bioreduction of propiophenone (**2**) with IDCC gave the corresponding (*S*)-alcohol **2a** with an optical purity of 89% ee in a 63% chemical yield. Unfortunately, the analogous transformations of **1** and **2** with immobilized *N. tabacum* cells (INTC) stopped before reaching 100% conversion, resulting in the (*S*)-alcohols **1a** and **2a** in a low chemical yield. Immobilized *G. jasminoides* cells (IGJC), in the biotransformations of **1** and **2**, yielded **1a** and **2a** with the opposite stereochemistry at the asymmetric carbon in an optical yield of 91 and 63%, respectively (Table 1).

IDCC also enantioselectively reduced 1-acetonaphthone (**3**) and 2-acetonaphthone (**4**) to the corresponding (*S*)-alcohols **3a** and **4a** in an excellent optical yield of 99 and 97% and in an acceptable chemical yield of 60 and 70%. Of interest is that IDCC reduction of **3** required 13 days to reach *ca* 80% conversion, while that of **4** was complete within 48 hr. Although the biotransformations of **3** and **4** with INTC showed a very low conversion, (*S*)-**3a** and (*S*)-**4a** were each produced in a high optical yield. In the case of IGJC reduction of **3**, (*S*)-**3a** was obtained in a 99% optical yield; for a similar reduction of **4**, no alcohol product was isolated.

An attempt was made to reduce **1** and **2** with freely suspended cells of *D. carota*. Although the chemical and optical yields in the case of the free cells were comparable with those of the immobilized cells, the free cells required longer reaction times and failed to be reused consecutively [5, 6]. Alternatively, microbial reductions of **1** and **2** by free baker's yeast had been investigated by MacLeod *et al.* [10]. The chemical and optical yields of the alcohol products obtained by the yeast reduction were generally lower than those obtained by the present IDCC system, although the yeast reduction proceeded at a faster rate than the IDCC one. Similar yeast reductions of **1** and **2** were run in this laboratory by using immobilized baker's yeast entrapped in calcium alginate beads, according to the procedures described previously [11]. The attempts were unsuccessful (20% conversion for **1** and only 3–5% conversion for **2**, 12 days) and led to a very low chemical yield of (*S*)-**1a**.

It is noteworthy that of the present three bioreduction systems tested, IDCC reduction exceeds 50% in the chemical yield of the chiral alcohol product and the rates of IDCC reduction are generally greater than those of both INTC and IGJC.

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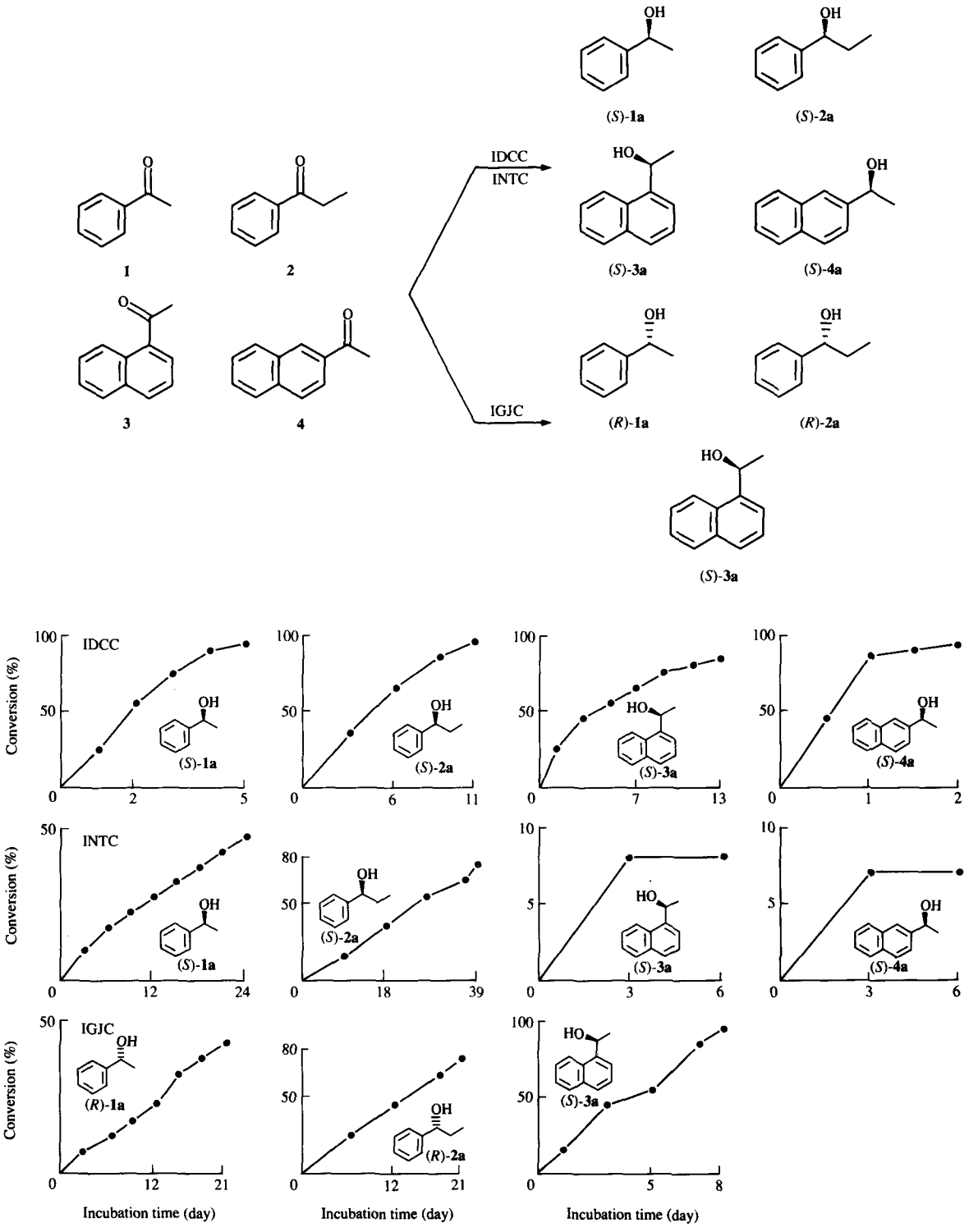

Fig. 1. Conversion of aromatic ketones (**1–4**) to aromatic alcohols (**1a–4a**) by immobilized cells of *D. carota* (IDCC), *N. tabacum* (INTC) and *G. jasminoides* (IGJC) as a function of incubation time.

EXPERIMENTAL

Cultivation of cells. Suspension cells of *D. carota* [12] and *N. tabacum* [5, 8] were cultivated as previously described and those of *G. jasminoides* cultivated in B5 medium containing 2 ppm naphthylacetic acid (pH 5.5) on a rotary shaker at 95 rpm and 25°.

Preparation of immobilized cells. Suspension cells (150 g) of *D. carota* and *G. jasminoides* and those (60 g) of *N. tabacum* were immobilized with a 5% aq. soln of Na alginate (600 ml) and a

Table 1. Biotransformation of aromatic ketones **1–4** with IDCC, INTC and IGJC

Immobilized plant cell culture	Substrate	Product	% Yield	% ee	$[\alpha]_D^{20}$ ($CHCl_3$, *c*)
IDCC*	**1**	(*S*)-**1a**	54	99	−57.06° (2.74)
	2	(*S*)-**2a**	63	89	−44.94° (2.65)
	3	(*S*)-**3a**	60	99	−51.90° (2.85)
	4	(*S*)-**4a**	70	97	−51.58° (2.95)
INTC†	**1**	(*S*)-**1a**	21	88	−52.86° (2.16)
	2	(*S*)-**2a**	37	61	−34.37° (2.35)
	3	(*S*)-**3a**	7	99	−51.21° (0.81)
	4	(*S*)-**4a**	6	95	−50.45° (1.21)
IGJC‡	**1**	(*R*)-**1a**	13	91	+53.37° (2.28)
	2	(*R*)-**2a**	25	63	+33.72° (2.21)
	3	(*S*)-**3a**	20	99	−51.70° (2.07)

*Immobilized *D. carota* cells.
†Immobilized *N. tabacum* cells.
‡Immobilized *G. jasminoides* cells.

0.6% aq. soln of $CaCl_2$ according to the procedures described in refs [5, 13]. The resulting IDCC and INTC beads, *ca* 4–5 mm diam, were allowed to stand for 30 min, washed with Murashige and Skoog's (MS) medium, and stored in MS medium; the IGJC beads were washed with B5 medium.

Biotransformation of aromatic ketones with immobilized plant cell cultures. IDCC prepd from suspension cells (150 g) of *D. carota* as described was added to freshly prepd MS medium (1 l) containing 2 ppm naphthylacetic acid and 0.1 ppm kinetin, and the medium was shaken for 2 days. An aromatic ketone (**4** dissolved in 5 ml EtOH) (200 mg) was administered to the precultured MS medium containing IDCC and the mixt. incubated at 25° on a rotary shaker in the dark. The reaction mixt. was filtered, and IDCC beads were washed with MS medium. The filtrate (the cultured medium from IDCC beads) and washings were combined and extracted with EtOAc. Work-up of the extracts gave a crude alcohol product, which was purified by column chromatography to give a chiral aromatic alcohol [**1a** (110 mg), **2a** (127 mg), **3a** (121 mg), **4a** (142 mg)].

INTC reduction was carried out in MS medium containing 2 ppm 2,4-dichlorophenoxyacetic acid and 3% sucrose. IGJC reduction was run by the same procedure as described for IDCC, except for the use of B5 medium containing 2 ppm naphthylacetic acid.

For the time-course experiments on these biotransformations (Fig. 1), at a regular time, a part of the incubated mixt. was removed and extracted with EtOAc. Each extract was analysed by GC (FID) with a PEG-20 M 25 m × 0.25 mm WCOT fused silica capillary column (GL Sciences, Tokyo, Japan) at 130° (carrier gas, He 0.48 ml min^{-1}; split ratio, 1/55). The conversion ratios were determined on the basis of the peak areas of ketone substrates and alcohol products.

Identification of the products. All alcohol products were fully characterized by comparing their spectra with those of authentic samples. Chemical yields were referred to products purified by silica gel CC.

Determination of optical purity. The optical purities (% ee) of **1a–4a** were determined by their HPLC analyses in comparison with racemic ones [column, Chiralcel OB 4.6 × 250 mm; eluent, hexane-2-propanol (9:1); flow rate, 1 ml min^{-1}; detection, 254 nm light]. The alcohol products were separable under these conditions and each showed two peaks; racemic alcohols were each sepd into two equal peaks. The ee of the chiral alcohols was, therefore, estimated on the basis of relative peak areas of the two enantiomers.

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REFERENCES

1. Reinhard, E. and Alfermann, A. W. (1980) *Adv. Biochem. Engng* **16**, 49.
2. Dixon, R. A. (ed.) (1985) *Plant Cell Culture: A Practical Approach.* IRL Press, Eynsham.
3. Prenosil, J. E. and Pedersen, H. (1983) *Enzyme Microb. Technol.* **5**, 323.
4. Hulst, A. C. and Tramper, J. (1989) *Enzyme Microb. Technol.* **11**, 546.
5. Naoshima, Y., Akakabe, Y. and Watanabe, F. (1989) *Agric. Biol. Chem.* **53**, 545.
6. Naoshima, Y. and Akakabe, Y. (1989) *J. Org. Chem.* **54**, 4237.
7. Galun, E., Aviv, D., Dantes, A. and Freeman, A. (1985) *Planta Med.* **51**, 511.
8. Suga, T., Hirata, T. and Izumi, S. (1986) *Phytochemistry* **25**, 2791.
9. Hamada, H., Umeda, N., Otsuka, N. and Kawabe, S. (1988) *Plant Cell Rep.* **7**, 493.
10. MacLeod, R., Prosser, H., Fikentscher, L., Lanyi, J. and Mosher, H. S. (1964) *Biochemistry* **3**, 838.
11. Naoshima, Y., Nakamura, A., Nishiyama, T., Haramaki, T., Mende, M. and Munakata, Y. (1989) *Chem. Letters* 1023.
12. Brodelius, P. and Nilsson, K. (1983) *Eur. J. Appl. Microbiol. Biotechnol.* **17**, 275.
13. Jones, A. and Veliky, I. A. (1981) *Eur. J. Appl. Microbiol. Biotechnol.* **13**, 84.