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Asymmetric reduction of 3-oxo-3-phenylpropionic acid ethyl ester by undifferentiated cells of white turnip in phosphate buffer/organic solvent

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Abstract−Ethyl (S)-3-hydroxy-3-phenylpropionate was synthesized by asymmetric reduction of 3-oxo-3-phenylpropionic acid ethyl ester with undifferentiated cells of white turnip in phosphate buffer/organic solvent. The conversion increased with the $LogP_{oct}$ of organic solvent increase. The phosphate buffer (0.2 mol/L, pH 7.0)/dodecane was selected as optimum medium for reduction. The optimal content of dodecane in medium is 10% (v). The conversion decreased with initial substrate concentration increase. Addition of more biomass of plant cells and 10% ethanol as co-substrate can improve conversion. The plant cells can be reused well for three times. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate reached 100% with 1% allyl bromide as inhibitor.

Key words: Ethyl (S)-3-hydroxy-3-phenylpropionate, Undifferentiated Cells of White Turnip, Phosphate Buffer/Organic Solvent, Asymmetric Reduction

INTRODUCTION

Ethyl (S)-3-hydroxy-3-phenylpropionate is an important chiral building block for synthesis of (S)-fluoxetine [1]. Fluoxetine is a potent serotonin-uptake inhibitor [2] that shows promise not only as an antidepressant but also for treatment of anxiety, alcoholism, chronic pain, and eating disorders such as obesity and bulimia [3]. Serotonin uptake was inhibited longer in mice after injection of (S)-fluoxetine [4], due to the persistence of parent drug and the metabolite, (S)-norfluoxetine, than it was after injection of (R)-fluoxetine which is N-demethylated more rapidly to a relatively inactive compound, (R)-norfluoxetine [5,6]. (R)-fluoxetine appeared less potent as a serotonin uptake inhibitor in vivo than did (S)-fluoxetine [7]. Ethyl (S)-3-hydroxy-3-phenylpropionate can be prepared by asymmetric reduction of 3-oxo-3-phenylpropionic acid ethyl ester (Fig. 1).

Plant cells have asymmetric reduction activity. Many plants such as *daucus carota* [8], *apium graveolens* [9], *allium schoenoprasum*, *coriandrum sativum* [10], *caragana chamlagu* [11], *catharanthus roseus*, *nicotiana tabacum* [12], *brassica oleracea botrytis*, *cucurbita maxima* [13], *marchantia polymorpha, glycine max* [14], *pouteria sapota*, *pouteria sapota*, *prunus persica* [15], *annona muricata* [16], were used in asymmetric reduction of carbonyl groups of some ketones and α-keto esters or β-keto esters. The configuration of product is influenced not only by regioselectivity [17] and stereoselectivity of cells from different plants even different parts of the same plant, but also by steric effect and electronic effect of substitute group of the substrate [18]. Biotransformation of carbonyl compound can be carried out in different medium including aqueous, organic solvent, aqueous/organic solvent two phase system, supercritical carbon dioxide and ionic liquid. For reduction happening in an aqueous/organic two phase system, plant cell activity can be preserved perfectly in aqueous and substrate or product can dissolve easily in organic solvent so that the inhibition of substrate or product on reduction can be eliminated. In this paper, asymmetric reduction of 3-oxo-3-phenylpropionic acid ethyl ester with undifferentiated cells of white turnip in aqueous/organic two phase system was investigated and ethyl (S)-3-hydroxy-3-phenylpropionate was synthesized successfully.

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EXPERIMENTAL SECTION

1. Chemicals

Ethyl (S)-3-hydroxy-3-phenylpropionate and Ethyl (R)-3-hydroxy-3-phenylpropionate were purchased from J & K Chemical Ltd. 3-oxo-3-phenylpropionic acid ethyl ester was supplied by Maxim Group of China.

2. Preparation of Undifferentiated Cells of White Turnip

After the epidermis was cut off and washed by water, the taproot of white turnip was immersed in 75% ethanol for 5 min, followed by 0.1% mercury bichloride for 10 min and finally with sterile distilled water for sterilization. The cambium of taproot was cut into cubes (1×1×1 mm) and added to the MS solid medium amended with 1.0 mg/L 2,4-dichlorophenoxy acetic acid and 0.5 mg/L kinetin for incubation. The callus obtained after cultivation for 18 d was suspended in 100 ml MS liquid medium amended with 30 g/L sucrose and 1.0 mg/L 2,4-dichlorophonoxacetic acid in a triangular flask (250 ml) kept at 27 °C and 100 rpm. After 18 d, undifferentiated cells were harvested by centrifugation at 3,000 rpm for 20 min at room temperature.

3. Reduction of 3-Oxo-3-phenylpropionic Acid Ethyl Ester with Undifferentiated Cells of White Turnip in Phosphate Buffer

2 g (dry weight) of undifferentiated cells and 3-oxo-3-phenylpropionic acid ethyl ester (0.108-0.846 mmol) were suspended in a triangular flask (50 ml) containing 30 ml phosphate buffer (0.2 mol/L) kept at 27 °C and 100 rpm. After 10-15 days the cells were filtered

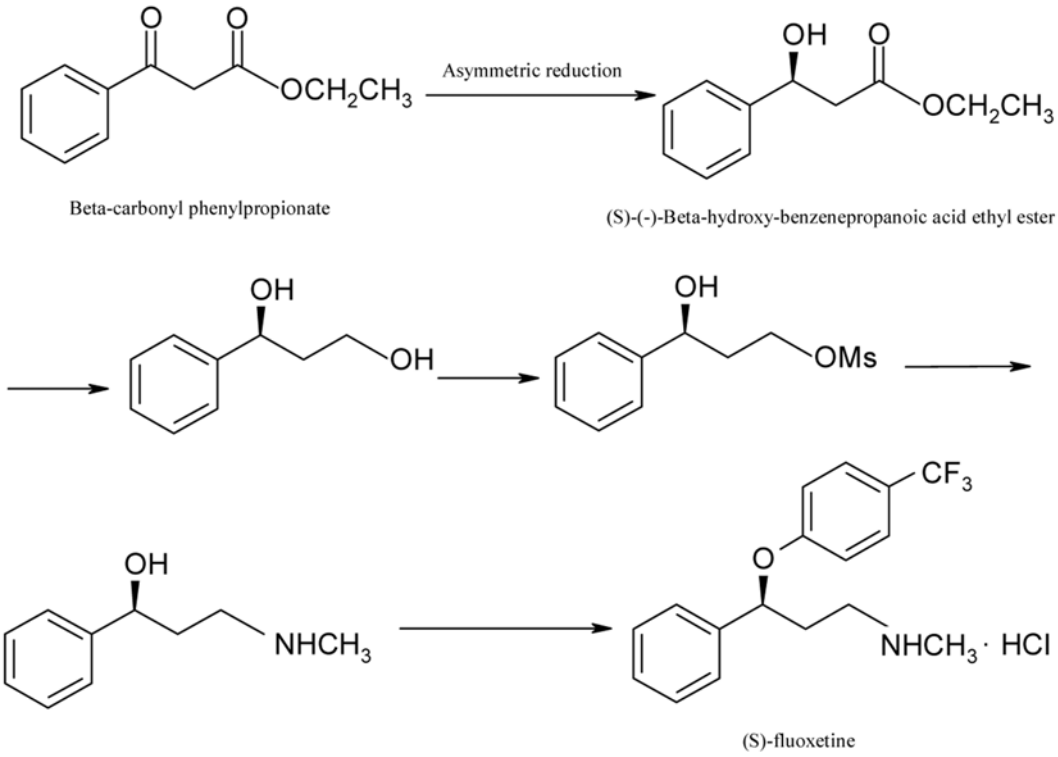

Fig. 1. Preparation of (S)-fluoxetine made from 3-oxo-3-phenylpropionic acid ethyl ester.

off and the product was extracted from the filtrate with n-hexane. The extract liquor was detected by HPLC.

4. Reduction of 3-Oxo-3-phenylpropionic Acid Ethyl Ester with Undifferentiated Cells of White Turnip in Phosphate Buffer/ Organic Solvent

Different organic solvent, such as n-hexane, cyclohexane, n-heptane, n-octane, dodecane, benzene, toluol, ethyl acetate, propanol, isopropanol and n-butanol, was respectively mixed with phosphate buffer. 2-6 g (dry weight) of undifferentiated cells and 3-oxo-3-phenylpropionic acid ethyl ester (0.108-0.846 mmol) were suspended in triangular flask (50 ml) contained 30 ml phosphate buffer/organic solvent. The reaction was carried out at 20-35 ºC and 100 rpm. After 13-17 days the cells were filtered off. The product was extracted from the filtrate and obtained by distilling. The product dissolved in n-hexane was detected by HPLC.

5. Analytical Method

Agilent 1200 series HPLC was used to detect the content of substrate and product. Chiral column Daicel OB-H was purchased from Daicel Chemical Industries Co., Ltd. and used to analyze the enantiomeric excess of Ethyl (S)-3-hydroxy-3-phenylpropionate. Mobile

Table 1. Reduction of 3-oxo-3-phenylpropionic acid ethyl ester in different medium

Reaction medium	Organic solvent $LogP_{oct}$	Conversion (%)	*e.e.* (%) of Ethyl (S)-3-hydroxy-3-phenylpropionate
Buffer	/	96.2	95.8
Phosphate buffer/n-hexane	3.4	62.7	96.2
Phosphate buffer/cyclohexane	1.5	50.4	96.0
Phosphate buffer/n-heptane	4	72.8	97.3
Phosphate buffer/n-octane	4.5	83.2	98.2
Phosphate buffer/dodecane	6.6	92.1	99.2
Phosphate buffer/benzene	2	0	/
Phosphate buffer/toluol	2.5	0	/
Phosphate buffer/ethyl acetate	0.68	0	/
Phosphate buffer/propanol	0.28	0	/
Phosphate buffer/isopropanol	0.28	0	/
Phosphate buffer/n-butanol	0.8	0	/

Reaction conditions: The content of organic solvent is 10% (v) in 30 ml phosphate buffer (0.2 mol/L, pH 7.0)/organic solvent. The initial substrate concentration is 3.6 mmol/L. The dry weight of undiferentiated cells is 2 g. 27 ºC. 100 rpm. 15 d

phase was composed of n-hexane and isopropanol (80 : 20). The flow rate of mobile phase was 1 ml/min. The injection volume was 5 μl. Column temperature was 30 ºC and UV wave length was 220 nm.

RESULTS AND DISCUSSION

1. Comparison of Reduction in Phosphate Buffer and in Phosphate Buffer/organic Solvent

The plant cells have different reduction activity in different medium (Table 1). The higher enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate can be acquired in phosphate buffer/organic solvent than in phosphate buffer. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate reached 99.2% in phosphate buffer/dodecane. The optimal phosphate buffer/organic solvent as reaction medium can improve the stereoselectivity of reduction. The conversion gained in buffer is higher than in phosphate buffer/organic solvent. The conversion increased with the $LogP_{oct}$ of organic solvent increase. Benzene and toluol is probably so toxic for plant cells that the substrate cannot be reduced. When $LogP_{oct}$ of organic solvent is too low, the polarity of organic solvent is so high that the plant cells probably lose the reduction activity.

2. Effect of Dodecane Content in Phosphate Buffer/Dodecane on Reduction

Dodecane content in phosphate buffer/dodecane has significant effect on reduction (Fig. 2). The conversion decreased with the dodecane content increase. The conversion reached 92.1% at 10% (v) dodecane in phosphate buffer/dodecane. More amount of dodecane in phosphate buffer/dodecane is not a benefit for high conversion because the substrate has better solubility in dodecane than in phosphate buffer so that the substrate cannot contact with plant cells well and be reduced. The enantiomeric excess increased with the dodecane content increase. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate gets to 100% at 30% (v) of dodecane. It is good for higher enantiomeric excess to raise the content of dodecane because the reduction activity of plant cells that reduce substrate to (R)-configuration product probably can be inhibited partly by dodecane.

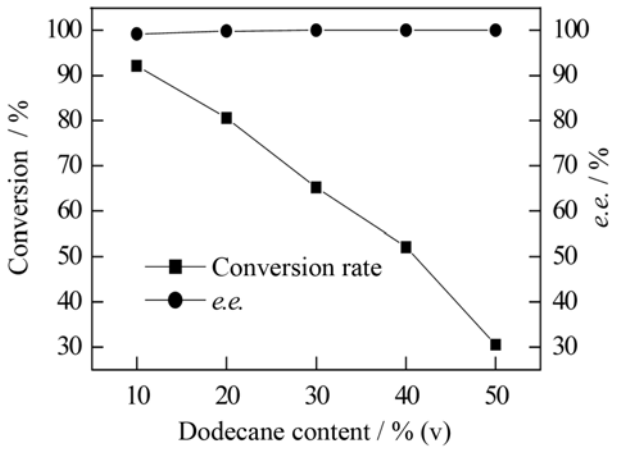

Fig. 2. Effect of dodecane content in phosphate buffer/dodecane on reduction of 3-oxo-3-phenyl propionic acid ethyl ester (Reaction conditions: The phosphate buffer is 0.2 mol/L and pH 7.0. The initial substrate concentration is 3.6 mmol/L. The dry weight of undiferentiated cells is 2 g. 27 ºC. 100 rpm. 15 d).

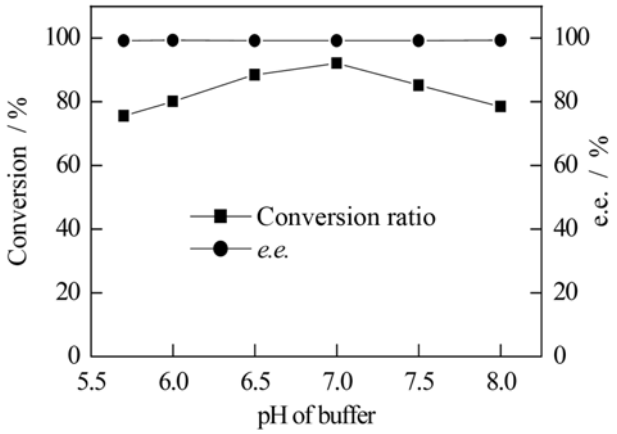

Fig. 3. Effect of phosphate buffer pH on reduction (Reaction conditions: The initial substrate concentration is 3.6 mmol/L. The content of dodecane is 10% (v) in 30 ml phosphate buffer (0.2 mol/L)/dodecane. The dry weight of undiferentiated cells is 2 g. 27 ºC. 100 rpm. 15 d).

3. Effect of pH Value of Phosphate Buffer on Reduction

The plant cells should be suspended in optimal buffer to ensure reduction activity effectiveness (Fig. 3). The optimal pH of phosphate buffer in phosphate buffer/dodecane is 7.0. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate is about 99.2± 0.5% at pH 5.6-8.0 of phosphate buffer. The pH of phosphate buffer has not significant effect on the configuration of product.

4. Effect of Co-substrate on Reduction

Appropriate co-substrate was favored for high conversion (Fig. 4). Different concentrations of methanol, ethanol, propanol and butanol were selected as co-substrate to study the effect of co-substrate on reduction. The result showed that addition of propanol and butanol

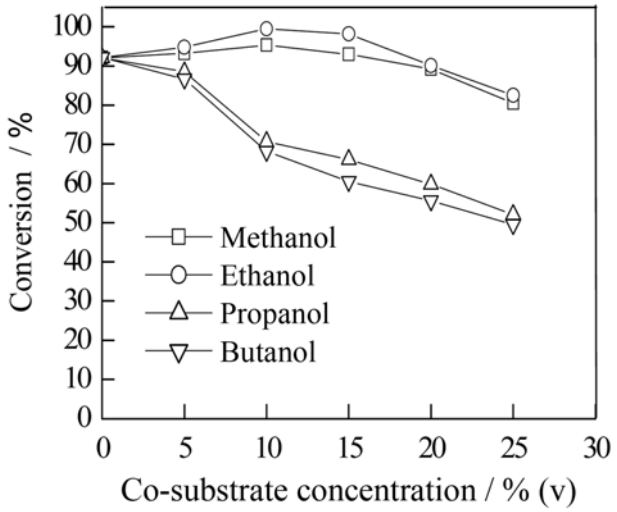

Fig. 4. Effect of cosubstrate on reduction (Reaction conditions: The content of dodecane is 10% (v) in 30 ml phosphate buffer (0.2 mol/L, pH 7.0)/dodecane. Initial substrate concentration is 3.6 mmol/L. The dry weight of undiferentiated cells is 2 g. 27 ºC. 100 rpm. 15 d).

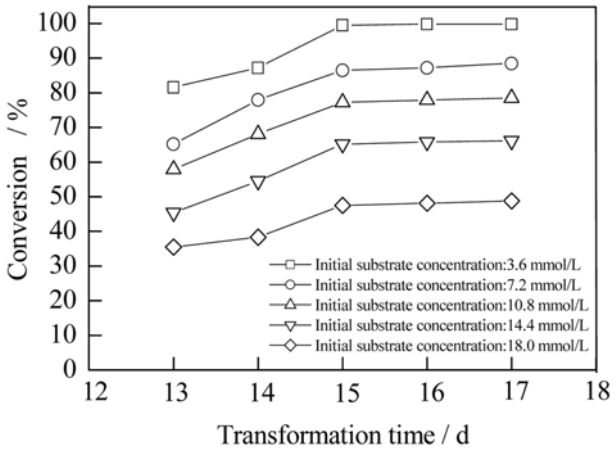

Fig. 5. Effect of initial substrate concentration on reduction (Reaction conditions: The content of dodecane is 10% (v) in 30 ml phosphate buffer (0.2 mol/L, pH 7.0)/dodecane. Cosubstrate is 10% (v) ethanol. The dry weight of undiferentiated cells is 2 g. 27 ºC. 100 rpm).

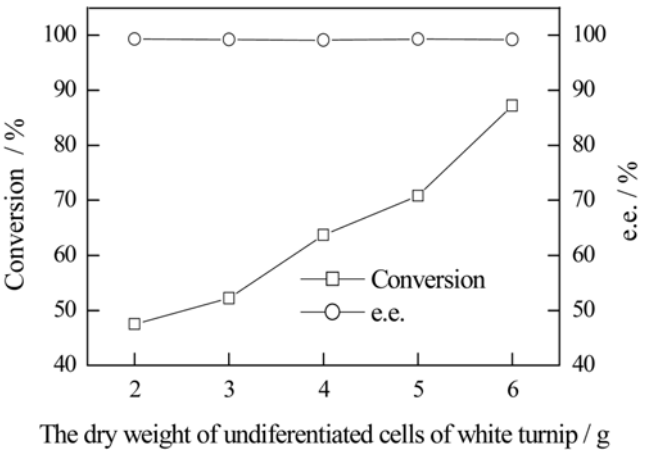

Fig. 6. Effect of biomass of undiferentiated cells of white turnip on reduction (Reaction conditions: The content of dodecane is 10% (v) in 30 ml phosphate buffer (0.2 mol/L, pH 7.0)/ dodecane. Cosubstrate is 10% (v) ethanol. Initial substrate concentration is 18.0 mmol/L. 27 ºC. 100 rpm. 15 d).

cannot improve the conversion. Higher conversion was achieved with 5-15% methanol and ethanol as co-substrate than without addition of co-substrate. The conversion reached 99.5% with 10% ethanol as co-substrate. Ethanol can promote the permeability of plant cells and greater amount of substrate and product can easily permeate the cell membrane. The content of NADH is enough for reduction and contributes to improve the conversion because NADH as hydrogen donor for reduction can be regenerated by alcohol dehydrogenase existing in cells with ethanol as substrate. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate is about 99.2±0.5% with addition of different co-substrate. The configuration of product was not influenced observably by co-substrate in the experiment.

5. Effect of Initial Substrate Concentration and Transformation Time on Reduction

The conversion increased with the transformation time increase (Fig. 5). It was enough to get good conversion for 15 days. The conversion did not rise too much after 15 d probably because the reduction activity of plant cells was lost. The conversion decreased with the initial substrate concentration increase. The inhibition of substrate on reduction was not observed at 3.6-18.1 mmol/L of initial substrate concentration. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate was 99.2±0.2% at experimental initial substrate concentration range.

6. Effect of Biomass of Undifferentiated Cells of White Turnip on Reduction

The biomass of undifferentiated cells of white turnip used in reduction has significant effect on conversion (Fig. 6). More addition of plant cells can improve conversion. The content of reduction enzyme and coenzyme NAD(P)H increased with more biomass of plant cells added in reduction so that the higher conversion could be acquired. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate was 99.2±0.5% at 2-6 g (dry weight) of undifferentiated cells.

7. Effect of Temperature on Reduction

The higher conversion can be achieved at optimal temperature

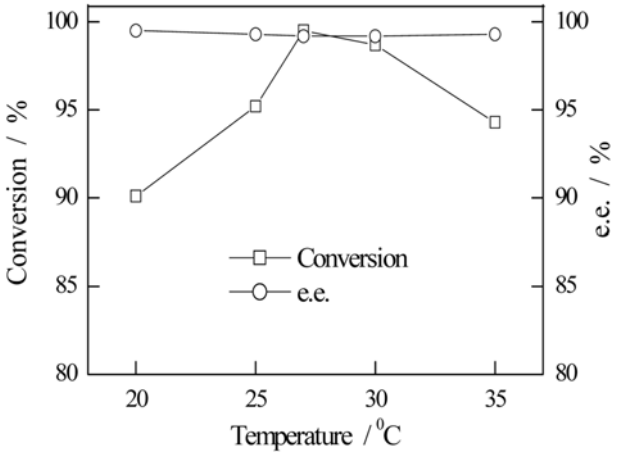

Fig. 7. Effect of temperature on reduction (Reaction conditions: The content of dodecane is 10% (v) in 30 ml phosphate buffer (0.2 mol/L, pH 7.0)/dodecane. Cosubstrate is 10% (v) ethanol. Initial substrate concentration is 3.6 mmol/L. The dry weight of undiferentiated cells is 2 g. 100 rpm. 15 d).

(Fig. 7). The optimal temperature is 27 ºC. The catalytic activity of plant cells decreased when the reduction happened at above 30 ºC. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate was 99.2±0.5% at 20-35 ºC.

8. Reutilization of Undifferentiated Cells of White Turnip

The plant cells were harvested by centrifugation to reuse in reduction for next time (Fig. 8). The plant cells lose catalytic activity with the increase of reuse time and can be reused well in reduction for three times. After the third time, the conversion dropped sharply. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate was 99.2±0.5% every time.

9. Effect of Allyl Bromide on Reduction

It was reported that addition of allyl bromide can improve the selectivity of reduction [19]. 1% (v) of allyl bromide can effectively inhibit R-enzyme activities in plant cells (Fig. 9). The conversion

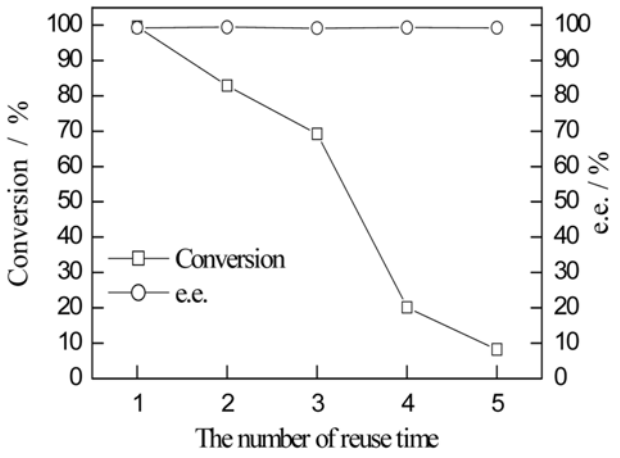

Fig. 8. Effect of reuse time on reduction (Reaction conditions: The initial substrate concentration is 3.6 mmol/L. The content of dodecane is 10% (v) in 30 ml phosphate buffer (0.2 mol/L, pH 7.0)/dodecane. The dry weight of undiferentiated cells is 2 g. Cosubstrate is 10% (v) ethanol. 27 °C. 100 rpm. 15 d).

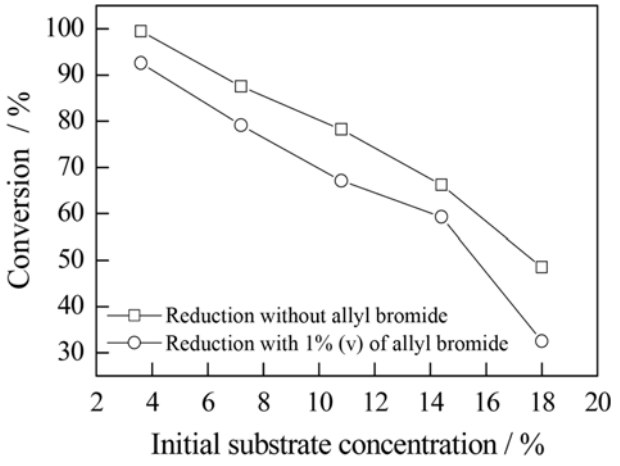

Fig. 9. Effect of allyl bromide on reduction (Reaction conditions: The initial substrate concentration is 3.6 mmol/L. The content of dodecane is 10% (v) in 30 ml phosphate buffer (0.2 mol/L, pH 7.0)/dodecane. The dry weight of undiferentiated cells is 2 g. Cosubstrate is 10% (v) ethanol. 27 °C. 100 rpm. 15 d).

gained in reduction with addition of allyl bromide was lower than that gained without allyl bromide. The enantiomeric excess of ethyl (S)-3-hydroxy-3-phenylpropionate reached 100% with 1% (v) of allyl bromide as inhibitor.

CONCLUSIONS

Synthesis of chiral compounds with plant cells as catalyst is low cost and environmentally friendly. Ethyl (S)-3-hydroxy-3-phenylpropionate was synthesized by reduction of 3-oxo-3-phenylpropionic acid ethyl ester with undifferentiated cells of white turnip in phosphate buffer/organic solvent. The enantiomeric excess of (S)-(−)-Beta-hydroxy-benzenepropanoic acid ethyl ester gets to 100%. The type and content of organic solvent in phosphate buffer/organic solvent have a great effect on reduction. The organic solvent of high $LogP_{oct}$ and low toxicity shows preferable biocompatibility and can be used well in biotransformation with plant cells as catalyst. The 10% (v) of dodecane in phosphate buffer (0.2 mol/L, pH 7.0)/dodecane is beneficial for reduction.

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