

Stereoselective Reduction of Ketone and Enone using Plant Cell Cultures

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Abstract

The biotransformation of a ketone and enone by plant cell cultures was investigated. It was found that the cultured cells of *Marchantia polymorpha* reduced preferentially the carbonyl group of 2-pentylcyclopentanone to produce the corresponding *anti*-2-pentylcyclopentanol. Hence, 2-pentylcyclopent-2-enone was hydrogenated by the cultured cells of *Catharanthus roseus* to give (*R*)-2-pentylcyclopentanone with high stereoselectivity.

Keywords: plant cell culture, stereoselective, stereoselective reduction.

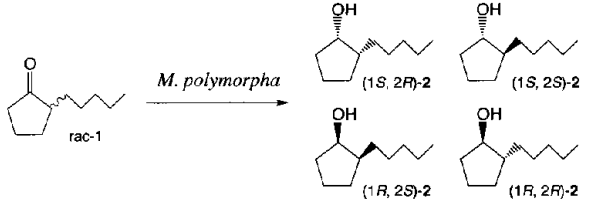
We have previously investigated the biotransformation of various carbonyl compounds and their derivatives by cultured plant cells (Hamada, 1988; Hamada *et al.*, 1988, 1994; Nakamura *et al.*, 1995). It has been reported that immobilized carrot, tobacco, and gardenia cells reduced the carbonyl group of aromatic ketones to the corresponding alcohols (Naoshima and Akakabe, 1991). However, there are few reports detailing the mechanism of the bioreduction of the carbonyl group by plant cultured cells. Carbonyl reductases, which catalyze the reduction of carbonyl compounds, are of considerable importance in drug metabolism (for a review, see Felsted and Bachur, 1980). Furthermore, there have been many studies on the reduction of the carbonyl group by the livers of rats, rabbits, and humans (Culp and McMahon, 1968; Moreland and Hewick, 1975; Tanaka *et al.*, 1984; Maser and Netter, 1989; Falguyret *et al.*, 1990; Oppermann *et al.*, 1991; Imamura *et al.*, 1992).

To clarify stereoselectivity of the reduction of the carbonyl group, we carried out the biotransformations of cycloalkanone and an α, β -unsaturated ketone by cultured plant cells. In this paper, we describe the stereochemistry of the biotransformations of 2-pentylcyclopentanone (**1**) and 2-pentylcyclopent-2-enone (**3**) by two cultured plant

cells.

The calli of *Marchantia polymorpha* (liverwort) and *Catharanthus roseus* were induced and maintained as previously reported (Hamada *et al.*, 1993, 1997). The experiments were carried out as follows. Some of the cultured cells of *C. roseus* and *M. polymorpha* were transferred to 100 ml of SH medium (Schenk and Hildebrandt, 1972) in a conical flask (300 ml) and grown with continuous shaking for 1 week at 25 °C in the light (approximately 2,000 lx). The substrate (20 mg) was administered to the precultured cells (about 30 g) in the flask and the cultures were incubated at 25 °C in a rotary shaker (120 rpm) in the light. At regular intervals, a portion of the reaction mixture was pipetted out under sterile conditions and extracted with ethyl acetate, and then the extract was subjected to GC analysis (Hamada *et al.*, 1988a; Hamada *et al.*, 1997). The enantiomeric excess (e.e.) and the absolute configuration of 2-pentylcyclopentanol (**2**) were determined by a GC analysis with an optically active capillary column (Chirasil-DEX CB; 0.25 mm x 25 m; column temperature at 120 °C; carrier gas: He at 1.0 kg cm⁻²) with optically pure standards. The e.e. and the absolute configuration of **1** were determined by GC analysis after derivatization to **2** by the reduction with NaBH₄. The individual

Table 1 Time course during the biotransformation of 2-pentylcyclopentanone (**1**) by the cultured cells of *M. Polymorpha*



Time (h)	2 Yield (%)	Syn / Anti	e.e. (%)	
			Syn-(1S, 2R)	Anti-(1S, 2S)
12	11.5	21 / 79	26.8	54.9
24	20.0	20 / 80	32.5	62.9
48	25.1	24 / 76	34.1	65.2
72	32.3	26 / 74	80.3	65.1
120	48.3	25 / 75	86.9	73.3

The precultured cells of *M. polymorpha* (about 30 g) was incubated in SH medium with the substrate (racemic **1**, 10 mg) at 25°C under light (2000 lx).

product yields were determined on the basis of the peak areas from GC chromatograms, and are expressed as percentages of the total product yields.

The time course and stereochemical results in the biotransformation of the racemic mixture of **1** by the cultured cells of *M. polymorpha* are shown in **Table 1**. The amount of **2** increased with the incubation time, and the enantioselectivity of *syn*-(1*S*, 2*R*) and *anti*-(1*S*, 2*S*) alcohol was also improved. However, the *syn*-to-*anti* ratio of the produced alcohol did not change. This result showed that the cultured cells of *M. polymorpha* mainly reduced **1** to the corresponding *anti*-2-pentylcyclopentanol, and the stereoselectivity of the reduction at the carbonyl group in the substrate was for the (*S*)-form preferentially.

We also studied the hydrogenation of the C=C double bond of **3** by cultured plant cells. Substrate **3** was hydrogenated by the cultured cells of *C. roseus* (see **Fig. 1**), whereas the cultured cells of *M. polymorpha* did not perform the hydrogenation. The conversion ratio of the hydrogenation was >99% after 48 h incubation, and the absolute configuration of the pentyl group at the 2-position was the (*R*)-form with high optical purity (89.0% e.e.). No reduced product of the carbonyl group of **1** was found in this biotransformation.

In conclusion, the cultured cells of *M. polymorpha* stereoselectively reduced the carbonyl group of **1** to the corresponding (*S*)-alcohol. Hence, the C=C double bond of **3** was preferentially hydrogenated by the cells of *C. roseus* to (*R*)-2-pentyl-

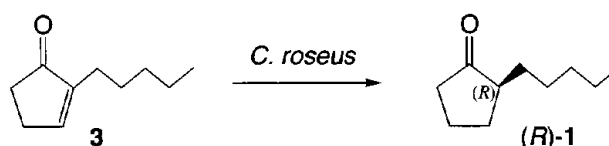


Fig. 1 The hydrogenation of 2-pentylcyclopent-2-enone (**3**) to (*R*)-2-pentylcyclopentanone by the cultured cells of *C. roseus*.

cyclopentanone with high stereoselectivity.

We are currently isolating the enzymes involved in this process, and the results will be reported in a future paper.

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