

# Asymmetric reduction of prochiral ketones to chiral alcohols catalyzed by plants tissue

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**Abstract** As an important organic compound, chiral alcohols are the key chiral building blocks to many single enantiomer pharmaceuticals. Asymmetric reduction of the corresponding prochiral ketones to produce the chiral alcohols by biocatalysis is one of the most promising routes. Asymmetric reduction of different kinds of non-natural prochiral ketones catalyzed by various plants tissue was studied in this work. Acetophenone, 4'-chloroacetophenone and ethyl 4-chloroacetoacetate were chosen as the model substrates for simple ketone, halogen-containing aromatic ketone and  $\beta$ -ketoesters, respectively. Apple (*Malus pumila*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), onion (*Allium cepa*), potato (*Solanum tuberosum*), radish (*Raphanus sativus*) and sweet potato (*Ipomoea batatas*) were chosen as the biocatalysts. It was found that these kinds of prochiral ketones could be reduced by these plants tissue with high enantioselectivity. Both R- and S-form configuration chiral alcohols could be obtained. The *e.e.* and chemical yield could reach about 98 and 80% respectively for acetophenone and 4'-chloroacetophenone reduction reaction with favorable plant tissue. And the *e.e.* and yield for ethyl 4-chloroacetoacetate reduction reaction was about 91 and 45% respectively.

**Keywords** Asymmetric reduction · Chiral alcohol · Plant catalysis · Acetophenone · 4'-Chloroacetophenone · Ethyl 4-chloroacetoacetate

## Introduction

Because of safety, therapeutics and regulatory concerns, there has been increasing interests in the development of processes capable of producing enantiomerically pure drugs [1, 2]. The enantiomerically pure pharmaceuticals are ordinarily synthesized from the chiral building blocks, which is usually produced through chemical catalysis or biocatalysis. Chiral alcohols are one kind of the most important chiral building blocks for numerous chiral pharmaceuticals due to their unique structure property [2–5]. Asymmetric reduction of the corresponding prochiral ketones is one of the effective and promising routes to manufacture chiral alcohols [6]. Biocatalysis, involving either isolated oxido-reductases or living organisms, is always regarded as one of the most promising method due to its outstanding enantioselectivity, mild reaction conditions and environment-friendly [7–9]. In this case, whole cell is an excellent alternative to the isolated enzyme, since the oxido-reductase, cofactor (NAD(P)H) and its regenerate system all locate within cell, and the addition of the expensive cofactor can be avoided [4, 10, 11]. There are extensive works on the asymmetric reduction reaction catalyzed by whole cell with excellent enantioselectivity and yield [4, 6, 11]. However, the previous reported works mainly focus on application microbial organism as the biocatalyst [11–16].

Moreover, plant cell is another potential biocatalyst, since diverse oxido-reductases and the cofactor regeneration system exist in the plant cell. Unfortunately, there are

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few reports about the biotransformation catalyzed by plant cell [17–22]. But the previous reported works is mainly concentrated on natural substrates. Excellent results are obtained in natural ketones reduction, since they are easily accepted by plant cell [18–22]. However the asymmetric reduction reaction of non-natural prochiral ketone catalyzed by plant tissue or cell was rarely reported, which is very valuable to both theoretical research and practical application. The objective of this study is to explore the asymmetric reduction of different kinds of simple prochiral ketones catalyzed by various plants tissue. Acetophenone, 4'-chloroacetophenone and ethyl 4-chloroacetoacetate was chosen as the model substrate for simple ketones, halogen-containing aromatic ketones and  $\beta$ -ketoesters, respectively. Apple (*Malus pumila*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), onion (*Allium cepa*), potato (*Solanum tuberosum*), radish (*Raphanus sativus*) and sweet potato (*Ipomoea batatas*) were chosen as the biocatalyst.

## Materials and methods

### Chemicals

Acetophenone and benzaldehyde were purchased from China Medicine Shanghai Chemical Reagent Corporation (China), analytical reagent. R- and S-1-phenylethanol, ethyl 4-chloroacetoacetate, ethyl S- 4-chloro-3-hydroxybutyrate, and ethyl R- 4-chloro-3-hydroxybutyrate were purchased from ACROS Organic In. (New Jersey, USA), lab reagent grade. 4'-chloroacetophenone, R- and S-1-(4-chloro-phenyl)ethanol was purchased from Fluka Chemia In., analytical reagent. Ethyl acetate and other reagents were of analytical reagent and commercially available.

### Plant tissue

Fresh apple (*M. pumila*), carrot (*D. carota*), cucumber (*C. sativus*), onion (*A. cepa*), potato (*S. tuberosum*), Radish (*R. sativus*) and sweet potato (*I. batatas*) were obtained from a local market. To increase the contact of the substrate with the biocatalyst, the external layer of the plants was removed and the rest was carefully cut into small thin pieces (approximately 1 cm long slice).

### General procedure for the asymmetric reduction of ketones with various plants tissue

A certain amount of substrate (the final concentration was 10 mmol/L) were added to a suspension of freshly cut plant tissue (15 g) in 40 mL of water, and the reaction mixture was incubated in an orbital shaker operating at 150 r/min and 30 °C for a certain period (for example 50 h) to obtain

a appropriate conversion. Finally, the reaction mixture was extracted with ethyl acetate (1:1). The organic phase was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. Then chemical yield and enantioselectivity were determined. Each experiment was parallelly repeated at least three times. Then the average value and standard deviations were given.

### Analysis

The concentrations of acetophenone, R- and S-1-phenylethanol, 4'-chloroacetophenone, and R- and S-1-(4-chloro-phenyl)ethanol were determined with a gas chromatograph (Model 6890, Agilent Technologies Co., Ltd) equipped with a chiral Cyclodex-B capillary column (0.25 mm × 30 m, Agilent Technologies Co., Ltd). Benzaldehyde was applied as the internal standard substance. The conditions of gas chromatograph were: N<sub>2</sub> as the carrier gas at 3.5 mL/min of flow rate, splitting ratio 50:1, flame ionization detector (FID). The temperature of injector and FID were both 250 °C. The oven temperature for acetophenone, and R- and S-1-phenylethanol was 90 °C for 5 min, increasing from 90 to 180 °C at speed of 15 °C/min and keeping at 180 °C for 2 min. And it for 4'-chloroacetophenone, and R- and S-1-(4-chloro-phenyl) ethanol was 120 °C for 5 min, increasing from 120 to 190 °C at speed of 9 °C/min and keeping at 190 °C for 5 min. The retention time of acetophenone, R-1-phenylethanol and S-1-phenylethanol was 13.08, 14.43 and 14.59 min, respectively. And the retention time of 4'-chloroacetophenone, R-1-(4-chloro-phenyl)ethanol and S-1-(4-chloro-phenyl)ethanol was 15.41, 15.74 and 15.93 min, respectively.

The analytical approach of ethyl 4-chloroacetoacetate reduction reaction was the same as our previous work [14].

The reaction degree and the enantioselectivity were indicated by “yield” (chemical yield) and “*e.e.*” (enantiomeric excess) respectively, which were defined as:

$$\text{Yield} = \frac{C_P}{C_0} \times 100\%; \quad (1)$$

$$e.e. = \left| \frac{C_S - C_R}{C_S + C_R} \right| \times 100\% \quad (2)$$

$C_0$  initial substrate concentration

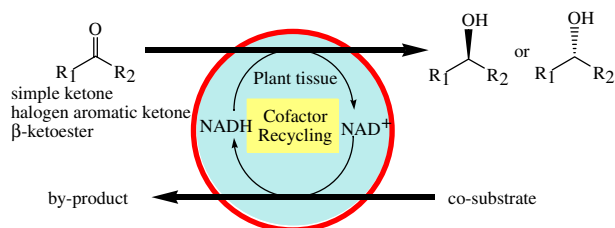
$C_P$  final product concentration

$C_S$  final S-form product concentration

$C_R$  final R-form product concentration.

## Results and discussion

Asymmetric reduction reactions of acetophenone, 4'-chloroacetophenone and ethyl 4-chloroacetoacetate by various



**Scheme. 1** Asymmetric reduction of ketones catalyzed by plants tissue

plants tissue were investigated. Apple (*M. pumila*), carrot (*D. carota*), cucumber (*C. sativus*), onion (*A. cepa*), potato (*S. tuberosum*), radish (*R. sativus*) and sweet potato (*I. batatas*) were selected as the biocatalysts. The reaction was shown in Scheme 1. And the reaction time was 50 and 100 h.

### Acetophenone

Acetophenone is a preferred model substrate of simple ketone and aromatic ketone for asymmetric reaction. The results of asymmetric reduction of acetophenone by various plants tissue was given in Table 1, indicated that acetophenone could be reduced to chiral 1-phenylethanol with attractive enantioselectivity and chemical yield. S-form and R-form product (1-phenylethanol) were all obtained by the asymmetric reduction reaction catalyzed by different plants tissue. Except for potato (*S. tuberosum*), the reaction reached the equilibrium within 50 h. The best results can be obtained by the action of carrot (*D. carota*) and potato (*S. tuberosum*) in terms of the yield and *e.e.*. The yield and *e.e.* are 79.2 and 96.4% respectively catalyzed by carrot (*D. carota*). And that are 51.4 and 92.1% for potato (*S. tuberosum*). The product of the reaction catalyzed by carrot (*D. carota*) is in S-form configuration, which is in agreement with Prelog's rule. It is consistent to Yadav's results in their work of reduction of different ketones with carrot [17]. On the contrary, it is R-form configuration catalyzed by potato (*S. tuberosum*), which

follows anti-Prelog's rule. Compared with the results of catalyzed by microbe cell (such as yeast cell), the results are attractive [4, 12, 23].

### 4'-chloroacetophenone

The single enantiomer of halogen-containing aromatic alcohols is one of the most important kinds of chiral building blocks for many enantiomerically pure pharmaceuticals, such as L-chlorprenaline, R-tomoxetine, S-fluoxetine, R-salbutamol, and R-denopamine [24]. These chiral alcohols can be synthesized by asymmetric reduction of the corresponding prochiral halogen-containing aromatic ketones. To investigate the asymmetric reduction of the halogen-containing aromatic ketones catalyzed by plants tissue, 4'-chloroacetophenone was chosen as the model substrate, since it possesses the general peculiarities of this kind of ketones. Moreover, the products, R- or S-1-(4-chloro-phenyl)ethanol, are key chiral intermediates for many chiral drugs [24]. The results were shown in Table 2. It was observed that the reaction results of asymmetric reduction of 4'-chloroacetophenone by these plants tissue is similar to that of acetophenone. Even more interesting is that the enantioselectivity and yield are  $\geq 90$  and  $\geq 50\%$  respectively to most plants tissue, remarkably higher than that of the acetophenone reduction reaction. That indicates that halogen-containing aromatic ketone is more acceptable to plant cells than simple aromatic ketone. And the chlorine bond on the phenyl group enlarges the difference between the two groups on both sides of the carbonyl, therefore improving the enantioselectivity of this reduction reaction. The results show that it is with potential practical application value.

### Ethyl 4-chloroacetoacetate

Reduction of  $\beta$ -ketoesters is probably the most extensively studied in asymmetric reduction of prochiral ketones to chiral alcohols catalyzed by microbe organism [25]. Ethyl 4-chloroacetoacetate was chosen as the model substrate for

**Table 1** Asymmetric reduction of acetophenone by various plants tissue

Entry	Plant tissue	50 h		100 h		Config.
		Yield/%	<i>e.e.</i> /%	Yield/%	<i>e.e.</i> /%	
1	Apple ( <i>M. pumila</i> )	38.7 ± 2.1	82.5 ± 2.5	40.9 ± 1.5	81.5 ± 2.7	R
2	Carrot ( <i>D. carota</i> )	78.4 ± 2.6	95.0 ± 2.9	79.2 ± 2.0	96.4 ± 1.9	S
3	Cucumber ( <i>C. sativus</i> )	50.5 ± 1.5	75.2 ± 3.1	55.5 ± 1.8	75.8 ± 3.2	S
4	Onion ( <i>A. cepa</i> )	52.7 ± 1.8	74.2 ± 2.6	54.3 ± 1.4	73.8 ± 2.9	S
5	Potato ( <i>S. tuberosum</i> )	28.0 ± 2.3	93.7 ± 2.8	51.4 ± 2.2	92.1 ± 3.4	R
6	Radish ( <i>R. sativus</i> )	71.9 ± 1.7	70.6 ± 3.8	82.3 ± 2.5	72.8 ± 2.8	S
7	Sweet potato ( <i>I. batatas</i> )	42.5 ± 2.4	80.0 ± 3.4	43.5 ± 1.9	80.2 ± 2.9	R

**Table 2** Asymmetric reduction of 4'-chloroacetophenone by various plants tissue

Entry	Plant tissue	50 h		100 h		Config.
		Yield/%	<i>e.e.</i> /%	Yield/%	<i>e.e.</i> /%	
8	Apple ( <i>M. pumila</i> )	59.2 ± 1.2	92.4 ± 1.6	65.2 ± 1.9	93.1 ± 1.9	R
9	Carrot ( <i>D. carota</i> )	41.7 ± 1.6	99.0 ± 0.8	42.6 ± 1.1	98.2 ± 1.7	S
10	Cucumber ( <i>C. sativus</i> )	70.4 ± 1.3	94.1 ± 1.6	72.4 ± 2.0	93.1 ± 1.8	S
11	Onion ( <i>A. cepa</i> )	57.7 ± 2.0	89.0 ± 1.8	74.5 ± 2.6	90.0 ± 2.9	S
12	Potato ( <i>S. tuberosum</i> )	50.1 ± 1.7	99.1 ± 0.9	62.6 ± 1.9	98.6 ± 1.4	R
13	Radish ( <i>R. sativus</i> )	55.1 ± 1.8	89.2 ± 2.6	72.4 ± 2.1	90.2 ± 2.1	S
14	Sweet potato ( <i>I. batatas</i> )	53.1 ± 1.9	93.6 ± 1.2	53.1 ± 2.4	93.0 ± 1.8	R

**Table 3** asymmetric reduction of ethyl 4-chloroacetoacetate by various plants tissue

Entry	Plant tissue	50 h		100 h		Config.
		Yield/%	<i>e.e.</i> /%	Yield/%	<i>e.e.</i> /%	
15	Apple ( <i>M. pumila</i> )	25.4 ± 2.6	85.3 ± 2.1	28.4 ± 2.8	88.3 ± 3.6	S
16	Carrot ( <i>D. carota</i> )	39.6 ± 2.3	90.1 ± 2.2	45.5 ± 2.2	91.0 ± 2.1	S
17	Cucumber ( <i>C. sativus</i> )	35.7 ± 2.9	75.3 ± 2.9	37.7 ± 2.7	73.3 ± 2.9	S
18	Onion ( <i>A. cepa</i> )	48.8 ± 2.1	70.6 ± 3.6	56.0 ± 3.4	76.6 ± 3.4	R
19	Potato ( <i>S. tuberosum</i> )	24.6 ± 3.4	58.4 ± 2.4	29.6 ± 2.9	60.4 ± 3.2	S
20	Radish ( <i>R. sativus</i> )	21.0 ± 3.6	68.7 ± 2.9	31.2 ± 3.1	65.7 ± 2.7	S
21	Sweet potato ( <i>I. batatas</i> )	36.0 ± 2.7	82.4 ± 3.6	46.7 ± 2.4	80.4 ± 3.3	S

$\beta$ -ketoesters, and its asymmetric reduction catalyzed by plants tissue was investigated. The product chemical yield and enantioselectivity of the reduction reaction were shown in Table 3. The results coincide with Yadav's results in investigation on the reduction of other  $\beta$ -ketoesters with *D. carota* root [17]. The results indicated that the product is mainly S-form configuration excessive (i.e. ethyl (S)-(-)-4-Chloro-3-Hydroxybutanoate) except catalyzed by onion (*A. cepa*). The best result was obtained with using carrot (*D. carota*) as the biocatalyst. The yield and *e.e.* are not so excellent compared with aromatic ketone reduction, but yield and *e.e.* are comparable to those of catalyzed by microbe [26]. The yield and *e.e.* could reach to 45.5 and 91.0% respectively.

## Conclusions

This work indicates that like natural ketones the non-natural prochiral ketones (simple ketones, halogen-containing aromatic ketones and  $\beta$ -ketoesters) can also be reduced to chiral alcohols by various plants tissue with excellent enantioselectivity. Acetophenone, 4'-chloroacetophenone and ethyl 4-chloroacetoacetate as the corresponding model substrates can be effectively reduced to the corresponding chiral alcohols by the applied plants tissue, i.e. apple (*M. pumila*), carrot (*D. carota*), cucumber (*C. sativus*), onion (*A. cepa*),

potato (*S. tuberosum*), radish (*R. sativus*) and sweet potato (*I. batatas*). Moreover, both R- and S-form configuration chiral alcohols could be obtained through these asymmetric reduction reactions. The reaction properties of reduction acetophenone and 4'-chloroacetophenone are similar. However, the results of reduction 4'-chloroacetophenone is more attractive in term of the chemical yield and *e.e.*. The reason is that halogen-containing aromatic ketone is more acceptable to plant cells than simple aromatic ketone. The favorable plants tissue to acetophenone and 4'-chloroacetophenone are carrot (*D. carota*) and potato (*S. tuberosum*) based on the chemical yield and *e.e.*. The configurations of the corresponding chiral alcohol are S-form and R-form respectively. To ethyl 4-chloroacetoacetate reduction reaction, the *e.e.* and yield were not so satisfactory compared with acetophenone and 4'-chloroacetophenone. However, with the favorable plants tissue, carrot (*D. carota*), the *e.e.* and yield can reach about 91 and 45%. This provides a new route to produce chiral alcohols, as the platform chemicals for enantiomerically pure pharmaceuticals, through asymmetric reduction of the corresponding simple prochiral ketones.

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