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Stereoselective determination of 2-benzamidomethyl-3-oxobutanoate and methyl-2-benzoylamide-3-hydroxybutanoate by chiral high-performance liquid chromatography in biotransformation

Xiang Chen[a,b], Yu-Guo Zheng[a,b,*], Zhi-Qiang Liu[a,b], Li-Hui Sun[a,b]

[a] Institute of Bioengineering, Zhejiang University of Technology, Hangzhou 310014, PR China
[b] Engineering Research Center of Bioconversion and Biopurification, Ministry of Education, Zhejiang University of Technology, Hangzhou 310014, PR China

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ABSTRACT

(*2S, 3R*)-methyl-2-benzamidomethyl-3-hydroxybutyrate (MBHB) is a key intermediate in the synthesis of 4-aceoxyazetidinone, a building block for the production of penems and carbapenems. More attentions have been paid to screen biocatalysts achieving asymmetric preparation of (*2S, 3R*)-MBHB. In this study, an improved chiral high-performance liquid chromatographic (HPLC) method was developed for the stereoselective determination of 2-benzamidomethyl-3-oxobutanoate (BMOB) and MBHB, and further employed into the biotransformation of BMOB. Chiral separation was achieved within 12 min on Chiralpak AY-H column, which was faster and more suitable for screening biocatalysts exhibited reduction activity and (*2S, 3R*)-stereospecificity toward BMOB than on other columns. Ultimately, a new strain, *Burkholderia gladioli* ZJB-12126 capable of reducing BMOB to (*2S, 3R*)-MBHB was successfully isolated based on this newly constructed HPLC method. Samples were prepared by liquid–liquid extraction system using ethyl acetate as the extractor solvent. The extraction recoveries of BMOB and MBHB isomers ranged from 91.6 to 94.1% with relative standard deviation (RSD) below 10%. Linear calibration curves were obtained in the concentration range of 50–5000 μg/mL for both BMOB and MBHB isomers, respectively. Intra-day and inter-day precisions and accuracy were below 15% for all isomers evaluated by RSDs and relative errors (REs), respectively. This novel method was demonstrated to be suitable for assessing the biotransformation process of BMOB.

1. Introduction

Chiral stationary phases (CSPs) based chromatography technology for enantioseparation is widely used for the preparation of chemicals. In the past decades, the chiral separation materials used for analysis in chromatographic technology have developed rapidly. Various derivates of amylose and cellulose had been used as CSPs in the high-performance liquid chromatography (HPLC). Recently, many derivatives of classical CSPs were developed as new CSPs due to their high specificity and enantioselectivity [1–5].

Methyl-2-benzamidomethyl-3-hydroxybutyrate (MBHB) (Fig. 1) has two chiral centers and four diastereomers consisting of (*2S, 3R*)-MBHB, (*2R, 3S*)-MBHB, (*2R, 3R*)-MBHB and (*2S, 3S*)-MBHB. Among these four diastereomers, (*2S, 3R*)-MBHB is the only optically active isomer used as a key intermediate in the synthesis of 4-aceoxyazetidinone (4-AA) (Fig. 1) [6,7], a building block for the production of penems and carbapenems [8,9], which are classified as a new-generation β-lactams antibiotics and considered the "first-line agents" for the treatment of severe nosocomial infections due to their antibacterial spectrum, antibacterial activity and stability to the most β-lactamases.

The traditional method to produce the (*2S, 3R*)-MBHB is the asymmetric reduction of prochiral ketone BMOB using chiral metal ligand catalysts such as (*R*)-BINAP-Ru complex, in which the chiral catalysts are costly and cause trace metal contaminations in the product as well as require harsh reaction conditions, limiting its industrial application [7,10–15]. In the past decade, biocatalysis has been an alternative for the synthesis of chiral chemicals compared to the traditional chemical method due to its instinctively high chemo-, region- and enantioselectivity under mild reaction conditions, in which biocatalysts displace reagents and chemocatalysts. Up to now, only a few biocatalysts are capable of catalyzing

* Corresponding author. Tel.: +86 571 88320630; fax: +86 571 88320630.
E-mail address: zhengyg@zjut.edu.cn (Y.-G. Zheng).

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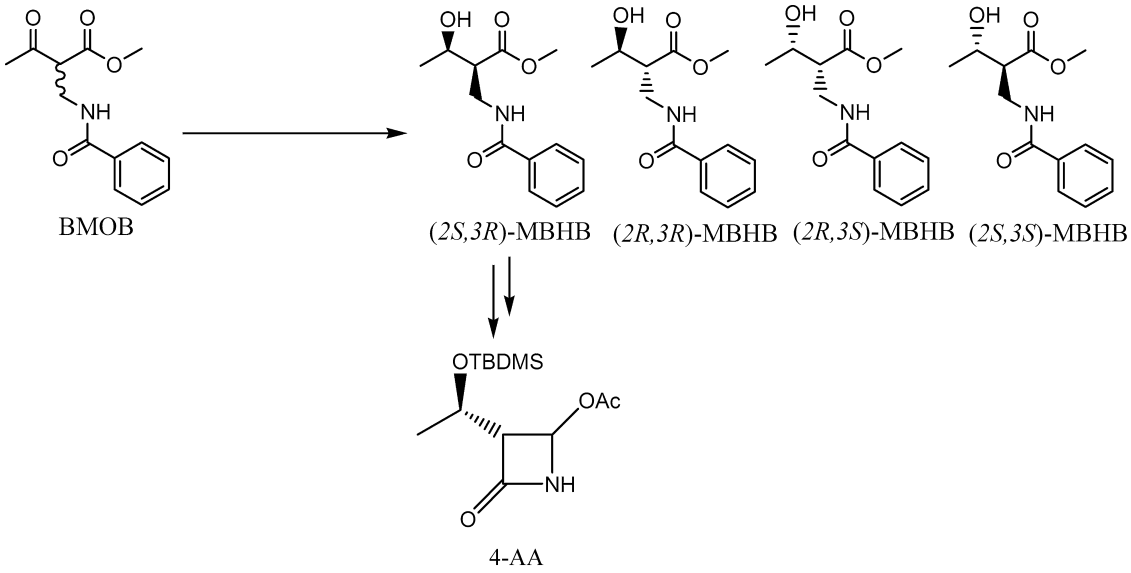

Fig. 1. Chemical structures of 2-benzamidomethyl-3-oxobutanoate (BMOB), four methyl-2-benzamidomethyl-3-hydroxybutyrate (MBHB) isomers and 4-aceoxyazetidinone (4-AA).

BMOB to give the single enantiomer of MBHB. Whole cells *Parthenocissus tricuspidata* and *Gossypium hirsutum* could catalyze the BMOB to produce (*2R, 3S*) and (*2S, 3S*)-MBHB, respectively, while these two isomers are useless in the synthesis of 4AA [16,17]. The *Lactobacillus brevis* JCM 1059 showed enantio- and diatereoselectivity for the production of (*2S, 3R*)-MBHB, whereas the enantiomeric excess (*ee*) and diasteromeric excess (*de*) were unsatisfactory (71.7% *ee*, 92.2% *de*). Using a short-chain alcohol dehydrogenase cloned from *L. brevis* JCM 1059, the enantioselectivity was improved significantly (99% *ee*) [18]. Thus exploiting novel biocatalysts for the synthesis of (*2S, 3R*)-MBHB is necessary and urgent.

Several chromatographic methods for the diastereo- and enantioselective separation of MBHB isomers have been reported [7,16,19,20]. Schneider and co-workers firstly described the diastereoselective resolution of (*2S, 3S*)-MBHB and (*2R, 3S*)-MBHB isomers by thin-layer chromatography (TLC) using toluene/ethyl acetate (1:2, v/v) as eluent [19]. Then indirect HPLC using sample derivatization with (+)-(*R*)-α-methoxy-α-(trifluoromethyl) phenylacetic acid (MTPA) esters of MBHB prior to injection onto achiral chromatography columns, Nucleosil 100-3 and Deverosil 100-3 [16,20], has been described for the enantioselective separation of MBHB; however, it is inconvenient and time consuming thus not suitable for screening biocatalysts. Subsequently, direct HPLC methods have been developed for the enantioselective separation of MBHB [7] and structural analogs involved ethyl-2-benzoylamide-3-hydroxybutanoate [15,21,22], ethyl-2-benzamidomethyl-4-fluoro-3-hydrobutanoate [6] and ethyl-2-(benzamidomethyl)-3-oxo-phenyl propanoate [15], using Chiralcel OD column, Chiralpak AD column or Chiralpak IA column. Although the *ee* and *de* of single enantiomer MBHB could be determined on Chiralpak AD and Chiralpak IA columns, there are no reports on the elution order and retention times of MBHB isomers as well as the chiral simultaneous determination of four MBHB isomers and two BMOB enantiomers in biotransformation. In this study, we tested five CSPs involving Chiracel OD-H, Chiralpak IC, Chiralpak IA, Chiralpak AD-H and Chiralpak AY-H columns in the chiral separation of two BMOB and four MBHB isomers in order to establish an improved direct chiral HPLC method for the isolation of biocatalysts exhibiting reduction activity and (*2S, 3R*)-stereospecificity toward BMOB. Amongst them, the Chiralpak AY-H column exhibited the fastest and accurate ability of separation. Using this method, a new strain, *Burkholderia gladioli* ZJB-12126, was successfully isolated, and the biotransformation process of BMOB by this strain was also assessed and optimized.

2. Materials and methods

2.1. Materials and reagents

BMOB was synthesized as racemates by nonstereoselectively synthetic method [23], MBHB was obtained by reduction of BMOB with $NaBH_4$. *n*-Hexane, methanol, isopropanol and ethanol (Merck, Darmstadt, Germany) were used for preparation of mobile phases or solutions. Water was purified with a Milli-Q plus system (Millipore, Bedford, MA, USA). All other chemicals used were analytical grade without purification.

2.2. Solutions

The stock solutions of BMOB and MBHB were prepared by transferring 500 mg of each to the mobile phase at the concentration of 5 mg/mL. The working solutions were obtained by dilution of stock solutions with the mobile phase at the concentrations of 1500, 500, 100 and 50 μg/mL for BMOB and MBHB, respectively.

2.3. Apparatus and conditions

Analytical HPLC was performed on five chiral columns including Chiracel OD-H (cellulose *tris*-(3,5-dimethylphenylcarbamate), 250 × 4.6 mm^2, 5 μm), Chiralpak IC (cellulose *tris*-(3,5-dichorophenylcarbamate), 250 × 4.6 mm^2, 5 μm), Chiralpak AD-H (amylose *tris*-(3,5-dimethylphenylcarbamate), 150 × 4.6 mm^2, 5 μm), Chiralpak IA (amylose *tris*-(3,5-dimethylphenylcarbamate), 250 × 4.6 mm^2, 5 μm) and Chiralpak AY-H (amylose *tris*-(5-chloro-2-methylphenylcarbamate), 250 × 4.6 mm^2, 5 μm) (Daicel Chemical IND., Japan). HPLC apparatus consisted of a Model LC-20AT HPLC system (Shimadzu, Kyoto, Japan) equipped with an SPD-20A ultraviolet (UV) detector operating at 254 nm. Data analysis was carried out on LC solution software (Shimadzu, Kyoto, Japan) [24]. Typical operating temperature, pressure and flow rate of the above-mentioned columns are 0–40 °C, 5 MPa (maximum) and 1.0 mL/min, respectively, following the manufacturer's instructions. The mobile phase is based on different quantitative ratio of alkane and alcohol. In this study, mobile phase