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Epoxide Hydrolase-catalyzed Resolution of Ethyl 3-Phenylglycidate

Cong Fa LI[1, 2], Qiao LIU [1], Hong Jie CUI[3], Di DING [1], Ai Guo JI [3], Yin Bo QU [1,*]

[1] State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100
[2] College of Engineering, South China University of Tropical Agriculture, Hainan 571737
[3] College of Pharmacy, Shandong University, Jinan 250012

Abstract: Epoxide hydrolase-catalyzed resolution of ethyl 3-phenylglydidate was investigated using resting cells of *Pseudomonas* sp. BZS21. Under the present conditions 26.2 % of (2R, 3S)-ethyl 3-phenylglycidate with ee value of 94.6 % was obtained from the racemic mixture.

Keywords: Epoxide hydrolase, ethyl 3-phenylglycidate, resolution, asymmetric hydrolysis.

Optically active glycidates are extremely important compounds in the synthesis of biologically active products. In particular,（2R, 3S）-3-phenylglycidate is an efficient and practical intermediate for taxol and taxotere side chains synthesis[1, 2]; (2S, 3R)-4-phenyl-2, 3-epoxybutanoate is an important intermediate for the synthesis of aminopeptidase N inhibitors with potent anticancer activity, including bestatin, phebestin, probestin, MR-387 and so on.

Epoxide hydrolases are ubiquitous in nature, and require no cofactors for their activity. Depending on the substrates, microbial epoxide hydrolases may show high enantioselectivity as well as high activity. As a consequence, they maybe enable to use for the preparation of enantiopure epoxides and/or vicinal diols in a very simple way from cheap and easily available racemic epoxides[3].

With this in mind, we attempted to screen microbial strains producing epoxide hydrolase with high enantioselectivity towards glycidates. And finally an epoxide hydrolase-producing bacterium was isolated from oil-contaminated soil samples. The bacterium was identified as *Pseudomonas* sp. BZS21. It enantioselectively catalyzed the hydrolysis of *trans* ethyl 3-phenylglycidate with retaining（2R, 3S）- ethyl 3-Phenyl-glycidate, and it also catalyzed the asymmetrical hydrolysis of (2S, 3R)-4-phenyl-2, 3-epoxy butanoate (the figuration of the retained glycidate was unidentified). As far as we know, this is the first report on obtaining enantiopure （2R, 3S）- ethyl 3-phenyl glycidate through asymmetrical hydrolysis of racemic *trans*-ethyl 3-phenylglycidate by using epoxide hydrolase.

The isolated bacterium was cultivated in 300 mL of fermentation medium

* E-mail: quyinbo@sdu.edu.cn

containing 1 % sucrose, 1.5 % urea, 0.2 % beef extract , 0.2 % peptone, 0.155 % K_2HPO_4, 0.085 % NaH_2PO_4，0.02 % $MgSO_4 \cdot 7H_2O$，and 10 mg/L $CaCl_2 \cdot 2H_2O$，1.0 mg/L $FeSO_4 \cdot 7H_2O$，0.1mg/L $ZnSO_4$，pH 7.0. The cells were harvested by centrifugation and washing after cultivation of 30 h at 30 ℃ and 130 r/min. The cells were resuspended in 18 mL of 100 mmol/L phosphate buffer (pH 8.0). Then 2 mL of 2 % (104.2 mmol/L) ethyl 3-phenyl glycidate in acetone was added. The mixture was shaken at 130 rpm and 30 ℃ for 12 h. Then some sodium chloride was added into the reaction system and the mixture was extracted with 2 times volume of *n*-hexane (containing styrene as internal standard)[4]. The organic phase was dried over anhydrous sodium sulfate and then centrifuged before injection into the chiral column.

The samples were assayed by HPLC. The optical purity was determined on Chiralcel OD column (250 x 4.6 mm, Daicel Co., Japan.). The mobile phase was *n*-hexane/2-propanol (98.7/1.3) and the flow rate was 1 mL/min. The column temperature was 25 ℃. Detection was made at wavelength of 220 nm using DAD detector. The retention times of（2R, 3S）ethyl 3-phenylglycidate and its enantiomer（2 S, 3 R）ethyl 3-phenylglycidate were 16.4 min and 18.3 min, respectively.

According to the results of HPLC assay, 26.2 % of(2R, 3S)ethyl 3-phenyl glycidate was recovered with ee value of 94.6 %.

In conclusion, the present investigation provides a new method for the resolution of racemic glycidates. Further studies to expand the application scopes of epoxide hydrolase from *Pseudomonas* sp. BZS21 and improve the yields of desired products are in progress.

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