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Journal of Molecular Catalysis B: Enzymatic 43 (2006) 133–136
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
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Preparation of (2*S*, 3*R*)-methyl-3-phenylglycidate using whole cells of *Pseudomonas putida*

GuoJun Zheng [a], Qipeng Yuan [a], Liu Yang [b], Xu Zhang [a], JianJun Wang [b,*], WanRu Sun [b]

[a] *State Key Laboratory of Chemical Resource, Beijing University of Chemic al Technology, Beijing 100029, China*
[b] *State Key Laboratory of Microbial Resource, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, China*

Available online 1 September 2006

Abstract

Preparation of (2*S*, 3*R*)-methyl 3-phenylglycidate via enantioselective hydrolysis of racemic phenylglycidate was carried out using whole cells of *Pseudomonas putida*. Under optimal conditions (2*S*, 3*R*)-methyl-3-phenylglycidate could be got with ee value 99 and 48% chemical yield.

Keywords: Clausenamide; (2*S*, 3*R*)-methyl-3-phenylglycidate; Kinetic resolution; Lipase

1. Introduction

Enantiomerically enriched phenylglycidates, which can be used as the building blocks of diltiazem and taxol have drawn much attention during the past decade [1–6]. Most of those researches were focused on the enantioselective synthesis of the 2R, 3S isomer via chemical or enzymatic methods [7–12]. Recently (2*S*, 3*R*)-methyl phenylglycidate has reportedly been prepared by enzymatic transesterification resolution methods in organic solvent, but with low to moderate ee value and it was difficult to separate the product from the reaction [13,14]. However, till now no report about enantioselectively hydrolysis of racemic 3-phenylglycidate to give its 2*S*, 3*R* isomer was found in the literature.

Racemic clausenamide which showed remarkable nootropic activity was isolated from dry leaves of Chinese traditional medicine *Clausena lansium* (lour) skeels [15]. The desired pharmacological effect resided only in its levo optical isomer [16]. Chemical enantioselective synthesis of (−) clausenamide was carried out employing (2*S*, 3*R*)-3-phenylglycidate as the starting material [17] (Fig. 1).

Herein we want to report the kinetic resolution of racemic 3-phenylglycidate via enzymatic enantioselective hydrolysis using the free cells of *Pseudomonas putida*. It can enantioselectively catalyze the hydrolysis of methyl 3-phenylglycidate to (2R, 3S)-3-phenylglycidic acid which is unstable and decompose to phenylacetaldehyde, meanwhile (2*S*, 3*R*)-methyl 3-phenylglycidate is left behind and can be recovered easily [1].

2. Materials and methods

Racemic and authentic (2*S*, 3*R*)-methyl 3-phenylglycidate were prepared according to literature [17]. Methyl phenylcinnate was purchased from ACROS Company. All other chemicals were all in analytical grade and obtained from commercial sources. Chiral GC analysis was performed on HP25890 serial equipped with 3365 workstation, and G-TA column which modified by 2,6-dipentyl-3-trifluoroacetyl cyclodextrin was purchased from American Advanced Separation Technologies Co.

Optical rotations were determined on a Perkin-Elmer automatic polarimeter at room temperature.

2.1. Enantiomer separation

The optical purity (ee value) was determined by chiral GC on HP25890 serial (Hewlett-Packard Company USA) equipped with 3365 workstation, using methyl phenylcinnamate as internal standard; G-TA (Advanced Separation Technologies Co. Ltd.) column length 10 m; nitrogen as carrier gas; fluid rate 2.5 ml/min; sample concentration 2 μg/ml; oven temperature 105 °C; temperature of the injection chamber 160 °C and

* Corresponding author. Tel.: +86 10 62 587 206
E-mail address: wangjj@sun.im.ac.cn (J. Wang).

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doi:10.1016/j.molcatb.2006.07.010

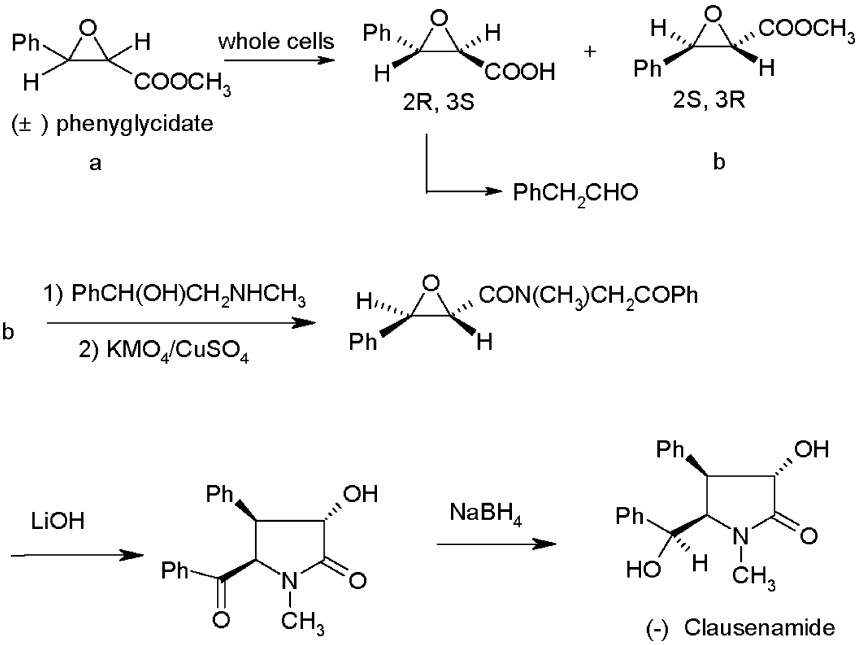

Fig. 1. Synthesis of (−) clausenamide starting from (2*S*, 3*R*)-methyl 3-phenylglycidate.

detector temperature 200 °C. Their retention times were 8.451 min for (2R, 3S) isomer, and 9.285 min for (2*S*, 3*R*) isomer.

2.2. Typical procedure for hydrolysis of racemic methyl 3-phenylglycidate

The producing medium consisted of glucose 20 g/L, peptone 20 g/L, olive oil 0.2%, Tween 2% in 1 L distilled water; pH was adjusted to 6.5. 5 mL sterilized (120 °C for 30 min) medium was inoculated with *P. putida* and grown for 16 h at 30 °C, then the 0.2 mL culture was added to 30 mL same medium and grown for a further 24 h at 30 °C, after the culture the microorganism was centrifuged, the cells were collected and suspended in 0.1 M phosphate buffer(pH 6.5), 50 mg substrate was added to the suspension and incubated for 24 h. After incubation the suspension was extracted three times with ethyl acetate. The organic extract were gathered and washed with saturated sodium bisulfate and then dried over with $MgSO_4$. After removing the solvent, the product was obtained as colorless oil.

3. Results and discussion

In a preliminary experiment, several commercially available lipases were used for resolution by stirring racemic methyl 3-phenylglycidate in a biphasic solution (phosphate buffer pH 7.0 and Pr–I_2O) at 30 °C. it was disappointed that no hydrolytic reaction was observed with lipases such as PPL (porcine pancreas lipase), PLE (pig liver esterase), WGL (*wheat germ* lipase), etc.

Then around 300 isolates were employed for the hydrolysis, these isolates were screened from soil samples collected in Beijing, by the enrichment culture technique in minimal salt medium containing 1% olive oil as the sole source of carbon. One bacterial strain which showed high selectivity was selected for further catalysis after initial screening, and it was identified as *P. putida*. It was grown on a producing medium containing in (g/L) dextrin (1.5), peptone (2.5), Tween-80 (3), and olive oil (0.2) in phosphate buffer (pH 6.5) at 30 °C for 18 h, and then the cells were collected and washed with phosphate buffer (pH 6.5) and used for the next step.

The transformation was proceeded in 5 mL phosphorate buffer with pH 6.5 at 30 °C for 12 h. The cell of *P. putida* occupies high activity and stereoselsectivity. After the reaction, the ester was recovered with 48% yield and 99% ee value. $[\alpha]_D^{20} = +171$ (*c* 1.0 $CHCl_3$) (lit. [17] $[\alpha]_D^{20} = +171.2$ (*c* 1.13 $CHCl_3$)). Compared with the authentic sample on chiral GC, the absolute configuration of the ester was assigned as 2*S*, 3*R*.

Then the effects of pH and temperature on the enantioselectivity of the lipase were studied. pH was optimized by running reactions at a range of pH from 6 to 8. Finally pH 6.5 was determined to be the optimal value for this reaction (Fig. 2). Hydrolysis of methyl 3-phenylglycidate catalyzed by this lipase was investigated at different temperature (20–42 °C). The highest ee (>99%) was obtained at 30 °C; meanwhile the yield can reach 46.2%. Considering the yield and ee value of the product, 30 °C was chosen for further study (Fig. 3).

Next, the concentration of substrate was investigated to find out the optimal concentration for this transformation reaction. As depicted in Fig. 4, the highest ee and yield were obtained at concentration of 1%.

The effects of solvents on the selectivity of the whole cell in the transformation of methyl 3-phenylgyliate were studied as shown in Table 1. Sometimes the enzyme enantioselectivity in organic solvent has a correlation between enantioselectivity and the physicochemical properties such as log *P* (octanol–water partition coefficient) and dielectric constant of the solvent [18]. There are complete lacks of the correlation for this *P. putida* lipase and that it might be caused by the interaction between the

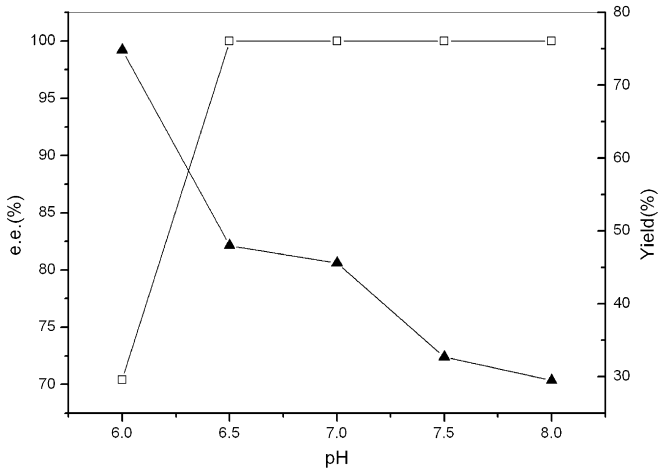

Fig. 2. Effect of pH on ee and yield (the transformation was carried in 0.1 M phosphate buffer pH range 6–8, at 30 °C, with substrate concentration 1%). Others were same as the procedures in Section 2.2. (▲) ee; (□) conversion.

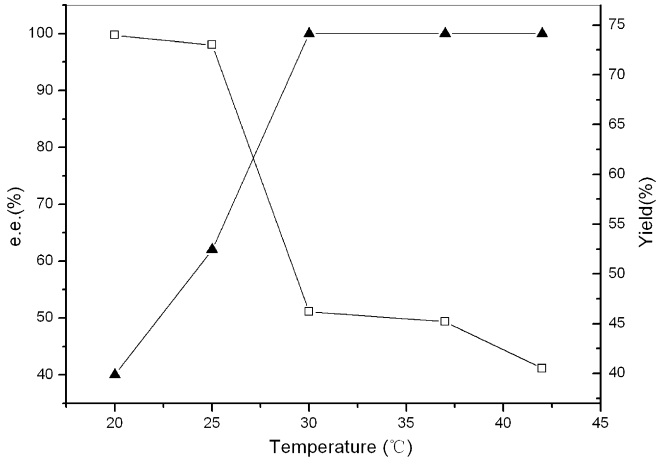

Fig. 3. Effect of temperature on ee and yield (the transformation was carried in 0.1 M phosphate buffer pH range 6.5, with substrate concentration 1% at temperature range from 20 to 42 °C). Others were same as the procedures in Section 2.2. (▲) ee; (□) conversion.

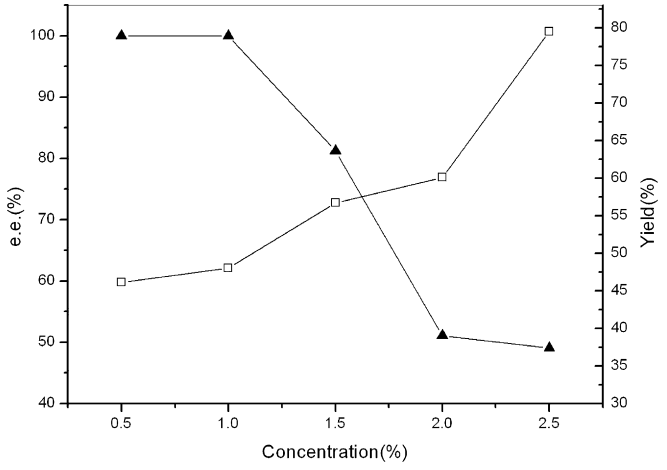

Fig. 4. Effect of substrate concentration on ee and yield (the transformation was carried in 0.1 M phosphate buffer pH range 6.5, at 30 °C). Others were same as the procedures in Section 2.2. (▲) ee; (□) conversion.

Table 1
Effect of different organic solvents on resolution

Solvent	Yield (%)	ee (%)	log *P*	Dielectric constant
Ethyl acetate	91	9.6	0.73	6.0
Toluene	41	41.0	2.5	2.4
Benzene	70	67.0	2.0	2.3
Isopropyl ether	48	99	1.9	3.9
Dichloro methane	0	0	1.25	10.3
1,2-Dichloro ethane	95	0	1.48	10.7
Xylene	0	0	3.15	2.4

enzyme and the solvent aside from the interaction of enzyme and substrate [18]. Among all the solvents tested, isopropyl ether was the only solvent of choice.

Since the substrate and product are insoluble in water, addition of surfactants was tested. Surfactants could upgrade the selectivity of the lipase by providing a large interfacial area [19], adding 1% Tween-80 to the reaction system could enhance the dissolving ability of the substrate significantly, meanwhile the ee and yield were improved and no side effects observed.

4. Conclusion

We had developed a new procedure for the preparation of optically active (2*S*, 3*R*)-methyl 3-phenylglycidate, via the microbial enantioselective hydrolysis of the corresponding ester. The lipase of the cell from *P. putida* showed high enantioselectiviy to racemic methyl 3-phenylglycidate. The ee and yield of (2*S*, 3*R*)-methyl 3-phenylglycidate can reach 99 and 48%, respectively, at 30 °C, in phosphate buffer (pH 6.5) with the substrate concentration of 1%. Due to the potential of its application in the production of chiral drugs, immobilized of the whole cell and purification of the lipase are underway.

Acknowledgements

This work is supported by 973 program and foundation of State Key Laboratory of Microbial Resource, CAS.

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