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Letter

Preparative synthesis of chiral alcohols by enantioselective reduction with *Daucus carota* root as biocatalyst

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Abstract

The enzymatic reduction of (±)-2-methylcyclohexanone with fresh carrot root as biocatalyst occurred in a complete diastereoisomeric way giving a 1:1 mixture of enantiomerically pure (1*S*,2*R*)- and (1*S*,2*S*)-2-methylcyclohexanol. The analogous reaction carried out on the racemic 2-hydroxycyclohexanone afforded a 1:2 mixture of (1*S*,2*R*)- and (1*S*,2*S*)-1,2-cyclohexanediol with an enantiomeric excess > 95%. The low cost and the easy availability of the biocatalyst besides the very simple reaction conditions suggest the possible use of the present method for large scale preparations of important chiral alcohols.

Keywords: Biocatalytic transformation; Carrot root; Enantioselective reduction; Chiral alcohols

1. Introduction

Great attention has been paid to enantioselective syntheses of enantiomerically pure compounds or chiral synthons that are increasingly in demand for the development of modern drugs and agrochemicals. Asymmetric synthesis carried out with chiral metal complexes as catalysts has been successfully used [1,2]; however, some difficulties often remain in attaining high optical purity and practical usage in comparison with the ones performed by enzymatic catalysis. In fact, in most cases the use of enzymes as biocatalysts dramatically improves stereochemical quality, while reducing equipment requirements and, in addition, simplifying significantly separation and disposal steps [3]. Moreover, unlike heavy metals, biocatalysts are environmentally acceptable, being susceptible to complete degradation after use. Enzymatic reactions have been carried out by employing either whole cells, cell organelles or isolated enzymes. Biotransformation systems involving baker's yeast [4] and lipase [5] have been widely used as useful means in obtaining optically active compounds.

Baker's yeast is by far the most widely used microorganism for reduction of prochiral ketones yielding the corresponding optically active alcohols with fair-to-excellent enantioselectivity [6–10]. More recently, plant cell cultures have been considered as suitable biochemical systems able to transform enantioselectively important foreign synthetic substrates, as well as natural and endogenous compounds [11–15].

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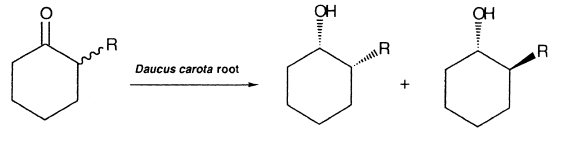
The biotransformation of organic xenobiotics, e.g. ethyl 3-oxobutanoate [14] and acetophenone [15] by immobilized plant cell cultures of tobacco, gardenia and carrot have been investigated. In particular, excellent enantioselectivity has been demonstrated in the reduction of prochiral ketone substrates such as keto esters, aromatic and heterocyclic aromatic ketones with immobilized carrot (*Daucus carota*) cell cultures [16,17].

The use of whole plant cells, instead of isolated dehydrogenases, has many advantages, the first of which is that the biological material would not have to be handled in order to obtain a more or less purified enzyme. In fact, this relatively simple handling requires ultracentrifugation and chromatographic apparatus, and the use of low amounts of starting material. Furthermore, unlike the purified enzyme, the biotransformation carried out with whole cells does not require costly co-factor recycling since it is automatically done by the cell. On the other hand, although the whole cells are able to accept non-natural substrates, the productivity of cellular conversion is usually low due to the toxicity of these substrates for living organisms or to a dramatic modification of the cellular system. Thus, the need of a large amount of biomass to obtain reasonable yields requires adequate and careful growing operations and a more difficult large-scale set.

In order to avoid the time consuming and care-requiring preparations of plant cell cultures, we have been investigating the possibility to directly use parts of plants as biocatalysts in the reduction of prochiral ketones. In this case, indeed, the starting material used for the preparations of cell cultures is directly obtained from a portion of the vegetable and consists of a large amount of functionally intact cells. Fresh carrot root has therefore been used as the biocatalyst and racemic 2-methylcyclohexanone (**1**) and 2-hydroxycyclohexanone (**4**) were chosen as model compounds.



(±)-**1**: R = CH_3
(±)-**4**: R = OH

(1*S*,2*R*)-**2**: R = CH_3 (1*S*,2*S*)-**3**: R = CH_3
(1*S*,2*R*)-**5**: R = OH (1*S*,2*S*)-**6**: R = OH

2. Experimental

Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The ^{1}H and ^{13}C NMR spectra were registered in $CDCl_3$ with a Bruker AC 200 instrument using TMS as the internal standard. The e.e.'s of **1**, **2**, **3** and **6** were determined by GLC analysis using a Carlo Erba HRGC 5300 instrument equipped with a 20 m Chiraldex G-TA (ASTEC) column, 80°C for 10 min, at 6°C/min, and 110°C for 10 min; **6**, 110°C. The e.e. of recovered **4** was calculated on the basis of the last reported $[\alpha]_D$ value [18,19]. The conversions and yields were determined by GLC analysis, under the same conditions used for the determination of the e.e.'s by adding benzylmethylketone as an internal standard.

2.1. Materials

Racemic 2-methylcyclohexanone (**1**) and 2-hydroxycyclohexanone (**4**) are commercially available substrates and were used without further purification. To increase the contact of the substrate with the biocatalyst, a thin portion of the external part of carrot root was removed and the rest was carefully cut into about 2 mm thread-like pieces with an ordinary mechanical kitchen utensil. This handling avoids the increase in temperature during cutting that could denature the cellular proteins.

2.2. Biocatalyzed transformations

Ketones **1** and **4** (1 g, 8.93–869 mmol) were added to a stirred suspension of freshly cut carrot root (200 g) in 500 ml of water and the reaction mixtures were stirred at room temperature for the time necessary to obtain the appropriate conversion (40–58 h). The final suspensions were then filtered off and the filtrates were extracted with AcOEt (500 ml). The organic phases (dried with $MgSO_4$) were then evaporated in vacuo. The conversions were determined by GLC by dilution to an exactly known volume (100 ml) with ethyl acetate and by addition of a proper amount of internal standard to an aliquot (10 ml) of these solutions and analyzed by GLC. The organic solutions were then evaporated and the residue chromatographed on a silica gel column and

the optical rotation of the recovered products **1**, **2**, **3**, **4** and **6** were measured. In typical experiments, at complete conversion of (±)-**1** (after 48 h), (1*S*,2*S*)-(+)-2-methylcyclohexanol **2** (e.e. > 99%) and (1*S*,2*R*)-(+)-2-methylcyclohexanol **3** (e.e. > 99%) were obtained. After extraction and chromatography, **2** (385 mg, 3.38 mmol) and **3** (380 mg, 3.33 mmol) were recovered in a 75% overall yield. When the reaction was stopped at 85% conversion (after 36 h) optically pure (*R*)-(−)-**1** (110 mg, 0.98 mmol), (1*S*,2*S*)-(+)-**2** (370 mg, 3.25 mmol, e.e. > 99%) and (1*S*,2*R*)-(+)-**3** (280 mg, 2.46 mmol, e.e. > 99%) were recovered in a 11, 36.4 and 27.5% yield, respectively. At complete conversion of (±)-2-hydroxycyclohexanone (**4**) (after 54 h), (1*S*,2*R*)-(**5**) (233 mg, 2.01 mmol) and (1*S*,2*S*)-(+)-1,2-cyclohexanediol (**6**) (433 mg, 3.74 mmol, e.e. > 95%) were recovered in a 65% overall yield. At incomplete conversion of (±)-**4** (40 h), (*R*)-(+)-**4**, $[\alpha]_D^{25} = +10.0$ ($c = 0.6$, $CHCl_3$) (128 mg, 1.11 mmol, 77% e.e. [18,19]), (1*S*,2*R*)-**5** (192 mg, 1.66 mmol) and (1*S*,2*S*)-(+)-**6** (392 mg, 3.38 mmol, e.e. > 95%) were recovered in a 70% overall yield.

3. Results and discussion

As shown in Tables 1 and 2, the incubations of ketones **1** and **4** with fresh carrot in water at room temperature gave the corresponding alcohols with a high product enantioselectivity. In particular, the racemic 2-methylcyclohexanone (**1**) was completely reduced affording a 1:1 mixture of the diastereoisomers (1*S*,2*R*)-(+)-2-methylcyclohexanol (**2**) (e.e. > 99%) and (1*S*,2*S*)-(+)-2-methylcyclohexanol (**3**) (e.e. > 99%). When the same reaction was stopped at 85% conversion, in addition to the enantiomerically pure alcohols **2** and **3**, the unreacted (*R*)-(−)-2-methylcyclohexanone (e.e. > 99%) was recovered. The complete absence of compounds having (*R*) configuration at C-1 in the reaction products indicates that the carrot catalyzed reduction of racemic **1** occurs in a diastereospecific way. The diastereoisomers (1*S*,2*R*)-**2** and (1*S*,2*S*)-**3** were obtained in a 1:1 ratio by the reduction of (*R*)- and (*S*)-2-methylcyclohexanone **1**, respectively. This behavior is confirmed by the recovery of pure (*R*)-(−)-**1** at incomplete conversion, in connection with the formation of a lower amount of (1*S*,2*R*)-**2**, with respect to that of (1*S*,2*S*)-**3**. Furthermore, the results indicate that: (1) the reaction occurs with substrate enantioselectivity, the (*S*) enantiomer being reduced before the (*R*) one; (2) the enzyme delivers the hydride exclusively from the *re* face of carbonyl of both enantiomers to give the (*S*) alcohols. The selectivity of dehydrogenases is dependent upon the size of the carbon chains attached to the carbonyl group. The size of carbon chain however, is not necessarily in accordance with the CIP-rules [20].

In analogy with the results observed for **1**, the reduction of racemic 2-hydroxycyclohexanone **4** (Table 2) gives a diastereoisomeric mixture of the meso (1*S*,2*R*)-**5** and optically active (1*S*,2*S*)-(+)-1,2-cyclohexanediol **6** (e.e. > 95%) and, at incomplete conversion, the (*R*) enantiomer of **4** was recovered. However, the 1:2 ratio of **5** to **6** obtained at complete conversion clearly indicates the formation of (1*S*,2*S*)-1,2-cyclohexanediol **6** both from the (*S*) and from the (*R*) enantiomer. This may be explained considering that, in the presence of electron-withdrawing substituents adjacent to the carbonyl group, the enolization process can cause a relatively rapid interconversion of the enantiomers of the substrate, the resultant diastereoisomeric ratio being determined by the substrate enantioselectivity of the enzyme involved in the reduction process [21].

Table 1
Carrot root catalyzed reduction (%) of (±)-2-methylcyclohexanone (**1**)

Conversion	(*S*)-**1**	(*R*)-**1**	e.e.	(1*S*,2*R*)-**2**	e.e.	(1*S*,2*S*)-**3**	e.e.
85[a]	–	15	> 99	35	> 99	50	> 99
100[b]	–	–	–	50	> 99	50	> 99

[a] Substrate 1 g; carrot root 200 g; time 36 h.
[b] Substrate 1 g; carrot root 200 g; time 48 h.

Table 2
Carrot root catalyzed reduction (%) of (±)-2-hydroxycyclohexanone (**4**)

Conversion	(*R*)-**4**	e.e.	(1*S*,2*R*)-**5**	(1*S*,2*S*)-6	e.e.
82[a]	18	77	27	55	> 95
100[b]	–	–	35	65	> 95

[a] Substrate 1 g; carrot root 200 g; time 40 h.
[b] Substrate 1 g; carrot root 200 g; time 54 h.

In conclusion, the present results show that the use of the root of *D. carota* in the catalytic reduction of prochiral ketones is a practical route to produce chiral alcohols, providing an attractive environmentally acceptable option which could be used on an industrial scale. Studies are in progress in order to improve the efficiency of this process both by increasing the substrate–catalyst contact surface and by evaluating the influence of other parameters such as pH, temperature and different carrot cultivar on the reaction course, besides the possible use of the biocatalyst in an immobilized form.

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