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Enantioselective reduction of acetophenone analogues using carrot and celeriac enzymes system

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Abstract

The enantioselective reduction of acetophenone analogues catalyzed by carrot and celeriac was performed in moderate conversions and excellent enantiomeric excesses. The steric factors and electronic effects of the substituents at the aromatic ring were found to significantly affect the efficiency of the enantioselective reduction of acetophenone analogues, while they had a little effect on the enantioselectivity of acetophenone analogues reduction. It was also found that the conversions of acetophenone analogues reduction at 33 °C by means of both biocatalysts were three times as great as those at room temperature.

Keywords: Enantioselective reduction; Acetophenone analogues; Plant cells; Biocatalysis

There is a considerable interest in efficient routes to obtain chiral alcohols since they are useful building blocks for the synthesis of drugs, agrochemicals, flavors and pigments. These alcohols can be obtained from the enantioselective reduction of prochiral ketones by biological methods [1–4]. Among these biological methods, microbes and plant cell cultures are more useful than isolated enzymes due to the existence of many enzymes that catalyze various reactions [5,6]. In addition, the use of microbes and plant cell cultures is particularly advantageous for carrying out the desired reduction since they do not require the addition of cofactors for their regeneration. It has been reported that reduction of a carbonyl group may be performed using various plant biocatalysts [7–10]. Acetophenone is a kind of very interesting model xenobiotic substrate for bioreduction, because it may give rise to both enantiomers of 1-phenylethanol. These compounds, as well as their simple analogues, have been effectively used as building blocks for asymmetric synthesis of drugs [11–14].

Here we would like to report the results of the reduction of acetophenone analogues catalyzed by carrot (*Daucus carota* L.) and celeriac (*Apium graveolens* L.) and evaluate the substituent effects on activity and enantioselectivity in the enzymatic reduction of these ketones. Aromatic ketones **1a**–**1g** were used as the substrates for the biotransformations. The bioreduction of the carbonyl group by the carrot's and celeriac's enzymes system proceeded enantioselectivity, following the Prelog's rule [15], which resulted in formation of (*S*)-alcohols predominantly (Scheme 1).

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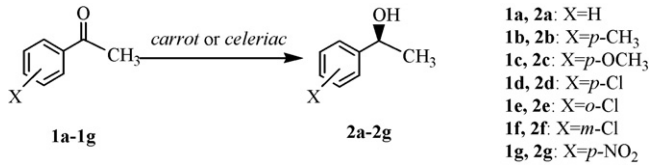

Scheme 1. Enantioselective reduction of **1a–1g** catalyzed by carrot or celeriac.

Table 1
Bioreduction results of **1a–1g** using carrot or celeriac as biocatalysts.

Entry	Substrate	Product	Biocatalyst and condition[a]	Conversion (%)[b]	*e.e.* (%)[b]
1	**1a**	**2a**	A	97	96
2	**1b**	**2b**	A	26	99
3	**1c**	**2c**	A	12	99
4	**1d**	**2d**	A	39	95
5	**1e**	**2e**	A	18	94
6	**1f**	**2f**	A	43	96
7	**1g**	**2g**	A	48	93
8	**1a**	**2a**	B	100	99
9	**1b**	**2b**	B	37	99
10	**1c**	**2c**	B	19	99
11	**1d**	**2d**	B	58	92
12	**1e**	**2e**	B	28	90
13	**1f**	**2f**	B	65	93
14	**1g**	**2g**	B	71	94

[a] A: Reductions were carried out at 33 °C for 28 h in distilled water (pH 7.0) using carrot as biocatalyst. B: Reductions were carried out at 33 °C for 28 h in 0.1 mol/L phosphate buffer (pH 6.2) using celeriac as biocatalyst.
[b] The conversion of **1a–1g** and the "*e.e.*" (enantiomeric excess) of **2a–2g** were determined by chiral GC analysis of crude extracts.

The bioreduction results of **1a–1g** were presented in Table 1. The results obtained showed that **1a** was reduced to the corresponding chiral alcohol with excellent conversion and enantioselectivity using both biocatalysts (entries 1 and 8 in Table 1), while the presence of a substituent at the aromatic ring could reduce the conversion of **1b–1g** (entries 2–7 and 9–14 in Table 1), especially the presence of *o*-substituent (entries 5 and 12 in Table 1). Therefore, the steric factors of these substituents were unfavorable to the conversion of **1b–1g**. Besides the steric factors, the electronic effects of these substituents played an important impact on the activity of both biocatalysts. The electron-withdrawing character of chloro and nitro group enhanced the activity of these biocatalysts and improved conversion of the substrates, especially these strong electron-withdrawing such as *m*-chloro and *p*-nitro group (entries 6, 7, 13 and 14 in Table 1). On the other hand, the electron-donating character of methyl and methoxy group had an opposite impact on this activity and made against conversion of the substrates (entries 2, 3, 9 and 10 in Table 1). The results of electronic effects were in accordance with the results reported by Zhu et al. [16] and Uwai et al. [17]. However, there were few changes in the enantiomeric excesses of **2b–2g**, no matter whether the aromatic ring of **2b–2g** possessed an electron-withdrawing or electron-donating substituent. This observation was in accordance with the results of the bioreduction of methoxyacetophenone and bromoacetophenone by means of plant cells [18].

Our research also focused on the influence of reaction temperature on the activity of acetophenone analogues bioreduction. It was observed that the conversions of **1a–1g** at 33 °C by means of both biocatalysts were three times as great as those of **1a–1g** at room temperature (Table 2). The bioreduction of acetophenone analogues were also tested at other temperatures (27 °C, 30 °C and 36 °C) and the results obtained were not as good as those at 33 °C. This suggested that these ketoreducatases of whole cell systems existing in carrot and celeriac possessed higher activity at 33 °C, although a lot of bioreductions using whole plant cells (including carrot [19,20] and celeriac [18]) were carried out at room temperature. The reaction temperature was another important factor which affected the activity of these two biocatalysts.

The results obtained indicate that the reduction activity of acetophenone analogues by means of carrot's and celeriac's enzymes system depends mainly on the steric factors and electronic effects of the substituents at the

Table 2
The conversion of acetophenone analogues reduction catalyzed by carrot or celeriac at different reaction temperatures.

Entry	Substrate	Product	Reaction temperature (°C)	Conversion (%)	
				Carrot	Celeriac
1	1a	2a	33	97	100
2	1b	2b	33	26	37
3	1c	2c	33	12	19
4	1d	2d	33	39	58
5	1e	2e	33	18	28
6	1f	2d	33	43	65
7	1g	2g	33	48	71
8	1a	2a	r.t.[a]	35	36
9	1b	2b	r.t.	10	12
10	1c	2c	r.t.	5	6
11	1d	2d	r.t.	14	20
12	1e	2e	r.t.	6	9
13	1f	2d	r.t.	15	21
14	1g	2g	r.t.	16	22

[a] r.t.: room temperature. The other reduction conditions were the same as those in Table 1.

aromatic ring, and also depends on reaction temperature. The influence of these substituents is less important for enantioselelctivity.

1. Experimental

Fresh carrot roots and celeriac stems were purchased from a local market. The external layer of these roots or stems was removed and the rest was carefully cut into small thin pieces (approximately 5 mm × 5 mm × 2 mm). Aromatic ketones **1a**–**1g** were obtained from Fluka and the racemic alcohol standards were prepared by chemical reduction of the corresponding ketones with sodium borohydride in methanol. Other chemical reagents were of analytic purity and purchased from local suppliers.

The small thin pieces of healthy roots or stems (10.0 g) were placed in flasks with 50 mL of 0.1 mol/L phosphate buffer at pH 6.2 (celeriac) or distilled water, pH 7.0 (carrot). To this suspension, 50 mg of **1a** dissolved in 0.5 mL ethanol was added and shaken at 33 °C for 1 h or 28 h in an orbital shaker (120 r/min). The suspension was filtered and the filtrate was extracted with ethyl acetate (3 × 20 mL). The organic phases were dried over anhydrous sodium sulfate and evaporated in vacuum. The final products were purified by column chromatography. The enantiomeric composition of product was established by GC equipped with CP-Chirasil-Dex CB chiral capillary column (25 m × 0.32 mm × 0.25 μm, Varian). Configuration of product was determined by the sign of the specific rotation.

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